Equine Herpes Virus 1 and 4

Introduction

There are at least 5 types of equine herpes viruses, three alpha herpes viruses (1, 3 and 4) and two gamma herpes viruses (2 and 5). The alpha herpes viruses are clearly responsible for clinical disease with EHV-1 associated diseases including abortion, respiratory, neurological - equine herpes myeloencephalopathy (EHM) and pulmonary vasculotropic diseases; EHV-3 is the cause of coital exanthema, while EHV-4 causes respiratory disease in young horses. The true pathogenic significance of the gamma herpesviruses EHV-2 and 5 are poorly characterised, although recently a syndrome of multinodular equine pulmonary fibrosis has been associated with EHV-5 infection. Primary infection with either EHV-1 / EHV-4 occurs via direct horse-to-horse contact and inhalation of aerosolised virus. For suckling foals the primary source of infection are their dams and other mares. Following initial replication in the respiratory epithelium and drainage to regional nodes, a cell-associated viraemia develops. This cell-associated viraemia is of particular importance in EHV-1 infections with systemic spread from the respiratory system, within lymphocytes to placenta, foetal and CNS tissues. At these sites local vasculitis and thrombosis may lead to abortion, neonatal mortality and myeloencephalopathy. EHV-4 on the other hand tends to remain limited to the respiratory tract. Lifelong latent infection is well documented with the alpha herpes viruses.

Sample Guide

Equine Herpes Virus-1/4 – antigen detection

Polymerase Chain Reaction

This is the diagnostic test of choice due to high analytical sensitivity and specificity and rapid turn around time. Positive results are still possible in cases negative on virus isolation due to low viral load.
• **Abortion investigation**: Foetal post mortal tissues (liver, lung, thymus) plus nasal swabs in PBS and EDTA blood from the mare are submitted.

• **Respiratory / neurological disease**: Simultaneous running of PCR on nasal or nasopharyngeal swabs and EDTA blood from live horses enables assessment of disease stage. Nasal / nasopharyngeal positive indicates virus shedding; EDTA blood positive indicates active viraemia. *Real-time PCR* can determine viral load and is therefore applicable to better characterising disease stage, assessment of risk of exposure and monitoring response to therapy.

• Due to the low level of virus in the **CSF** of equine herpes myeloencephalopathy (EHM) cases, PCR on CSF fluid is usually unrewarding.

*Note*: PCR is reserved for cases with clinical grounds to suspect EHV-1 infection and random testing or screening of healthy horses to try and determine latency status should be avoided.

All samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996 and transported at 4°C to the laboratory. (See Reference Ranges – Section 3).

**Immunohistochemistry**

Extremely useful diagnostic technique performed on formalin-fixed foetal tissues (liver, lung, adrenal, thymus and placenta). Test with high sensitivity and specificity, plus counterstaining with haematoxylin enables visualisation of the virus within pathological lesions. In this image above there is multifocal hepatic necrosis with dark brown granular staining of viral antigen in necrotic areas.
**Virus isolation**

Performed on foetal post mortal tissues (liver, lung, thymus, CSF, brain / spinal cord), nasal swabs in PBS and heparin blood samples. Time delay for results limits utility of virus isolation in outbreak management.

**EHV Antibody Detection – Serology**

**Compliment Fixation / Serum Neutralising Antibody**

These assays demonstrate the presence of antibodies against the virus in serum collected from the mare. A serum sample, collected in a red or yellow stopper tube, is required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

A 4 fold or greater increase in antibody titre on acute and convalescent samples collected 7-21 days apart provides presumptive evidence of EHV-1 infection. In outbreaks antibody detection can be used to screen for horses that have been exposed to the virus. Serology does not distinguish between antibodies to EHV-1 and EHV-4.
Cerebrospinal fluid (CSF) examination

Although PCR analysis for EHV-1 on CSF is of little diagnostic use, cytological evaluation of the CSF can provide useful diagnostic information. CSF is collected aseptically into a sterile syringe / fluid container or serum tube (without clot activator). Analysis of the CSF fluid includes viscosity, colour, specific gravity, protein content and a cytological smear examination. Xanthochromia (presence of bilirubin) is typical in horses with EHM confirming in vivo haemorrhage and not traumatic haemorrhage. When observed together with increased protein and monocytic pleocytosis, there is a high level of suspicion for EHM and running of PCR on nasal swabs and blood (EDTA) would be indicated.

