

The 2007 outbreak of equine influenza in Australia: lessons learned for international trade in horses

J. Watson⁽¹⁾, P. Daniels⁽¹⁾, P. Kirkland⁽²⁾, A. Carroll⁽³⁾ & M. Jeggo⁽¹⁾

(1) Australian Animal Health Laboratory, Private Bag 24, Geelong VIC 3220, Australia

(2) Elizabeth Macarthur Agriculture Institute, Woodbridge Rd, Menangle NSW 2568, Australia

(3) Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra ACT 2601, Australia

Summary

In August 2007 Australia experienced its first outbreak of equine influenza. The disease occurred first in a quarantine station for imported horses near Sydney and subsequently escaped into the general horse population. After an extensive campaign the disease was eradicated and Australia is again recognised as free of this disease. Equine influenza was then, and is now, recognised to be the major disease risk associated with live horse imports into Australia and measures designed to mitigate this risk formed the basis of the quarantine protocols then in place. Subsequent investigations into the cause of the outbreak identified failures in compliance with these quarantine requirements as a contributing factor. It is also likely that the immunity of horses vaccinated as part of the import protocol was less than optimal, and that this had a significant role to play in the escape of the disease from quarantine.

Keywords

Equine influenza virus – H3N8 – Polymerase chain reaction – Quarantine – Serology – Trade – Vaccine.

The trade in horses

Although horses were imported into Australia in the early days of European settlement of the continent in the 18th Century, the first transport of horses by air, with attendant quarantine risks posed by a shorter travel time, did not occur until the 1970s. Over the last decade the numbers of horses travelling to Australia have increased significantly, as changes in the quarantine regime have allowed short-term entry of shuttle stallions for breeding purposes and of competition horses being prepared for major races.

Equine influenza (EI) is nearly ubiquitous in distribution globally, coexisting with horse populations worldwide except in New Zealand and Iceland. Prior to 2007, EI had not occurred in Australia. A serosurvey conducted in the late 1990s as part of a study of equine respiratory disease confirmed Australia's EI-free status (4). Vaccination was permitted only as part of preparation for export, where required by the importing country.

Since EI was recognised in Australia as one of the major disease risks associated with live horse imports, import quarantine protocols were principally designed to manage this risk, and Australian import protocols for horses were, and remain, rigorous. As EI is endemic in nearly all horse populations worldwide, it is typically managed by the use of vaccines. Quarantine requirements for trade in horses between other countries focus on risks other than EI and are usually less onerous than the requirements for importation into Australia.

Quarantine requirements in Australia

Prior to 2007, horses entering Australia were required to undergo 21 days pre-export quarantine (PEQ) in approved premises in the country of origin and 14 days post-arrival quarantine (PAQ) at a quarantine station in Australia. Pre-

export quarantine facilities were either government-owned or privately owned but supervised by government veterinarians. However, competition horses temporarily imported from approved countries were permitted to undergo a shorter PEQ period of 14 days and were not required to undergo PEQ testing for contagious equine metritis. Specific testing requirements for other diseases varied with country of origin. The PAQ period of 14 days was intended to span two incubation periods for EI.

All imported horses were required to have been vaccinated against EI with an approved vaccine in the four months (six months in the case of Japan) prior to entering PEQ. On arrival, all horses were blood sampled to provide serum for the National Serum Bank (NSB) at the Australian Animal Health Laboratory (AAHL) to provide a baseline sample for serological analysis if significant disease should arise during the PAQ period.

Policy and practice of quarantine in Australia

In 2007 Australia had in place a quarantine regime with a distinct separation of roles. The Australian Quarantine and Inspection Service (AQIS) was responsible for the operational enforcement of quarantine requirements, while Biosecurity Australia (BA) was responsible for risk analysis and policy development. While the assessment of quarantine risks associated with the trade in particular commodities is generally dealt with by BA in a formal import risk assessment, the quarantine system applying to importation of horses had grown incrementally over time and no formal import risk assessment had been carried out.

In the subsequent inquiry into the outbreak, the separation of these two groups was recognised to be an impediment to implementation of an effective quarantine regime (3).

