# Reflections on the 2007 equine influenza outbreak

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**In late August 2007, Australia experienced its largest animal disease emergency with an outbreak of equine influenza (EI). This followed the importation of one or more infected horses and the entry of the virus into the Australian horse population. There are a number of aspects of this event that are not only applicable to the diagnosis and control of other high-consequence animal diseases but there are also many elements of interest and relevance to public health. In particular, there were interesting insights into the speed and manner of virus dissemination in a naive population. The benefits and capacity of real-time PCR and associated technology to support an emergency disease investigation and response were demonstrated, while the value of using a combination of a 'marker' vaccine and serological test that would differentiate between infected and vaccinated animals was clearly proven. Ultimately, the virus was eradicated following an outbreak on a scale and in a time frame not previously achieved in any other country.**

## Clinical observations

The 2007 EI outbreak has some parallels to the great human pandemics, with the introduction of a highly contagious virus into what was essentially a fully susceptible population, without even any cross-protection from other strains of influenza. As a consequence, EI virus spread rapidly and widely. It was estimated that more than 75,000 horses were infected. While a very high proportion (perhaps more than 90%) showed clinical signs, the majority were relatively mildly affected. The signs most consistently observed were fever, nasal discharge and a distinctive severe cough[1], not previously noticed in horses in Australia. Disease was more severe in large, high-density populations, possibly as a result of stress, the opportunity for secondary infections and probably a higher viral load at the time of exposure. Heavily pregnant mares were also more severely affected and had more complications during parturition than uninfected mares. There were a number of stillborn foals, mostly due to foetal distress and hypoxia during prolonged delivery. In the general population, the mortality rate was very low, with deaths confined mainly to very young foals or immunocompromised animals.

Interspecies transmission of this H3N8 virus was observed with infection of dogs confirmed by serology in several locations[2]. There was very little evidence of virus shedding and no evidence of spread to dogs that had not had close contact with infected horses. About 25% of the infected dogs showed clinical signs with persistent coughing a feature. Nucleic acid sequencing showed complete homology with the virus circulating in horses and did not have the changes observed in viruses infecting and now endemic in dogs in North America.

## Epidemiology

EI virus spread rapidly through a naive population of horses, with the movement of horses being a major factor in the widespread, long-distance dissemination of the virus[3]. The horse population, like their owners, is highly mobile and can be moved for competition or breeding over many hundreds or thousands of kilometres, and even internationally, in less than one day. In this respect, apart from humans, they are probably the only animal population to be so mobile. Such movements can have significant consequences for the spread of a highly contagious disease. By the time the index case was detected in Sydney, there were already infected animals in much of northern New South Wales and also in southern Queensland (Figure 1). However, unlike most instances of influenza in humans, it was possible to implement quarantine measures including movement controls, which ultimately were critical in preventing the nationwide dispersal of the virus. Despite these restrictions, there were still many instances where movement of virus to a new group of horses was shown to be the result of movement of contaminated equipment or people[4]. Spread by people was considered to be mechanical, through contamination of hands, clothing and footwear. There was no evidence of active infection or illness in people due to infection with this virus. Although it remains controversial, there were convincing instances where wind-borne spread was likely to have occurred, in some cases over a distance of several kilometres to isolated properties[5,6]. Patterns of spread were consistent with airborne plumes and no other method of transmission could be identified[6]. The inappetance induced by influenza did provide opportunities for wild birds, with more feed being available. On occasions there was concern that wild birds could be mechanically spreading virus after feeding in contaminated feed troughs, but direct sampling of the feet of birds did not provide any evidence for their involvement.

## Diagnostic aspects

Following the rapid spread of H5N1 influenza in both wild birds and poultry in many countries of South-East Asia, sometimes with concurrent human infections and death, pathology laboratories in Australia were equipped with an enhanced capacity to undertake the confirmation of Type A influenza virus infections. State

