EHV-1 Neurological Disease
One case of neurological disease was seen in a welsh cob that developed severe ataxia and was euthanased. PCR on mixed tissues was positive for viral DNA.

EHV-1 Respiratory Disease
A donkey that died of necrotizing tracheitis and bronchitis was diagnosed with EHV-1 on PCR.

EHV-3 Coital Exanthema
One case of coital exanthema was seen in a thoroughbred stallion. The diagnosis was made by paired serology that showed a high titre on both samples, and clinical signs. Breeding activities were halted and the infection has been contained.

Equine Influenza
A single case of influenza was seen in February in an unvaccinated horse in Surrey recently imported from the Netherlands. Diagnosis was made by virus isolation on nasopharyngeal swab and seroconversion on paired serology. Vaccinated in-contacts were apparently unaffected.

FOCUS ARTICLE: REVIEW OF EQUINE INFLUENZA DIAGNOSTIC TESTS

Adam Rash, Equine Influenza Surveillance Programme, Virology Unit, Animal Health Trust

Serology
Influenza has the ability to bind to red blood cells via their haemagglutinin (HA) protein. This haemagglutination allows us to use a simple assay to check for the presence of the influenza virus.

Blood samples can be used in a variation of this assay called the Haemagglutination Inhibition (HI) assay. This assay not only allows us to determine levels of antibodies against equine influenza, but also to establish from which lineage the virus that infected the horse belongs to. Currently, three virus strains are used in the diagnostic HI assay, and they represent different regions of the equine influenza family.

Prague/56 is an H7N7 strain and can be used to distinguish between vaccination and natural infection as H7N7 viruses are no longer thought to be circulating but this strain is included in many vaccines. Miami/63 is the prototype H3N8 strain and Newmarket/2/93 is an H3N8 European lineage virus.
When samples arrive for diagnostic testing the blood is allowed to separate and the serum is removed. The serum is titrated across a v-bottomed plate in two fold dilutions from 1 in 8 to 1 in 1024. This is done on three separate plates, one for each of the virus strains used. Virus, at a standardised HA concentration, is added to each well and incubated with the serum for 30 minutes. A 1% suspension of chick red blood cells is added in equal volume to the serum-virus solution and the samples are incubated for a further 30 minutes before the plates are read.

When haemagglutination has occurred, the red blood cells remain in suspension, however if it has been inhibited the red blood cells sink to the bottom of the plate. When the plate is tilted to a 45° angle the blood in these wells will run in a line, whereas the blood in the haemagglutinated wells will not.

The HI titre for the sample is the least concentrated serum dilution that still inhibits haemagglutination. The higher the HI titre, the more that particular serum sample recognises that virus strain, and therefore the horse has been infected with a virus similar to that strain.

This assay allows us to monitor the type of virus strains currently circulating in the UK.

**Nucleoprotein (NP) ELISA**

The NP ELISA detects equine influenza virus (EIV) nucleoprotein in fluid extracted from a nasopharyngeal swab. An ELISA plate, consisting of six wells, is pre-coated with rabbit polyclonal antiserum raised against purified whole virus. Two wells are used for a negative control, two for a positive control and two for the sample being tested. The swab is squeezed using sterile forceps into a clean tube and then a small volume is pipetted into the wells of the ELISA plate. If EIV is present in the sample then the rabbit antiserum will bind to it.

After an incubation period the plate is washed before a horse-radish peroxidase labelled mouse monoclonal antibody is added. Whilst being incubated at 37°C the mouse monoclonal antibody will bind to any virus that has been captured by the rabbit antiserum.

The plate is then washed again and a TMB peroxidase substrate is added. The enzyme label attached to the mouse monoclonal antibody acts on the peroxidase substrate resulting in a colour change. The intensity of this colour change indicates how much virus is present in the swab sample, and this intensity is read at 490nm using a colorimeter. A printout of the results will confirm whether the sample is positive or negative for equine influenza virus.

The remaining fluid from a positive swab sample can be used to inoculate embryonated hen’s eggs to grow the virus to a titre that will allow further characterisation of the virus, both genetically and antigenically. This allows us to understand how the virus has evolved and to see how different it is from the current vaccine strains.
AWARENESS ARTICLE: AFRICAN HORSE SICKNESS – A POTENTIAL THREAT FOR THE UNITED KINGDOM?
Paul Jepson MRCVS, Chief Executive, The Horse Trust

The occurrence and rapid spread of Bluetongue in cattle and sheep in Belgium, Netherlands, Luxembourg, France and Germany in 2006 has highlighted the increased risk of types of insect borne disease spreading to the UK. The midge-borne African Horse Sickness (AHS) virus, closely related to Bluetongue virus, may strike the UK’s equine population in the future, in which case high mortality might be expected.