The inquiry also identified a number of deficiencies in the implementation of quarantine procedures. The quarantine stations in Australia used for PAQ were found to have deficient written procedures and poor staff-training programmes. Biosecurity requirements, particularly in relation to non-government staff caring for the horses in PAQ, including attending private veterinarians, were poorly enforced. In addition, audits of PEQ premises outside Australia had been carried out inconsistently and a number of warnings, both from experienced AQIS staff and from personnel in other countries, had produced little change (3).

Equine influenza in Japan in 2007

An outbreak of EI occurred in Japan in August 2007, the first recorded in that country since 1972. Japan notified the World Organisation for Animal Health (OIE) of the outbreak on 28 August. The initial notification recorded that the first outbreak was recognised on 14 August in Shiga prefecture, on the island of Honshu. Subsequent notifications described outbreaks occurring as early as 12 August, and on 14 August on the island of Hokkaido. Thirteen horses travelled to Australia from PEQ premises on Hokkaido, departing on 8 August. While the outbreak had not been recognised at that time, it is likely that the virus was already circulating. Epidemiological investigations suggested that the likely origin of the outbreak in Japan was the United States.

Initial events in the 2007 equine influenza outbreak in Australia

Between 3 and 11 August 2007 52 horses entered Eastern Creek Quarantine Station in Sydney, and 27 entered Spotswood Quarantine Station in Melbourne, to undergo post-arrival quarantine. The horses in Eastern Creek had been imported from Japan, the United States, the United Kingdom and Ireland, and the horses in Spotswood had been imported from Japan and the United States.

On 17 August three horses at Eastern Creek began to show pyrexia and respiratory signs (6). On 20 August, blood and nasal swab samples were obtained and sent to AAHL for exclusion of equine influenza. Haemagglutination inhibition serology (12) on these samples showed that one horse had clearly seroconverted to EI whilst in Eastern Creek (11). The sample taken from this horse on arrival in Australia (stored at the NSB) had been seronegative, despite a documented vaccination history.

On 21 August, blood and nasal swab samples from 15 horses, including the first three clinically affected, were submitted. Real-time polymerase chain reaction (PCR), targeting the influenza A matrix gene, detected viral RNA in swabs taken from five horses, including one of the three that had shown clinical signs. All of these horses had been imported from either Japan or Ireland and were housed in the same row of stables as the first horse that had seroconverted.

At the same time, serum and swabs were collected from horses undergoing PAQ at Spotswood. Testing of these serum samples and the original samples held in the NSB

revealed that two horses imported from Japan showed a rising titre, indicating probable exposure to EI in these animals as well. No PCR positives were detected in these or any other horses at Spotswood at that time despite extensive testing (11).

Within a few days, EI had also been diagnosed outside the quarantine station, with the earliest cases linked to an equestrian event at Maitland, New South Wales (NSW), on 17-19 August (7). An immediate standstill on all horse movements in Australia was implemented. This allowed a comprehensive situation assessment to be carried out, and the results showed that the disease had spread extensively across two states: NSW and Queensland. Despite extensive inquiries the exact route by which the virus escaped from the quarantine station was not established.

The Australian response: disease eradication

As soon as the disease was confirmed outside of quarantine an emergency animal disease response was initiated using agreed protocols (1). A decision was taken to attempt eradication of the virus. This effort was ultimately successful, with the last case of EI in Australia recorded in late December 2007. By that time over 8,000 properties had been declared infected and over 150,000 horses vaccinated as part of disease control efforts. Although calculating the economic impact of the outbreak with any accuracy is difficult, it is estimated that direct costs to government were over AU\$390 million (€289 million), and that the impact on the equine industries was at least AU\$400 million (€297 million) (3).

Epidemiological investigations

Epidemiological investigation of horses in PAQ in the animal quarantine stations at Eastern Creek (NSW) and Spotswood (Victoria) showed evidence of infection early in the PAQ period in horses transported from several countries, including Japan, which was experiencing an outbreak of EI at the time.

Analysis of the haemagglutinin gene sequence of *A/equine/Sydney/2007*, the initial virus isolated from the Australian outbreak, placed this virus in Clade 1, the *A/equine/South Africa/03*-like variant American lineage. The isolate was identical, at the amino acid level, to both *A/equine/Ibaraki/07* (the initial virus isolated from the contemporaneous outbreak of EI in Japan) and *A/equine/Pennsylvania/07* (isolated in the United States in August 2007). Isolates *Sydney/07* and *Ibaraki/07* were also

identical at the nucleotide level, with a single non-coding nucleotide difference to *Pennsylvania/07*. No Clade 1 viruses had been isolated in Europe at the time of the Australian outbreak (10).

