RECOVERY OF EI COUNTRY FREE STATUS

AUSTRALIAN REPORT TO THE WORLD ORGANISATION FOR ANIMAL HEALTH (OIE)

Submitted by the Australian Chief Veterinary Officer

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Executive Summary

1. Prior to the equine influenza (EI) outbreak in Australia in late August 2007, Australia was free from EI according to the historical freedom provisions of Article 3.8.1.6 of the OIE Terrestrial Animal Health Code (that is, disease had never been recorded in Australia).

2. In 2007 Australia experienced a large outbreak of EI associated with the escape of virus from imported horses in a quarantine station.

3. Australia has an effective national animal health system which ensured that the EI incursion was quickly recognised and a comprehensive emergency response strategy immediately implemented. EI is a notifiable disease in Australia. Australia has detailed contingency plans (AUSVETPLAN) for managing animal disease emergencies including the incursion of exotic diseases. The AUSVETPLAN Equine Influenza Strategy documents nationally agreed policy and control measures for managing an outbreak of EI.

4. Despite Australia's excellent animal health system, the highly contagious nature of the infection and the presence of a fully susceptible horse population meant that the disease had spread before detection. Several horse events and associated movements that occurred before EI was identified meant that EI had already infected horses over a wide area in central and northern New South Wales (NSW) and south eastern Queensland (Qld). An effective response contained the EI outbreak to these parts of the two states.

5. National and state/territory Governments and industry agreed on a national approach to contain and eradicate EI using Australia's AUSVETPLAN EI strategy. The response focused on containing and eradicating the infection whilst mitigating the socio-economic impacts. Elements of the response included an initial national horse movement standstill and prohibition on events; biosecurity measures within infected and non-infected areas; limited risk-based movement within infected areas; implementation of risk zones; risk-based movements between zones and jurisdictions; strategic vaccination in infected areas; and strategic vaccination in non-infected areas as business risk insurance.

6. The national approach was effective in controlling the outbreak. The EI epidemic peaked in October 2007, with the number of new cases declining over the following months. The last infected premises first showed clinical signs on 9 December 2007 in NSW and 25 December 2007 in Qld. By the time the last case of EI was infected, more than 10 600 premises and nearly 80 000 horses had been infected.

7. In 2008 the emphasis of the response shifted to demonstrating freedom from disease in previously infected areas, reclassifying zones to lower risk status and progressively removing horse movement restrictions in response to the improving disease situation. The progressive reclassification of previously infected areas of NSW and Qld to free status hinged on the time elapsed since the areas last reported case, the resolution of all infected, suspect and dangerous contact premises; and surveillance results from both targeted and random sampling.

8. Australia declared itself provisionally free of EI on 14 March 2008 following the completion of comprehensive disease control and surveillance programs in both states. Surveillance under nationally agreed guidelines saw extensive testing of more than 34 000 horses under targeted and random sampling programs.

9. Surveillance continued in all jurisdictions of Australia after the declaration of provisional freedom and focussed on the investigation of suspect clinical cases. No evidence has been found that EI is still circulating in the horse population, even after the lifting of all movement restrictions and significant movements of horses from formerly infected regions to naïve populations of horses. It is highly likely that naïve populations would show signs of disease were EI still present in Australia.

10. As part of the process of maintaining Australia's EI free status quarantine requirements for imported horses have been strengthened. This includes implementing the Australian Government's response to recommendations from an independent inquiry held to examine the circumstances that contributed to the EI outbreak.

11. Conclusion: An outbreak of EI in Australia in 2007 was successfully contained and eradicated. It is more than 12 months since the last case and a comprehensive surveillance program that exceeds OIE requirements provides a high degree of confidence that disease is no longer present. Consequently, in accordance with the OIE Terrestrial Animal Health Code 2008, Australia declares that it has regained its EI free status.

1. Introduction

1.1 Australia's animal health system

The animal health system in Australia relies on representation from both government and industry to develop and implement effective and coordinated policies.

The Australian Government is responsible for quarantine and international animal health matters, including disease reporting, export certification and trade negotiation. It also advises and coordinates national policy and, in some circumstances, provides financial assistance for national animal disease control programs. Australian state and territory governments are responsible for disease control and eradication within their own boundaries. Private veterinarians and industry also contribute to animal health management and disease control efforts. Table 1 details the number of veterinarians and government auxiliary personnel in Australia in 2007.

Table 1: Veterinarians and other animal health personnel in Australia's animal health system (AHA 2007a).