Further Reading

African Horse Sickness (AHS)

Introduction

African horse sickness is a controlled disease in South Africa caused by the African horsesickness virus belonging to the genus *Orbivirus*, family *Reoviridae*. Nine different serotypes of AHS virus are known. It affects all species of equidae and is an infectious but not contagious insect born disease.

*Note:* Please refer to the Controlled and Notifiable Animal Diseases section for the procedure of result reporting.

Sample Guide

**AHS - Serum antibody testing**

**Complement Fixation Tests (CFT), Virus Neutralisation Tests (VN) and ELISA**

These assays demonstrate the presence of antibodies against the virus. A serum sample, collected in a red or yellow stopper tube, is required. Following collection, allow samples to stand for 20-30 minutes at room temperature to form a clot, after which they should ideally be centrifuged and the serum poured off. Serum or un-spun clotted blood should then be transported at 4°C to the laboratory. Separated serum may be frozen and stored at -20°C for testing at a later date.

Rising antibodies in acute and convalescent serum samples taken 3-4 weeks apart (> 4 fold increase in antibody titre = seroconversion) indicate AHS exposure and probable infection. Animals may start to develop antibodies within 8 –12 days post infection and a single CFT result of >1:8 is suggestive of possible exposure, but demonstrating seroconversion provides more definitive diagnostic evidence of exposure to field / vaccine virus

*Note:* Serology cannot distinguish between field virus and vaccine virus, although seroconversion with a >4 fold increase in antibody titre, is more consistent with field virus challenge. Always take into consideration the vaccination date when interpreting serology.
The **ELISA** is suitable for screening large numbers of animals and a competitive ELISA (suitable for use on crude or impure samples) has also been described for use in wildlife species. CFT antibody titres rise rapidly post infection and drop fairly rapidly following clearance of the infection and so high CFT titres indicate infection within the previous few months. VN and ELISA antibodies on the other hand remain at high levels for months or even years post infection.

**Virus neutralisation (VN)** is the method of choice for serotyping of AHS virus isolates.

**AHS – Viral antigen detection**

**Virus isolation**

Virus can be isolated from unclotted whole blood samples, taken in heparin during the febrile stage; or fresh lung, spleen and lymph node samples collected in a sterile manner, at post mortem.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Samples are stored and transported chilled at 4°C but **must not** be frozen, as AHSV is an RNA virus and easily damaged/inactivated with freeze / thawing.

*Note* there is a long turnaround time for results (usually 1-2 months) and costs are high.
Polymerase chain reaction (PCR)

A quantitative real-time PCR (RT-PCR) technique has been developed to rapidly detect AHS virus genetic material. This assay is a multiplex PCR based on 2 conserved genes common to all serotypes and is group specific for all AHSV serotypes with no cross reaction with any other orbiviruses. To distinguish between the vaccine and field virus strains additional steps are required. Being a quantitative PCR the RT-PCR can evaluate the actual AHS viral load in a particular sample.

Blood samples in EDTA / heparin (preferably both) or organ samples / swabs from post mortem (lung, spleen) are the preferred specimens. AHSV is an RNA virus and so sterility at sample collection is vital. Swabs should be placed in a viral transport medium or PBS and not the bacteriology gel. Do not freeze samples, freeze/thawing compromises the integrity of the orbiviral RNA.

Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. Specimens must be stored at 4°C and transported to the lab on ice, as quickly as possible.

*Note: Turnaround times are rapid, and the test is also able to detect non-viable AHS viral antigen.