Two horses that had undergone PEQ in the same premises in Hokkaido had travelled to Australia on the same flight, together with other horses from Japan. One of these horses underwent PAQ in Eastern Creek and the other in Spotswood. The horse that went to Spotswood was the one in which evidence of EI infection was detected in that station. The individual horse(s) that were initially carrying the virus in Eastern Creek, however, remain undetermined (11).

Given the short incubation period of EI (usually two to five days), the initial clinical case identified in Eastern Creek must have acquired the infection from a previously infected horse whilst in quarantine. The subsequent identification of spread of the virus outside of quarantine at an equestrian event at Maitland, NSW, in mid-August indicates that the virus had also escaped into the Australian horse population before the first clinical case was identified in Eastern Creek.

It was concluded that EI had most probably arrived in Australia from Japan, having recently arrived there from the United States. However, the possibility of direct transport from the United States could not be entirely excluded.

As the initial clinical case identified in Eastern Creek was a horse that was seronegative on entry into quarantine and that accordingly had a more severe expression of the disease than an effectively vaccinated animal, it was likely to have shed larger amounts of virus. It would therefore have acted as an amplifier, contributing to the more extensive expression and spread of disease in Eastern Creek. As all horses in Spotswood had moderate to high initial antibody titres, it is likely that the infection would have been less able to establish and spread within the quarantine station, reducing the risk of its escaping quarantine.

Vaccination and quarantine protocols

Serological testing conducted on the horses in quarantine during the outbreak revealed several that had low or negative titres against EI, suggesting a lack of protective antibody titres and therefore an increased susceptibility to EI. The first diagnostic indication of EI in this outbreak was the seroconversion in quarantine of a febrile stallion

that showed a negative baseline titre despite a certificate indicating it had been vaccinated according to protocol.

Concern has been expressed that many EI vaccines are based on older strains of the virus that are no longer epidemiologically relevant to viruses circulating in the field (9). These vaccine viruses are likely to differ antigenically from field strains (8) and may not provide adequate protection. Vaccines formulated with recent, relevant strains show markedly higher levels of protection and considerably reduced shedding of virus when infection does occur in vaccinated animals (2).

Human influenza vaccines are reformulated on an annual basis because of their constant antigenic evolution. Equine influenza has not shown the same level of antigenic variation over time as human strains, nonetheless, the virus does evolve and new strains continue to emerge (5). The OIE Expert Surveillance Panel on Equine Influenza has recommended reformulation of vaccines on several occasions, but the pace of adoption of these recommendations is slow (13).

Vaccines play an important role in the management of EI; however, it is important to distinguish between management and control. As EI is widespread in most countries with significant horse populations, vaccines are used to manage the clinical effects of EI rather than to eradicate the virus. The disease is kept at a low prevalence, but sporadic outbreaks occur regularly. Populations particularly sensitive to the effects of disease, such as competition and breeding horses, have their exposure managed more intensively than recreational horses.

Equine influenza vaccines can reduce the likelihood of infection and also reduce the amount of virus shed by the horse when infection does occur. It is, however, seldom possible to achieve sterile immunity, where infection is entirely prevented, or if it occurs, there is no shedding of virus. Australia's quarantine regime was reliant on the efficacy of EI vaccination as the utility of the PEQ and PAQ periods depends on there being no ongoing circulation of virus within the quarantined population.

Vaccinated horses may still be infected with EI. When this occurs, they will usually shed virus to some degree while showing only reduced, or no, clinical signs of disease. This represents a real threat to the assumptions outlined above; the virus may circulate to some degree in a population of vaccinated horses without producing significant signs of disease. If only clinical observations are used and no testing is employed, it is possible that the presence of infection will be overlooked.

Since the 2007 outbreak, the EI vaccine requirements for entry of horses to Australia have been significantly revised. While the basic requirement of vaccination between six

and two months prior to entering PEQ remains, there is now a specification that animals should be tested for the presence of the virus. In addition, vaccines used must contain viruses of specified lineages in accordance with the recommendations of the OIE Expert Surveillance Panel for Equine Influenza. The current requirement is for an *A/eq/South Africa/4/2003* (H3N8)-like virus (American lineage) and an *A/eq/Newmarket/2/93* (H3N8)-like virus (European lineage). While the primary intent of this requirement is to provide effective protection of horses entering Australia, it may also provide some incentive to vaccine manufacturers to keep their products up to date in line with the OIE recommendations.