veterinary laboratories in most Australian states had developed a similar capability. Generally a real-time, reverse transcriptase polymerase chain reaction (qRT-PCR) assay was used. The usual approach was to use a pan-reactive Type A influenza assay, targeting a highly conserved sequence of the matrix gene. Positive samples were then tested in sub-type specific (for example, H5) assays. When samples from sick horses at a Sydney equestrian centre were received at the Virology Laboratory at the Elizabeth Macarthur Agriculture Institute (EMAI) on the evening of 24 August 2007, the standard qRT-PCR pan-reactive assay was employed and, within a few hours, the presence of an influenza virus (later confirmed as EI) had been detected for the first time in horses not under the containment of a quarantine station[7]. The following day these results were confirmed by the Australian Animal Health Laboratory at Geelong, Victoria. Although a H3 subtype-specific qRT-PCR became available, the pan-reactive assay continued to be used in veterinary laboratories throughout the course of the outbreak.
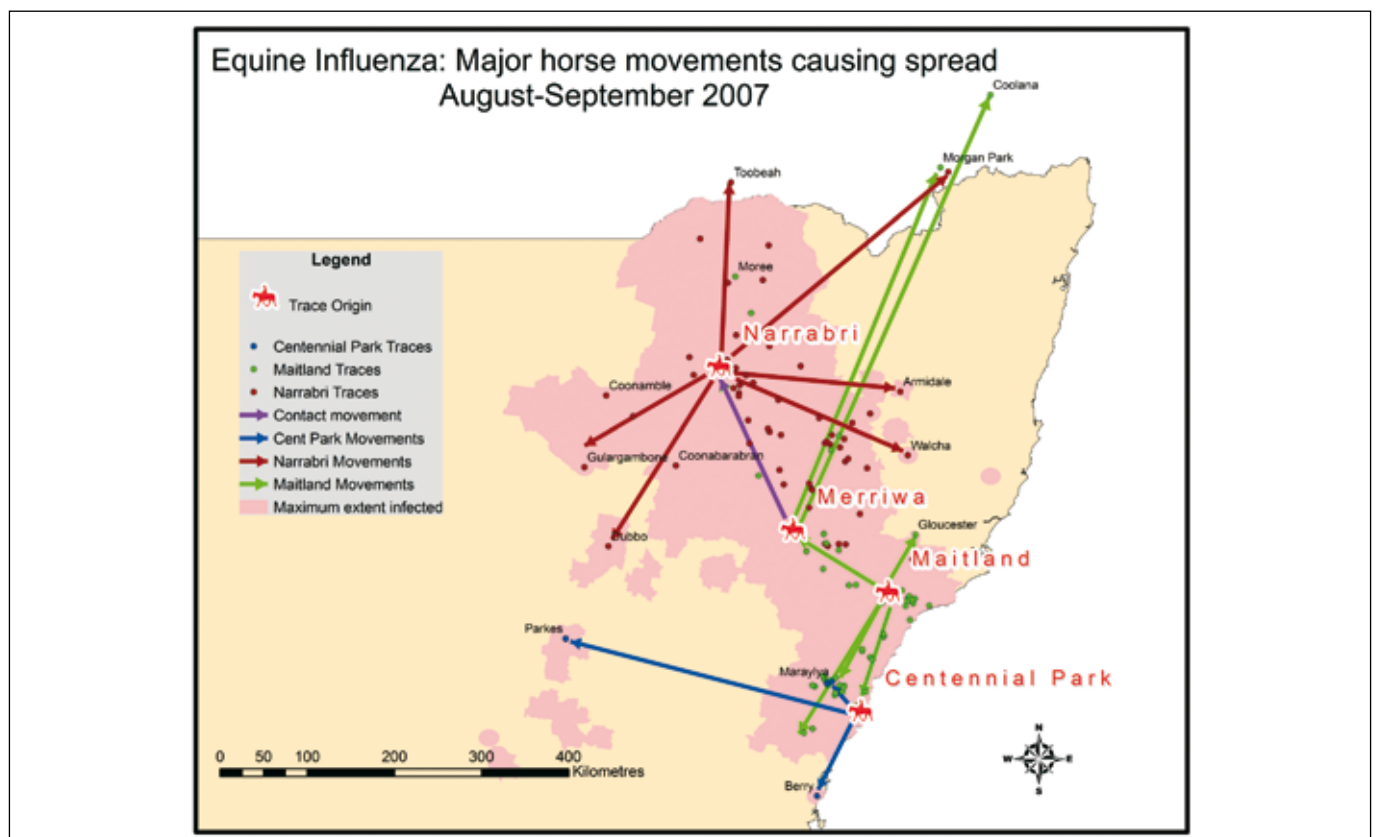
The speed and high sensitivity of the qRT-PCR, in conjunction with semi-automated, magnetic bead-based systems for nucleic acid extraction facilitated large-scale testing. Similar systems have since been successfully employed in human pathology laboratories, especially during the early stages of the H1N1 pandemic. Having an assay with very high analytical sensitivity did, however, create some challenges. Demonstration of an absence of infection was a requirement for the release of properties from quarantine. As most, if not all, animals were seropositive, serology was of no value to demonstrate freedom from infection. Therefore, qRT-PCR was employed on a large scale to test nasal swabs for evidence of virus shedding. It was generally believed that horses would cease shedding virus after about 14 days. However, longitudinal studies of horses showed that viral RNA could be detected for up to 35 days, albeit at low levels, and probably not infectious[8]. Nevertheless, RNA detection did have a finite limit and the qRT-PCR was later used extensively to demonstrate that horses in affected regions were now free from EI virus infection.