Immunohistochemistry (IHC) and histopathology

IHC staining is a test with diagnostic specificity of a 100 % in formalin-fixed tissues such as the heart and lung, and 90% in the spleen. This assay detects the presence of viral antigen of all 9 AHSV serotypes and there is no cross reactivity with other orbiviruses. Detection of AHSV antigen by IHC staining is not affected by the duration of formalin fixation with good results.
being obtained in tissues fixed from 24 hours up to 365 days in formalin. Heart and lung are essential samples of any organ histology pool and are placed in 10% buffered formalin.

*Note: as IHC stains are performed on formalin-fixed tissues, ambient temperature and time taken to reach the laboratory are not issues.

Combining IHC staining with histopathology enables demonstration of the AHSV antigen within histological lesions confirming the diagnosis. A full set organ samples (brain, lung, heart, liver, spleen, kidneys, mediastinal lymph nodes, gastrointestinal sections, muscle, adrenal, subcutaneous tissue) are collected in 10% buffered formalin to confirm or exclude the presence of lesions consistent with AHS and to rule in or rule out other disease conditions. Any suspicion of possible AHS related pathology could then be confirmed by IHC.

**OIE guidelines.**
- Both the CFT and indirect ELISA are the prescribed tests for international trade.
- In outbreaks, especially outside enzootic regions, a combination of tests is advised, especially in the index case. A more rapid ELISA or PCR may be followed by viral isolation and serotyping by VN or typing by RT-PCR.

**Further Reading**

Equine Encephalitis (EE)

Introduction

Equine encephalitis (EE) is caused by equine encephalitis virus (EEV) belonging to the genus *Orbivirus*, family *Reoviridae*. EE is an insect transmitted disease of equids (horses and donkeys), infection usually resulting in mild or sub-clinical disease closely resembling African Horse sickness. Seven different serotypes of EEV are currently known.

Sample Guide

**EE Antibody Detection – Serology**

**Complement Fixation Tests (CFT) / Agar-Gel Immunodiffusion Tests (AGID) / ELISA**

These serological assays are employed to demonstrate antibodies against the virus. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory. Separated serum samples can be frozen at -18°C for testing at a later stage. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3).

Demonstrating a 4 fold or greater increase in antibody titres in acute and convalescent serum samples collected 3-4 weeks apart provides fairly convincing evidence of infection. An indirect, group-reactive ELISA is used in seroepidemiological surveys, as it is more reliable than either the CF or the agar gel immunodiffusion (AGID) tests, for the detection of IgG in sera. The competitive ELISA assay determines end-point titres in individual serum samples. Both these ELISA’s are specific and there are no cross-reactions with other orbiviruses.
**EE Viral Antigen Detection**

**Virus isolation**

Virus can be isolated from heparin blood samples collected during the acute febrile stage of clinical disease; or aseptically collected fresh post mortal tissues (lung, spleen, lymph node, thymus and brain). Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C. (See Reference Ranges – Section 3).

**Polymerase chain reaction (PCR)**

PCR techniques detect EEV in EDTA blood samples or fresh tissue specimens (lung, spleen, lymph node, thymus and brain) and distinguish it from other orbiviruses. These techniques are also capable of determining the particular serotype of EEV involved. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported to the laboratory at 4°C.
Pathology

The pathology caused by EEV is not pathognomonic, but it should be used in conjunction with PCR and virus isolation to confirm a diagnosis. A full set of organ samples (brain, lung, heart, liver, spleen, kidneys, mediastinal lymph nodes, gastrointestinal sections, muscle, adrenal, subcutaneous tissue) should be collected in 10% buffered formalin. African horse sickness remains the most common and important differential for EEV.

Further Reading

Equine Influenza (EI)

Introduction

Equine influenza (EI) is a controlled disease in South Africa. It is a highly contagious acute respiratory disease of horses, donkeys, mules and zebras, caused by two distinct subtypes of influenza A virus namely H7N7 and H3N8. This virus belongs to the genus *Influenzavirus*, in the family *Orthomyxoviridae*. Mutations of these subtypes may be seen, resulting in antigenic drift of viruses isolated in certain parts of the world.