Conclusions

The 2007 outbreak of EI in Australia reinforced the view that EI is the greatest equine disease threat that the Australian quarantine system must manage. The outbreak also demonstrated that quarantine policy must be implemented fully in order to be effective.

In hindsight, the situation at Eastern Creek in August 2007, with a large group of 52 horses (several with dubious vaccination status) and poorly enforced biosecurity procedures (3), can be seen as an animal disease catastrophe waiting to happen. An instructive contrast can be made with the situation at Spotswood, which had a smaller intake of 27 horses (that probably had better vaccination status) and more strictly enforced biosecurity protocols (3). In this latter situation, despite documented introduction of an infected horse, there was minimal transmission of virus within the station and no release to the general population. The quarantine arrangements at the Spotswood facility delivered the desired outcome.

Australia has learned a great deal from the 2007 EI outbreak. The basic framework of the Australian quarantine protocol for the importation of horses from EI-infected countries remains substantially unchanged, which is appropriate as it was always designed to manage this risk. What has changed is the implementation of the protocol. Although Australian authorities do not mandate the use of specific commercial vaccine products, the specifications to be met by vaccines and vaccination have been more clearly stipulated, as outlined above. The requirements for pre-export certification are now more stringently enforced.

The assumption that a PAQ period would prevent disease transmission has been replaced by repeated PCR or antigen testing for the presence of virus during both PEQ and PAQ to ensure this is so. Biosecurity at quarantine stations is now strongly enforced. In addition to the blood collected

for storage in the NSB on arrival, a blood sample is also taken from each horse during PEQ. Half of this sample must be stored in the country of export in an approved laboratory. The other half of the sample is sent to AAHL for storage in the NSB.

This outbreak demonstrates that the regular international movement of live horses means that it is impossible to guarantee prevention of entry of a widespread and highly contagious disease. Quarantine protocols can be designed to manage the risks, but are sensitive to any imperfections

in their execution. Vaccines are a useful tool for managing disease, and especially the expression of disease, but are limited in their ability to absolutely prevent transmission of the virus, and should not be blindly relied upon to do so. Vaccines, like diagnostic tests, must be fit for purpose by being matched to the agents being transmitted in the target animal population. Testing for the presence of subclinical infection is a much more reliable screening tool than clinical observation for signs of disease.

Foyer de grippe équine survenu en 2007 en Australie : enseignements pour le commerce international de chevaux

J. Watson, P. Daniels, P. Kirkland, A. Carroll & M. Jeggo

Résumé

L'Australie a connu son premier foyer de grippe équine en août 2007. La maladie s'est d'abord déclarée dans une station de quarantaine pour chevaux importés, située près de Sydney, avant de se propager au sein de la population équine générale. Suite à la mise en œuvre d'une campagne de lutte extensive, la maladie a pu être éradiquée et l'Australie a recouvré son statut indemne de grippe équine. La grippe équine demeure encore aujourd'hui le principal risque sanitaire associé aux importations d'équidés vivants en Australie. Les protocoles de quarantaine en vigueur au moment du foyer reposaient déjà sur des mesures d'atténuation du risque. Les enquêtes approfondies visant à déterminer l'origine du foyer ont mis en évidence certains manquements aux exigences de quarantaine, qui ont pu favoriser l'apparition du foyer. Il est également probable que l'immunité conférée aux chevaux vaccinés dans le cadre des protocoles d'importation n'était guère optimale, ce qui a certainement contribué à la dissémination de la maladie en dehors de la station de quarantaine.

Mots-clés

Amplification en chaîne par polymérase – Commerce international – Quarantaine – Sérologie – Sérotype H3N8 – Vaccin – Virus de la grippe équine.