Veterinarians		**Auxiliary Personnel**	
Government	581	Stock inspectors, meat inspectors etc	1000
Laboratories, universities, etc	487		
Private practitioners	7042		
Other Veterinarians	1486		
Total	**9596**		**1000**

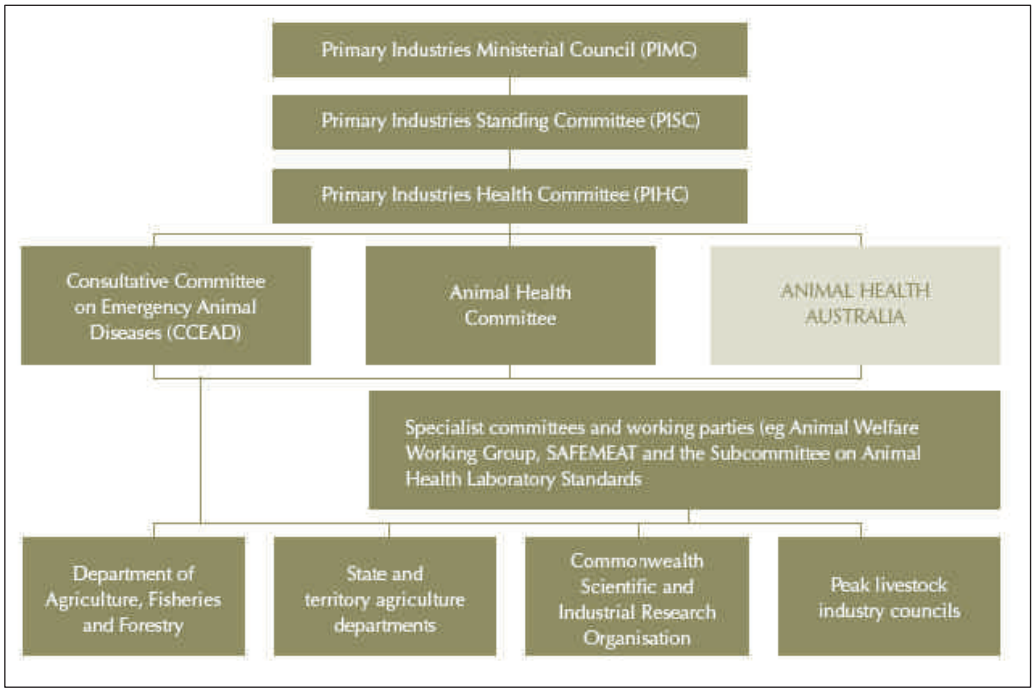
During animal health emergencies, coordination is provided by the Consultative Committee on Emergency Animal Diseases (CCEAD). Committee members are the Australian chief veterinary officer, state and territory chief veterinary officers, the head of the Australian Animal Health Laboratory, Biosecurity Australia, Australian Quarantine and Inspection Service, Animal Health Australia and technical representatives from the livestock industries. CCEAD initiates planning and

implementation of the most effective technical response while the National Management Group (NMG) endorses response plans and their resource requirements. NMG is a high-level committee comprising the heads of agriculture agencies (state and national), and senior representatives of the relevant industry or industries affected by an emergency animal disease, which has final responsibility for approving emergency response plans (including budgets), monitoring overall expenditure, and for making final determinations on eradication and the feasibility of eradication.

The capacity for dealing with emergency disease incidents in each state and territory is managed through each jurisdiction's emergency management committee. Figure 1 details the organisation of Australia's animal health system.

The Emergency Animal Disease Response Agreement (EADRA) provides a framework for managing and funding responses to animal disease emergencies. The agreement is between major industry groups and government parties. The Australian veterinary emergency plan (AUSVETPLAN) is the national technical plan for responding in a consistent manner to an outbreak, or suspected outbreak, of an emergency animal disease anywhere in Australia (Animal Health Australia 2007). AUSVETPLAN ensures that a prompt, efficient and effective response is implemented by providing a strategic framework and identifying roles, responsibilities and policies to be followed by agencies during an EAD response. AUSVETPLAN procedures are set out in 64 disease strategies, including Equine Influenza, and various procedural manuals (http://www.animalhealthaustralia.com.au/programs/eadp/ausvetplan_home.cfm).

Figure 1: Organisation of the Australian Animal Health System (AHA 2007a).