*Note:* Please refer to the Controlled and Notifiable Animal Diseases section for the procedure of result reporting.

Sample Guide

**EI Antibody Detection - Serology**

**Haemagglutination Inhibition (HI) / Single Radial Haemolysis (SRH)**

Serology should be performed by testing paired (acute and convalescent) serum samples. The acute sample should be collected as soon as possible after the first typical clinical symptoms are observed, followed by a second approximately 2 weeks later. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory. Separated serum samples can be frozen at -18°C for testing at a later stage. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996. (See Reference Ranges – Section 3).

The HI test is subtype specific and far more sensitive for antibody to H7 viruses than to the H3 virus. Four-fold or greater increases in antibody levels between the acute and convalescent-phase sera tested by HI is indicative of infection. In sub-clinically infected animals, which are an important source of infection, a four-fold increase in HI antibody levels is not always detected on acute and convalescent sera. With the SRH assay a two-fold increase in antibody levels is consistent with exposure.
In vaccinated populations interpretation of serological results is complicated by the presence of vaccine-induced antibodies. Most vaccines in use are trivalent vaccines that contain one strain of the H7N7 virus and two strains of the H3N8 virus. Vaccination usually leads to significant increases in antibody titres to both subtypes of virus. In contrast, antibody production following infection is usually subtype-specific. The use of antibody responses to non-structural (NS) equine influenza viral protein to differentiate between vaccination and natural infection has been reported.

**EI Viral Antigen Detection**

**Virus isolation**

Nasopharyngeal swabs and / or tracheal lavages are the preferred samples for virus isolation, although virus can also be isolated from post mortal tissues. Samples for virus isolation must be collected as early as possible in the disease syndrome, as EI virus may only be excreted for as short as two days. PBS must be used as transport medium, while any media containing foetal calf serum should be avoided. Tracheal lavages can be collected into sterile serum tubes (without clot activator), sterile urine containers or sterile syringes. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C. (See Reference Ranges – Section 3).

*Note:* a diagnosis of Equine influenza virus infection can be confirmed by virus isolation or by the demonstration of seroconversion in response to infection, but ideally both methods should be used.
Polymerase Chain Reaction

Nasal swabs in PBS are the samples of choice although the assay may also be performed on nasal / tracheal lavages collected into sterile serum tubes (without clot activator), sterile urine containers or sterile syringes. An EI H3-specific TaqMan real-time PCR assay targeting the haemagglutinin (HA) gene of H3 strains, has thus far proven the most sensitive of the PCR assays. Samples are packed using the prescribed ‘Triple Packaging System’ as per regulations SABS 0229:1996, stored and transported at 4°C. (See Reference Ranges – Section 3).

Antigen Capture ELISA

Nasal swabs in PBS are the samples of choice. The antigen-capture enzyme-linked immunosorbent assay (ELISA) employs a monoclonal antibody against the EIV nucleoprotein. These assays are however generally less sensitive than other methods and expensive for screening large numbers of animals.
Pathology

A mortality rate are usually very low in uncomplicated cases and so post mortal material is usually only collected in complicated cases with secondary bacterial infection. Pathological findings need to be correlated to virus isolation and serology. A full set organ samples including brain, lung, heart, liver, spleen, kidneys, lymph nodes (pharyngeal and thoracic) should be collected in 10% buffered formalin to confirm the presence of lesions consistent with equine influenza and to rule in / rule out other disease conditions.

Further Reading

**Equine Piroplasmosis (Equine Babesiosis)**

**Introduction**

Equine piroplasmosis results from infection with either *Theileria equi* or *Babesia caballi* and is a tick-borne protozoan disease of horses, mules, donkeys and zebras with zebras acting as an important reservoir of infection in Africa. Clinical disease is characterized by fever, anaemia, icterus, other signs arising from haemolytic anaemia and abortion. This is a tick transmitted disease with over 14 species of ticks in the genera *Boophilus*, *Rhipicephalus*, *Hyalomma* and *Dermacentor* acting as vectors.