As a result of the severity of the effects of African Horse sickness and its social and economic impact, it is listed as notifiable by the World Organisation for Animal Health (OIE) in Paris, by the European Commission in Brussels under Directive 92/25/EC and therefore also in UK legislation under the Infectious Diseases of Horses Order 1987. This means that, in practice, if there is any suspicion of AHS, a Defra Divisional Veterinary Manager must be notified immediately. Imported horses from countries outside the European Union are subjected to risk-based testing for AHS.

Clinical diagnosis
According to the OIE, the incubation period for AHS is usually 7-14 days, but may be as short as 2 days. The OIE also gives the following information about the disease:

- Subclinical form: fever (40-40.5°C) and general malaise for 1-2 days
- Subacute or cardiac form: fever (39-41°C), swelling of the supraorbital fossa, eyelids, facial tissues, neck, thorax, brisket and shoulders. Death usually within 1 week
- Acute respiratory form: fever (40-41°C), dyspnoea (difficulty breathing), spasmodic coughing, dilated nostrils with frothy fluid oozing out, redness of conjunctivae, death from anoxia (respiratory failure) within 1 week
- A mixed form (cardiac and pulmonary) occurs frequently: pulmonary signs of a mild nature that do not progress, oedematous swellings and effusions, death from cardiac failure, usually within 1 week
- In the majority of cases, the subclinical cardiac form is suddenly followed by marked dyspnoea and other signs typical of the pulmonary form
- A nervous form may occur, though it is rare

Lesions
- Interlobular respiratory form: oedema (fluid) of the lungs, hydropericardium (fluid around the heart), pleural effusion (fluid in the chest cavity), oedema of thoracic lymph nodes (swelling), petechial haemorrhages in pericardium (pin-point haemorrhages in the membrane surrounding the heart)
- Cardiac form: subcutaneous and intramuscular gelatinous oedema, epicardial and endocardial ecchymoses (bleeding into the tissues of the heart), myocarditis (inflammation of the heart muscle), haemorrhagic gastritis

Further information can be found on the OIE website at http://www.oie.int/eng/maladies/fiches/a_A110.htm
**Transmission and risk to Europe**

AHS virus is related to Bluetongue virus and is spread by the same Culicoides species of midge. In horses the mortality rate can be as high as 90%. In donkeys the mortality rate is much lower (about 10%) and there are concerns that donkeys and certain exotic species may act as subclinical carriers for a period of time following infection. Infected midges can be blown by the wind for more than 100km and transported long distances in farm vehicles.

There are multiple serotypes of AHS virus and the only vaccines currently available are live attenuated preparations manufactured in South Africa. These vaccines are not licensed for use in Europe, although they can be used as an emergency response when the disease has taken hold. Research institutes and vaccine manufacturers are already working to develop more effective and safe cattle and sheep vaccines for Bluetongue virus as it is anticipated that this disease could reach the UK in 2007. Similar research and development is urgently required for AHS.

AHS was diagnosed in Spain between 1987 and 1990 and in Portugal in 1989 but was eradicated using slaughter policies, movement restrictions, midge vector eradication and vaccination. Were AHS to break out in Europe again, under current vector and climate conditions it is probable that it would have a much better opportunity to establish itself - including in the UK.

**Future awareness and action**

The Horse Trust has recently launched a disease awareness campaign for AHS. Defra is already in the process of revising legislation on notifiable equine diseases and also contingency plans for measures to take in the event of an outbreak. A working group has been set up, initiated by the Horse Trust and involving Defra and others from the horse industry to look at how best Government and industry can work together to prepare for and manage any such outbreak. In particular this group is considering the difficulties that might be encountered and trying to provide options to prevent and resolve problems.

In 2007 The Horse Trust will spearhead:

- An education campaign to all horse owners to make them aware of the possibility of AHS striking the UK and the clinical signs associated with the disease;
- An information campaign throughout the equine veterinary profession to try and ensure early diagnosis;
- A research programme to evaluate the likely impact of the disease and to develop appropriate control measures in accordance with the aims of the Equine Health and Welfare Strategy.