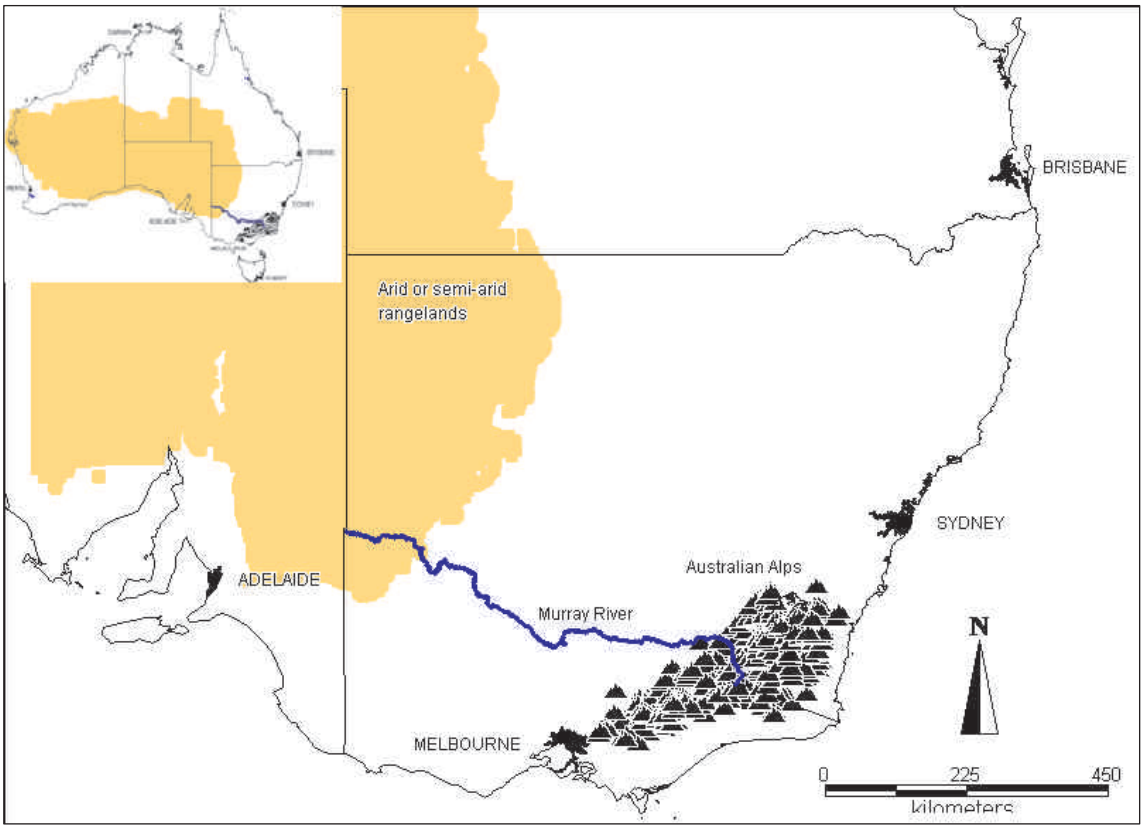
Further information on the Australian animal health system is available at: http://www.animalhealthaustralia.com.au/status/ahia.cfm

1.2 Australian geography relevant to the epidemic

Australia is a continental island, the sixth largest country in the world (Geoscience Australia 2005) and comprises six states and two territories. The physical size of Australia (approximately 70% of the size of Europe or the USA) and the distribution of animal populations make zoning a valuable approach to successfully managing animal diseases. Physical geography played a role in restricting the epidemic to smaller areas within Australia.

Figure 2: Geographical features limiting the spread of infection in the Australian EI epidemic.

The low density horse populations in western NSW and Qld associated with semi-arid and arid rangelands, along with the Murray River and Australian Alps helped contain EI.



Large distances and rapidly implemented movement restrictions also reduced the probability that horse owners from states remote from NSW and Qld could attend infected horse events and return home with potentially incubating horses. Furthermore, there is a semi-arid and arid region in western NSW and Qld with very low horse and human populations and few roads which helped to prevent the westward spread of infection to neighbouring states. Additionally, the Murray River and an adjacent alpine range form an extensive part of the border between NSW and Victoria. This border was readily policed at river crossings preventing the movement of potentially infected horses south to extensive horse populations in Victoria (see Figure 2).

