The initial laboratory diagnosis of equine influenza in Australia in 2007

Until August 2007, Australia had not recorded an outbreak of equine influenza; indeed, significant quarantine precautions exist to safeguard against such an event. This article outlines the lead up to virus confirmation and the procedures to first test, then contain it.

In early August 2007, 52 horses arrived at the Eastern Creek Quarantine Station (ECQS) in New South Wales from Japan, Ireland, UK and the USA. Following standard procedures, blood samples were collected from all animals soon after arrival and sent to the serum bank at the Australian Animal Health Laboratory (AAHL).

During the quarantine period, three horses developed mild fever and two of these developed abnormal respiratory signs. Blood samples were submitted to AAHL for serological testing by haemagglutination inhibition (HI) on 20 August, with a rising serum antibody titre between the first and second serum samples suggesting a possible equine influenza virus infection in the quarantine station. Further evidence of this was obtained using a polymerase chain reaction assay (PCR) on 23 August to test nasal swabs. Suspect clinical signs were reported in horses at the Sydney Centennial Park equestrian centre and stable complex on 24 August and were confirmed equine influenza virus positive that evening.

These results were considered by Australian veterinary authorities on 25 August who agreed that containment measures should be put in place around the suspect premises and to notify the World Organisation for Animal Health (OIE) of the suspicion of equine influenza in Australia. Follow-up investigations established that many other horses had been infected at events held at Maitland and Narrabri in NSW. More than 700km away, a horse at Morgan Park in Warwick, Queensland was confirmed positive on 26 August.

Of great significance is that the rapid diagnosis of the first case outside the ECQS, at Centennial Park, was confirmed by staff at Elizabeth MacArthur Agricultural Institute (EMAI) using an influenza A matrix gene real time qRT-PCR developed at AAHL for the detection of avian influenza viruses. Similarly, staff at the Yeerongpilly State Laboratory in Queensland obtained the first positive result on the 25 August that was confirmed by AAHL the following day. The rapid testing at EMAI and the subsequent ban on animal movements proved the key factors in restricting the spread of equine influenza to only the two States initially infected.

Equine influenza is an acute, highly contagious, respiratory viral disease of horses and other species of the family Equidae. The causative agent is equine influenza virus, a type A influenza virus in the family Orthomyxoviridae (genus Influenzavirus). Two distinct antigenic subtypes (H7N7 and H3N8) are known to infect equine species, although there may be others that in fact do infect horses. The H3N8 subtype is more pathogenic than the H7N7 subtype and has undergone periodic antigenic drift forming two distinct evolutionary lineages, designated ‘American-like’ and ‘European-like’ on the basis of geographic origin. This antigenic variability of the H3N8 subtype requires vaccine efficacy to be closely monitored.

Equine influenza is endemic in Europe, the Americas, North Africa and in some parts of Asia and routine vaccination is used to control disease. Australia and New Zealand have remained...
free. The major clinical signs are sudden onset of pyrexia, a dry hacking cough and nasal discharge. The disease spreads very rapidly to susceptible in-contact horses, with high morbidity. Vaccination can prevent clinical signs but does not prevent virus shedding.

At the onset of the outbreak the diagnostic tests available in Australia for equine influenza virus were limited to virus isolation in embryonated chicken eggs or tissue culture with virus growth determined by a haemagglutination (HA) test. The HA and neuraminidase (NA) type were classically determined by use of specific antisera. Both a conventional and an RT-PCR were also available for amplification and identification of specific viral RNA. Serological diagnosis was carried out by HI tests. An Influenza A group reactive enzyme-linked immunosorbent assay (ELISA) was also available but had not been validated for use on horse samples.

Whilst there were only the conventional tests available for the specific diagnosis of equine influenza at the AAHL, the ability to utilise the generic influenza A qRT-PCR provided an immediate and rapid test for virus detection, comparable to performance of any assays being used in endemic countries. Whilst there was a 5 day delay in gaining full laboratory confirmation using a range of equine influenza diagnostic assays, immediate disease control measures were instigated on the initial results.

During the outbreak, AAHL staff developed new or modified existing tests for use in the laboratory confirmation of equine influenza. The influenza A matrix gene qRT-PCR was validated as being suitable for equine influenza virus detection and became an invaluable assay for use by the State laboratories in supporting control and eradication activities. A qRT PCR to detect the H3 haemagglutinin gene was developed and used as a confirmatory test for Type A qRT-PCR positive samples. The competitive ELISA for avian influenza was modified as a blocking (b) ELISA for detection of equine influenza virus antibodies in horses and validated using 2000 negative and 500 positive sera. One significant advantage of the bELISA is that it allows differentiation of infected horses from horses vaccinated with a canary-pox vectored recombinant vaccine. The bELISA technology was distributed to State veterinary laboratories to support local State testing. An antigen capture ELISA was developed and validated for use in detecting equine influenza virus in nasal swabs. To further support the use of these assays in State laboratories, AAHL provided external proficiency testing panels of sera and viruses for the HI, bELISA and PCR.

Nucleotide sequence analysis of the matrix gene from the first Australian equine influenza virus isolate (A/Equine/Sydney/2888-8/2007 H3N8) carried out at AAHL showed that there was a high level of identity between the equine and avian matrix genes, with primer and probe sites well conserved. This provided a clear demonstration as to why the qRT-PCR was well suited to the diagnosis of equine influenza in the current outbreak.