*Theileria equi* zygotes do not multiply in ticks and thus transovarial transmission is not thought to occur. Ticks transmitting this protozoan become infected as larvae and transmit the infection as nymphs or become infected as nymphs and transmit the infection as adults (transstadial transmission). *Babesia caballi* on the other hand multiply within ticks and invade many of the tick’s organs including ovaries with *B. caballi* being readily passed to the next generation of ticks in the egg (transovarial transmission). Equine piroplasmosis can also be transmitted between animals by contaminated needles and syringes or by blood transfusions.

Equids infected with *T. equi* appear to remain permanently infected with recovered animals becoming asymptomatic carriers. Parasitaemia is often absent in carriers but can reoccur after immunosuppression or strenuous exercise. Treatment can suppress clinical signs but is ineffective in clearing *T. equi* from carriers. The incubation period is 12-19 days. Animals infected with *B. caballi* can remain carriers for up to 4 years but might be able to clear the organisms eventually. Transplacental infection with *B. caballi* is rarely reported. Incubation period is 10-30 days.

Infection of pregnant mares with *Theileria equi* at any stage in pregnancy is quite common. The consequence of infection depends on the stage of infection but includes reproduction failure (involved in up to 11% of all failures in South Africa), abortion, neonatal piroplasmosis and birth of asymptomatic carriers. Infection with *Theileria equi* can be persistent with development of carrier mares, which can produce further infected foetuses, randomly in subsequent pregnancies. Aborted foetuses usually demonstrate anaemia, moderate to marked icterus, petechiae on visceral and serosal surfaces, hydrothorax, splenomegaly and hepatomegaly. Parasitaemia is usually high.
**Sample Guide**

**Blood smears:**

Identification of parasites in blood smears is often successful with *Babesia caballi* (large babesia), but more difficult with *Theileria equi* (small “babesia”) especially with low parasitaemias. The exception to the rule is neonatal piroplasmosis where high *T. equi* parasitaemias are observed - image above-right.

**Piroplasmosis Antigen Detection**

**Molecular diagnostics (Polymerase Chain Reaction)**

In *live animals* blood collected into EDTA or heparin are the preferred samples. Various DNA probes exist and are used in polymerase chain reaction (PCR) assays for both *T. equi* and *B. caballi*.

Other molecular methods include nested PCR, multiplex PCR and loop-mediated isothermal amplification (LAMP).

PCR has emerged as a useful tool in clinical cases where blood smears are inconclusive and is the assay of choice for identifying carriers.
In dead animals or aborted foetuses fresh tissues organ smears, blood or fresh spleen can be submitted for PCR.

**Piroplasmosis Antibody Detection – Serology**

**Compliment Fixation (CFT) / Immunofluorescent Antibody (IFA) / Competitive ELISA / Western Blotting**

Serology can be performed using compliment fixation tests, various ELISA’s, indirect fluorescent antibody (IFA) tests and Western blotting. Preferably serum samples should be collected from a pyrexic animal. Following collection samples are allowed to stand for 20 minutes at room temperature to enable good clot formation. Blood specimens are then preferably centrifuged and the serum poured off. Centrifuged serum or clotted whole blood is then transported at 4°C to the laboratory. Separated serum samples can be frozen at -18°C for testing at a later stage.

The IFA and Western blotting procedures are able to distinguish between *Theileria equi* and *Babesia caballi* infections. Serology will not identify asymptomatic carriers; it can only confirm exposure.