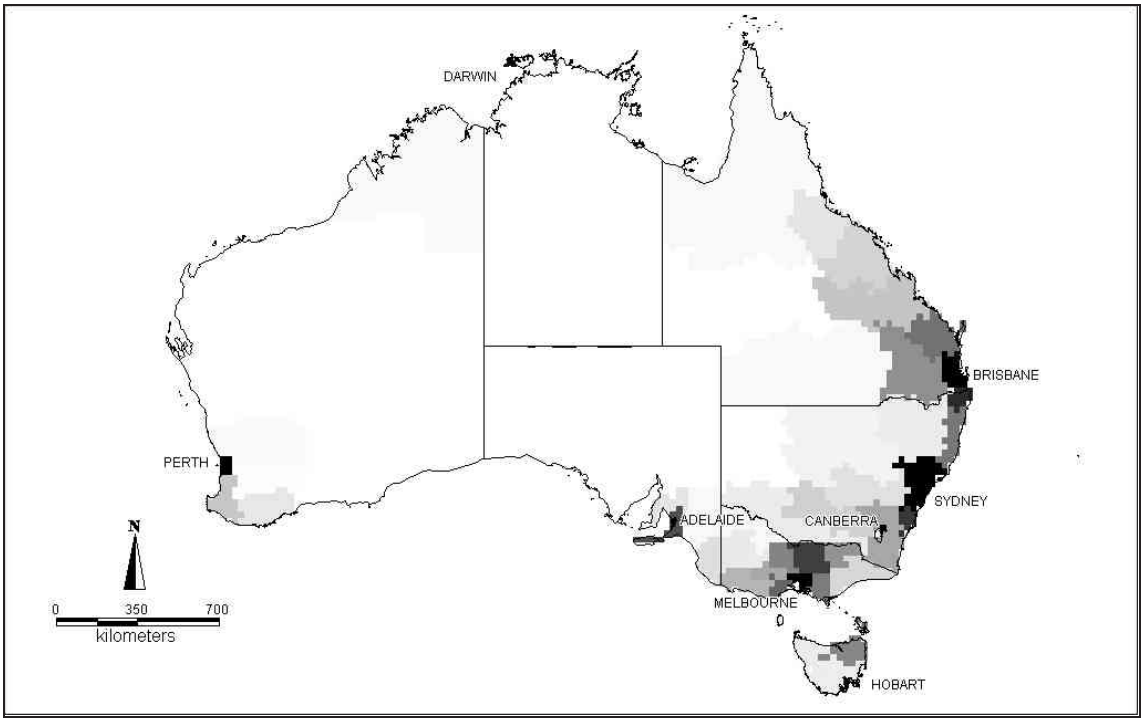
1.3 Australian horse population

The domestic horse population in Australia is estimated to number 932 000 (Gordon 2001; CIE 2007). The majority (85%) of these horses are found in the south east

portion of Australia in Qld, NSW and Victoria (Gordon 2001), where most of the human population is also situated (see Figure 3).

Figure 3: Density of domestic horses in Australia[1].

Extensive, high density horse populations are present near Brisbane in SE Queensland, Sydney (and the nearby Hunter Valley) and Melbourne.



Sectors of the domestic horse population include thoroughbred and standardbreds (the horse racing industry), eventing, working and recreational horses. The numbers of horses in each sector have been estimated (Gordon 2001; CIE 2007) and estimates are presented in Table 2.

Table 2: Estimated domestic horse numbers in Australia (adapted from ABARE 2007).

Sector		Population
Thoroughbred and Standardbred in 2005-06	Horses that raced	44 197
	Yearlings	23 584
	Stallions	1 178
	Mares served	37 233

[1] Raw data on stud and other horses collected by the Australian Bureau of Statistics (ABS 2006). This data was interpolated to give a qualitative national coverage of the density of domestic horses.

	Foals born	23 723
	All Thoroughbred and Standardbred	129 915
Total active racing and event horses		329 527
Recreational horses		602 473
Estimated total domestic horse population		**932 000**

There is also an estimated 300 000 to 400 000 wild horses (Dobbie *et al.* 1993; Dawson *et al.* 2006). These are mostly located in remote regions of Australia, in particular the tropical north (see Figure 4). Extensive wild populations were substantially remote from domestic horses involved in the EI epidemic, with only some small, isolated populations close to infected areas.

1.4 History of EI in Australia

Equine influenza is a highly infectious viral disease of equidae and is a major cause of equine respiratory disease in many countries worldwide (Hannat *et al.* 1996; Myers and Wilson 2006). Global transportation of horses has been responsible for numerous outbreaks of equine influenza virus (EIV) and susceptible populations in free countries are at risk if quarantine measures fail (Myers and Wilson 2006). Only a few geographically isolated countries such as New Zealand and Iceland have remained free of equine influenza.