Enseñanzas extraídas del brote de gripe equina de 2007 en Australia con respecto al comercio internacional de caballos

J. Watson, P. Daniels, P. Kirkland, A. Carroll & M. Jeggo

Resumen

En agosto de 2007, Australia sufrió por primera vez un brote de gripe equina. La enfermedad se manifestó en primer lugar en un centro de cuarentena para caballos importados cercano a Sydney, y posteriormente escapó y afectó a la población equina del país. Tras una vasta campaña, la gripe equina quedó erradicada y Australia ha sido de nuevo declarada libre de la enfermedad. Por entonces se consideraba (y sigue siendo el caso) que la gripe equina era el principal riesgo ligado a la importación de caballos vivos a Australia, y las medidas destinadas a mitigar ese riesgo formaban la base de los protocolos de cuarentena que se aplicaban en aquel momento. Las subsiguientes investigaciones sobre las causas del brote sirvieron para determinar que el incumplimiento de los requisitos de cuarentena fue un factor que contribuyó a su aparición. También es probable que el estado inmunitario de los caballos vacunados como parte del protocolo de importación no fuera el idóneo, y que ello influyera notablemente en el hecho de que la enfermedad escapase del centro de cuarentena.

Palabras clave

Comercio – Cuarentena – H3N8 – Reacción en cadena de la polimerasa – Serología – Vacuna – Virus de la gripe equina.



References

1. Animal Health Australia (2007). – Disease strategy: equine influenza. AUSVETPLAN Version 3.0. Primary Industries Ministerial Council, Canberra, ACT. Available at: www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/AUSVETPLAN/EFLU%203_0-17FINAL%2827Feb08%29.pdf (accessed in August 2010).
2. Bryant N., Rash A., Lewis N., Elton D., Montesso F., Ross J., Newton R., Paillot R., Watson J. & Jeggo M. (2008). – Australian equine influenza: vaccine protection in the UK. *Vet. Rec.*, **162**, 491-492.
3. Callinan A.C. (2008). – Equine influenza: the August 2007 outbreak in Australia. Report of the Equine Influenza Inquiry. Available at: www.equineinfluenza.gov.au/ei/exhibits/REP.0001.001.0001.pdf (accessed in August 2010).
4. Christley R.M., Hodgson D.R., Rose R.J., Wood J.L.N., Reid S.W.J., Whitear K.G. & Hodgson J.L. (2001). – A case-control study of respiratory disease in thoroughbred racehorses in Sydney, Australia. *Equine vet. J.*, **33**, 256-264.
5. Daly J.M., Lai A.C.K., Binns M.M., Chambers T.M., Barrandeguy M. & Mumford J.A. (1996). – Antigenic and genetic evolution of equine H3N8 influenza A viruses. *J. gen. Virol.*, **77**, 661-671.
6. Jeggo M.H., Hammond J.M. & Kirkland P.D. (2008). – The initial diagnosis of equine influenza in Australia in 2007. *Microbiol. Aust.*, **29**, 80-82.
7. Kirkland P.D., Davis R.J., Wong D., Ryan D., Hart K., Corney B., Hewitson G., Cooper K., Biddle A., Eastwood S., Slattery S., Evers M., Wright T., Halpin K., Selleck P. & Watson J. (2011). – The first 5 days: field and laboratory investigations during the early stages of the equine influenza outbreak in Australia. *Aust. vet. J.* (in press).
8. Lai A.C., Chambers T.M., Holland R.E. Jr, Morley P.S., Haines D.M., Townsend H.G.G. & Barrendeguy M. (2001). – Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. *Arch. Virol.*, **146**, 1063-1074.

9. Paillot R., Hannant D., Kydd J.H. & Daly J.M. (2006). – Vaccination against equine influenza: quid novi? *Vaccine*, **24**, 4047-4061.
 10. Watson J., Halpin K., Selleck P., Axell A., Bruce K., Hansson E., Hammond J., Daniels P. & Jeggo M. (2011). – Isolation and characterization of an H3N8 equine influenza virus from outbreaks in Australia in 2007. *Aust. vet. J.* (in press).
 11. Watson J., Selleck P., Axell A., Bruce K., Taylor T., Heine H., Daniels P. & Jeggo M. (2011). – Diagnosis of equine influenza virus infections in quarantine stations in Australia, 2007. *Aust. vet. J.* (in press).
 12. World Organisation for Animal Health (OIE) (2008). – Chapter 2.5.7. Equine Influenza. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, 6th Ed. OIE, Paris. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.05.07_EQ_INF.pdf (accessed in August 2010).
 13. World Organisation for Animal Health (OIE) (2008). – Expert Surveillance Panel on Equine Influenza Vaccines. Conclusions and recommendations. *OIE Bulletin*, **2**, 42-45.
-

