Between late February and May 2011 there was an increase in reports of horses displaying unusual neurological signs across NSW. Similar cases were reported in other states, particularly Victoria and South Australia. One or more arboviruses were immediately suspected in view of the very wet season across much of Australia.

Many arbovirus infections are asymptomatic, some horses may be concurrently infected with more than one virus and some of these viruses are closely related and may cross react in testing. Therefore, interpretation of laboratory test results for these Flaviviruses is difficult and time consuming, often requiring a range of tests to be conducted or repeated.

### Clinical Signs in horses

- **Video of horse affected by Flavivirus**

Initial clinical signs may include depression and mild colic. These are typically followed by the onset of neurological signs including increased responsiveness to touch and sound, facial paralysis and difficulty masticating, hypermetria in forelimbs and weakness in hindquarters and general ataxia. Most animals appear to be recovering slowly over one to three weeks, but 10-15% of horses have died, or been euthanased on welfare grounds.

There is no evidence yet on which animals are most at risk.

The weekly epidemic curve for horse cases is shown in figure 1 and demonstrates the trend towards declining case numbers as 8 June 2011.

![Weekly Epidemic Curve - Numbers of new cases of horses with neurological disease in NSW](image)

*Figure 1. Weekly epidemic curve for all horse neurological cases reported in NSW 2011*
Geographic distribution of cases as of 8 June 2011

Alternate version of map showing case reports by week
Testing and initial results
As of 8 June 2011 samples from 295 one cases have been received from NSW veterinarians at the virology laboratory at the Elizabeth MacArthur Agricultural Institute. Samples are being submitted from both new cases and convalescent horses. Testing includes:

- serology
  - MVE blocking ELISA
  - Kunjin blocking ELISA
  - West Nile IgM capture ELISA
  - MVE VNT
  - Kunjin VNT and
- agent detection by
  - West Nile Virus PCR (pan-reactive)
  - MVE PCR
  - Virus Isolation in cell culture

Preliminary results for the majority of cases to date are consistent with recent infection with Kunjin Virus. However infection with another related virus cannot be ruled out until testing of convalescent serum is completed.

In a number of cases where there is evidence that a horse has been infected with Kunjin virus, the MVE ELISA is also weakly positive or inconclusive early on in the infection, as the antibody response matures over several weeks this reaction disappears.

CSF and brain samples have been submitted from a small number of animals. Histopathology on formalin fixed brain samples show a non-suppurative encephalomyelitis, consistent with an acute viral infection.

Viral isolation from the brains of affected horses and subsequent sequencing of isolates will be the key to confirming the cause of this outbreak.

There has been no evidence of respiratory disease in affected horses and there is no suggestion that Hendra virus is involved in this syndrome. All cases tested to date in NSW have been negative for Hendra virus.

Testing in NSW has also ruled out infection with Japanese Encephalitis.

Information on cases in other states
For information on what is occurring in other states see:

- Victoria
- South Australia
- Queensland

Further investigation
Your cooperation in continuing to send in any new cases is appreciated. If any horses die or are euthanased, we would greatly appreciate submission of the brain so that we can attempt to isolate the virus. The virus cannot be isolated from blood, and all PCRs on blood have been negative, confirming that the horse is a dead end host, and that horse to horse and horse to human transmission is extremely unlikely.

It is vital that appropriate samples and a full history are collected to assist in building a full understanding of this disease.
History
Please record:
- The age and breed of the affected horse,
- The location of the horse, including the Property Identification Code (PIC) and GPS coordinates if available
- The clinical signs,
- Has the horse been recently moved and if so where from and
- Insect activity (are high numbers of mosquitoes or other biting insects present)

Personal Protection when sampling
Prevent mosquito bites in the field by:
- covering up with light-coloured, loose-fitting clothing and covered footwear and
- regularly applying an effective repellent on exposed skin. The best mosquito repellents contain Diethyl Toluamide (DEET) or Picaridin. Personal insect repellent needs to be re-applied regularly, especially if the concentration of the active ingredient is low or if you are perspiring. The product information should indicate how often you need to re-apply repellent.

There is no evidence that there are significant risks associated with blood collection however it is recommended that gloves are worn when collecting and handling blood.

Standard techniques should be used to minimise zoonotic risks during post mortems including careful technique, protection of mucous membranes and protection of cut or abraded skin. Minimum PPE includes: impervious overalls, sturdy gloves, goggles, a P2 or equivalent mask and solid footwear.

Sampling
Collect
- Blood in 10 mL heparin and plain (clotted sample) tubes (please do not use serum separator tubes)
- Blood free CSF if available, and
- both fresh and fixed brain and/or upper cervical cord samples from animals that die or are euthanased.

Keep samples chilled but do NOT freeze.

Both acute and convalescent blood samples (7 days and 3 weeks post first sampling) are important to establish the identity of the virus and to interpret all results.

Blood samples from up to five unaffected horses may be submitted for comparison. Young horses (less than 2 years old) that are less likely to have been previously exposed to flaviviruses are preferred.

Ring the duty veterinarian at the State Veterinary Diagnostic Laboratory on 02 4640 6325 during business hours or 0411 030 451 out-of-hours to alert them if you intend to dispatch samples or need further information on submissions.

While this event is being investigated there is no charge to the submitter for laboratory testing for investigation of these cases, nor for the associated courier costs with transporting the samples to the laboratory. Call Customer Service on 1800 675 623 to order pre-addressed consignment notes or to get the cost code to charge the cost of freight to the laboratory.
Packaging and submission of samples
Complete the Specimen submission form.

Pack samples as IATA 650 category B, biological substances UN 3373 as for routine diagnostic specimen submissions, i.e. small foam Esky inside a cardboard box. Include an ice-brick to keep samples cool but not frozen. Double bag the samples. See Vet Lab Manual - Packaging of specimens for further detail.

The Department has arrangements with both Metrostate (Ph. 02 9645 9700) and TNT couriers (Ph. 13 11 50 quote account number 21857635). Dispatch samples to:

State Veterinary Diagnostic Laboratory
Elizabeth Macarthur Agricultural Institute
'Camden Park'
Woodbridge Road
Menangle NSW 2568

Protecting unexposed horses
A range of products are available to protect horses from insect bites and should reduce the risk of horses being infected with an arbovirus by biting insects. They include both physical barriers e.g. rugs, fly veils and registered chemical treatments. Pyrethrum impregnated rugs are now being advertised.

Further information
- Flavivirus nervous disease in horses