**Further Reading**

Equine Respiratory Disease

Equine respiratory disease has emerged as a major concern in performance horses of various disciplines, especially those horses stabled in large numbers for training purposes. Various clinical syndromes have earned different names over the years, although these names described disease phenotypes rather than specific disease entities. Controversy exists to this day as to precise definitions of some of these phenotypes. One entity that does appear to have general acceptance is recurrent airway obstruction (RAO) or heaves. RAO tends to occur in older horses and is described as an organic particle-dust-induced, reversible airway obstruction in which the neutrophil is the predominant inflammatory cell in bronchoalveolar lavage (BAL) fluid. Horses with RAO have clinical symptoms of respiratory distress at rest, and measurable evidence of airway obstruction. This may not be a single disease entity either however, with suspected ‘subsets’ or subtypes such as Summer Pasture Associated Obstructive Pulmonary Disease (SPAOPD) which is indistinguishable from RAO clinically, but only affects horses that are grazing rich summer pastures. These horses tend to have more severe airway inflammation than those with RAO.

Inflammatory airway disease (IAD) is differentiated from RAO most notably by the absence of respiratory abnormalities at rest. IAD is a phenotype of respiratory disease characterised by coughing in work, increased tracheobronchial mucous secretions and increased proportions of inflammatory cells in these fluids but no evidence of respiratory distress at rest or acute respiratory conditions. Controversy exists however, and a precise definition of the IAD phenotype is not available as yet. Initially it was limited to young performance horses but now is recognised in older horses too. Part of the proposed definition includes reduced respiratory function, which is often measured by reduced performance rather than more invasive and reproducible techniques, resulting in a lack of standardisation. Also controversy reigns with respect to bacterial lung infections and their role in IAD, with the ACVIM consensus statement in 2007 excluding infectious causes or any cases with pyrexia or haematological changes suggesting an infection but other authors including infectious causes. At the current time, until more research identifies distinct aetiopathogeneses and separate clinical entities, IAD should be used as an umbrella term for various clinical entities with the afore-mentioned phenotype. As the role of infectious and non-infectious agents in the pathogenesis of IAD has not been definitively determined, it is recommended both causes should be included until this matter is resolved.

Regardless of which disease phenotype is suspected, the most common and available diagnostic techniques are trans-tracheal aspirate or wash / tracheal lavage or bronchoalveolar lavage (BAL). It is now recognised that many young performance horses have increased proportions of neutrophils in tracheal secretions, even if they do not have IAD, and that horses in training have higher inflammatory cell numbers in tracheal wash (TW) samples than those not in training. Therefore, for purposes of diagnosing respiratory disease in horses, BAL samples are preferred but may not always be practical. However, interpretation of results from these two sampling methods is currently one of the most contentious areas regarding this syndrome. Each technique samples different areas of the lung, which influences the results obtained. BAL samples target small bronchioles with diameters <0.5mm and alveoli mainly in the dorso-caudal or diaphragmatic lung lobes. TW however samples the distal trachea and larger bronchi in the
cranio-ventral lungs. Discrepancies have been reported when both techniques are performed on the same horses.

These techniques therefore are not inter-changeable, and airway inflammation as determined by one method may not equate to a diagnosis of inflammation with the other. They have different sensitivities and specificities for IAD and therefore the method of diagnosis will influence the reported prevalence of airway inflammation. **Additionally, controversy exists regarding the delineation of normality in terms of the cytological findings for the two methods.**

**Sample Guide**

In both methods the fluid retrieved from the lavages should be left in the syringes used to aspirate or placed into plain serum tubes (gel-separation tubes should be avoided).

Depending on the volume retrieved an aliquot of the sample can be placed in an EDTA tube for cytology. It must be remembered however that EDTA is bacteriostatic and these samples cannot be used for culture.

Preferably the sample should be refrigerated and kept below 4°C and delivered to the laboratory within 24 hours. If refrigeration is not possible or it will take longer than 24 hours to reach the laboratory, some direct smears should be made (using clean slides) and air-dried to be submitted with the samples. There is also evidence in the literature that suggests different cell types vary in the different aliquots aspirated. Therefore ideally all the syringes should be submitted or a pooled sample must be submitted.

This photo shows a good BAL sample with frothy layer indicating the presence of surfactant, which confirms good depth with the BAL.
Further Reading


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