## The role of vaccination

Like the practice in humans with type A influenza, vaccination is used widely in countries where EI virus is endemic. However, in Australia, the only horses that had been vaccinated were a small number that had travelled overseas or had been imported. In the early stages of implementing a control program, a decision was made to establish a capacity for vaccination. Initially horses were vaccinated in buffer zones that surrounded some of the heavily infected regions. While there are several inactivated or subunit vaccines that are registered for use in horses, a canary-pox vectored vaccine was selected. One of the key reasons for its selection was the capacity to support a "DIVA" strategy – to differentiate serologically between naturally infected and vaccinated horses[9]. This was possible because the recombinant canary poxvirus, which undergoes limited replication in mammals, contained only the gene encoding the haemagglutinin antigen. In contrast, for serological surveillance, a blocking ELISA that detected only antibodies to the nucleoprotein was used.

Figure 1. Equine influenza virus was found to be widely disseminated across central and northern New South Wales soon after the virus entered Australia[4], reproduced with permission of the *Australian Veterinary Journal*.

This allowed monitoring of the virus-free buffer zones, in which there were only vaccinated horses. The DIVA strategy proved to be very useful but of course would not be suitable for monitoring influenza in humans due to the use of inactivated vaccine and prior infections.

As this vaccine was a genetically modified organism (GMO), and also an imported biological, approval was required from three regulatory bodies: the Australian Quarantine and Inspection Service, Australian Pesticide and Veterinary Medicines Authority and the Office of the Gene Technology Regulator. These approvals were obtained relatively quickly and the vaccine was used without problem. While the vaccine was very effective and appeared to reduce levels of virus excretion within a short time of the initial dose, there were nevertheless some logistical issues. As a condition of use, all vaccinated horses had to be permanently identified, usually by microchip and, because of its GMO status, there was rigorous accounting for the distribution of the vaccine and return of used vials.

## Control and eradication program

The EI control program was dependent on movement controls as a foundation[10]. These were supported by the implementation of strict biosecurity measures, vaccination in buffer zones and, late in the course of the outbreak, adoption of strategies to maximise population immunity, including the relaxation of movement controls to deliberately encourage spread of the virus in contained, infected populations as well as vaccination of horses that were thought not to have been infected. However, handling of individual horses and visits to farms with only a few animals limited the rate of progress with vaccination. In some instances this was alleviated by the use of 'drive by' vaccination clinics, where horses were brought to a central location to be identified and vaccinated. Collectively, these measures proved to be highly effective with the last clinical case observed in late December 2007 (Figure 2). Intensive monitoring between January and March 2008 failed to detect any evidence of active infection. Data from this surveillance were used to support an

international claim for freedom from infection with EI virus. This was submitted in December 2008 and Australia subsequently regained its EI-free status. There appear to have been few long-term sequelae, although there is a need for ongoing vaccination and testing of horses for export. Vaccination of other horses is not permitted.

## Further reading

An account of all aspects of the 2007 equine influenza outbreak, ranging from the initial diagnosis to the final declaration of freedom, including clinical accounts, disease control strategies, epidemiology, pathology, planning and policy issues, selection and use of vaccine and virology investigations can be found in the May 2011 edition of the *Australian Veterinary Journal*.

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## Biography

**Dr Kirkland** is a veterinary virologist with special interest in the development of rapid diagnostic assays, vector-borne viruses and viruses causing reproductive disease in animals.

Figure 2. The epidemic curve for the EI outbreak in Australia, showing the number of newly identified infected properties (IP) on a daily basis[3], reproduced with permission of the *Australian Veterinary Journal*.