Although the State veterinary laboratories conducted routine diagnosis and associated serology, in parallel, nasal swabs and sera were sent to AAHL for confirmation of infection. At AAHL nasal swab samples were inoculated into SPF chicken eggs and onto Madin-Darby Canine Kidney (MDCK) cells. The first virus isolates were made on the third passage in eggs, and by the fourth passage most virus isolates were causing HA. In MDCK cells, cytopathic effect (CPE) was first observed in the second passage but by the third passage the CPE was clearly visible with most samples. Sequence analysis of the haemagglutinin gene
showed that it shared high sequence identity with an equine influenza virus from Wisconsin USA isolated in 2003 (A/equine/ Wisconsin/1/03(H3N8)).

Viruses were subsequently isolated from horses at Randwick Race Course, Centennial Park Equestrian Centre and Morgan Park, Queensland. Nucleotide sequence analysis of the HA genes from all these viruses showed very high sequence identities with each other, the index isolate and the Wisconsin virus. These initial comparisons clearly demonstrated that the Australian viruses showed no significant sequence changes and therefore would have no expected changes in pathogenicity compared with other isolates of equine influenza that have caused outbreaks elsewhere in the world.

The ability of AAHL and EMAI to rapidly confirm the initial diagnosis and the immediate response of State veterinary authorities to prevent movement of horses are probably the most important factors in preventing nationwide spread and allowing eradication to remain a feasible objective. The subsequent and considerable laboratory testing in affected States has proved highly effective in providing a sound scientific basis for the control and subsequent ‘proof of freedom’ activities. This outbreak has provided a unique opportunity to exploit new diagnostic tools on an unprecedented scale to effectively deliver veterinary laboratory services in support of a response to a major emergency disease event.

Acknowledgements
The enormous efforts of the dedicated staff at the NSW and Qld Departments of Primary Industries and AAHL are gratefully recognised and acknowledged.

References

Dr Martyn Jeggo is the Director of CSIRO’s Australian Animal Health Laboratory (AAHL) and has headed AAHL since September 2002. He brings a wealth of experience in controlling and detecting exotic and emerging animal disease to his role of Director of the Australian Animal Health Laboratory (AAHL). From 1996-2002, Dr Jeggo was the Head of the Animal Production and Health Science Section of the Joint Food and Agricultural Organisation/International Atomic Energy Agency (FAO/IAEA) Division of Agriculture, in Vienna, Austria. Dr Jeggo’s many achievements include that he developed an international external quality-assurance program for veterinary laboratories; is a leading member of the Foot and Mouth Disease (FMD) Global Research Alliance and a Member of the Royal College of Veterinary Surgeons (MRCVS). In the time that Dr Jeggo has been Director of AAHL, some A$55 million has been brought in to improve and upgrade the facility.

Dr Jef Hammond is a senior research scientist based at CSIRO’s Australian Animal Health Laboratory (AAHL) in Geelong, Victoria, Australia. He is a virologist with expertise in developing livestock viral vector vaccines and investigating livestock immune responses to viral diseases. At AAHL, he leads terrestrial animal exotic disease diagnostics and coordinates foot-and-mouth disease (FMD) research projects which impact on Australia’s preparedness to deal with an outbreak of the disease. Dr. Hammond is also the Australian coordinator of a global collaborative project (the Global FMD Research Alliance) that is developing a new generation of control measures for FMD. He is an expert on both the national FMD expert advisory group and the Australian FMD vaccine advisory panel. Recently he has also fulfilled the role of scientific response coordinator at AAHL for the emergency response to the equine influenza outbreak in Australia.

Dr Peter Kirkland is a Veterinary Virologist and Principal Research Scientist in the Virology Laboratory for the NSW Veterinary Laboratory Network at EMAI, Camden. He has expertise in diagnosis and research of a number of major viral diseases of animals and has been responsible for leading the laboratory’s response during a number of exotic disease incidents over the last decade, including the recognition of 2 new viral pathogens.

New gene technology certification guidelines

Office of the Gene Technology Regulator (OGTR)

Over the past 12 months, the Office of the Gene Technology Regulator (OGTR) has implemented a number of major reforms to the certification guidelines for physical containment (PC) facilities. The recent changes are more outcome-focused and are more aligned with other standards and regulations (www.daff.gov.au/aqis/import/general-info/qap/class-5/criteria, www.standards.com.au).

The revised PC4 guidelines focus on the highest level of containment with limited options due to the nature of work conducted in those facilities. By contrast, the recent PC2 laboratory, animal and plant guidelines allow facility managers to choose a range of approaches to contain genetically modified organisms (GMOs), depending on the type of work being undertaken.

People managing and working in laboratories or specialised facilities are expected to know about the work being conducted in their facilities and the risks associated with that work. With every facility being different, the new PC2 guidelines provide organisations with sufficient flexibility to tailor their own risk management measures to maintain containment. This outcome-focus has reduced the number of variation requests sent to the OGTR.

One of the most noticeable changes to the PC2 guidelines is their division into three sections:

- Requirements that must be met before certification can be granted.

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