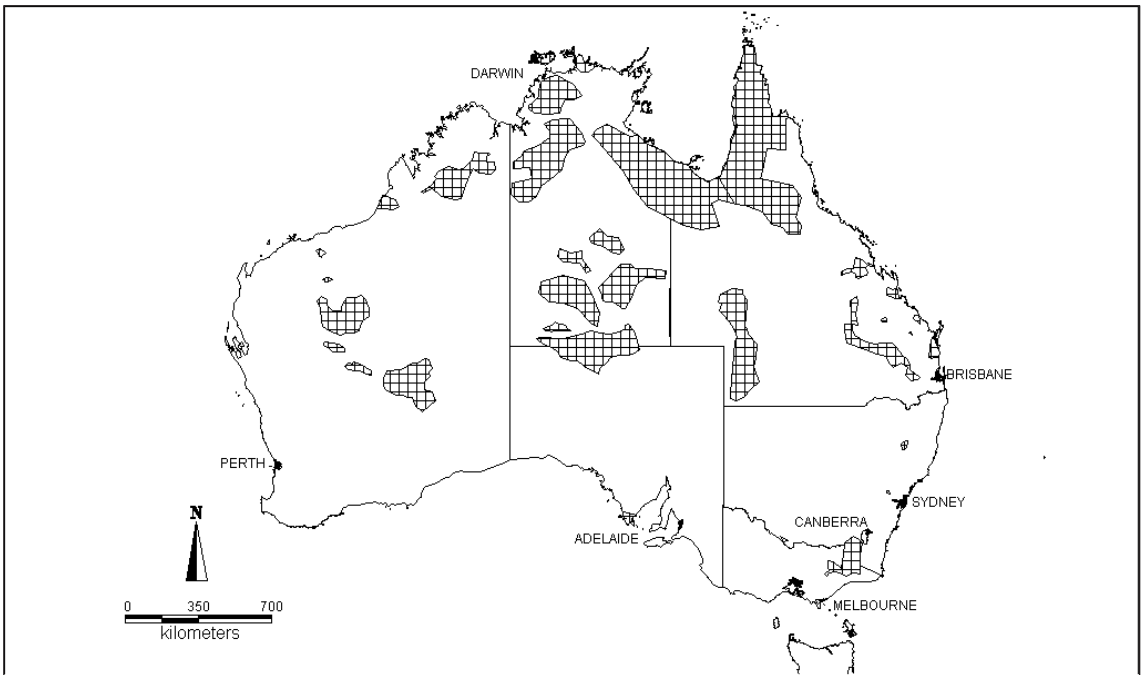


Figure 4: The distribution of feral horses in Australia (adapted from Dobbie *et al.* 1993).

Prior to August 2007, Australia was free from EI according to the historical freedom criteria of Article 3.8.1.6 of the OIE Terrestrial Animal Health Code (OIE 2007). However, clinical signs of EI were observed at Eastern Creek Quarantine Station (a quarantine station outside Sydney) from 17-20 August with disease confirmed on 23 August 2007. The following day, EI was confirmed at an equestrian centre about 40 km away. It soon became apparent that EI had spread to a number of sites in NSW and to Qld and this spread was associated with several equine events. A judicial enquiry found that EI had most likely been imported with infected horses from Japan to the Eastern Creek Quarantine Station where it escaped to the Australian horse population through fomite spread on people or equipment (Callinan 2008). This fomite spread was associated with inadequate biosecurity. Sequence analysis of the matrix gene and HA1 region of the haemagglutinin gene showed virus isolates to be almost identical to those of the Wisconsin strain of EI (A/equine/Wisconsin/1/03(H3N8)).

Following confirmation of EI, movement restrictions on all horses were implemented on 25 August 2007 and contained the epidemic to areas in NSW and Qld. Within NSW and Qld, the epidemic spread locally over the next three months with occasional long-distance movements. A disease management program using risk-based zoning, movement restrictions, tracing, laboratory testing, emergency vaccination, public education and industry engagement subsequently succeeded in eradicating the disease. During the epidemic, an estimated 10 651 premises were infected across an area of 283 000 km^2. Clinical signs were seen in the last known infected premises on the 25 December 2007 and following an extensive surveillance program, provisional freedom from EI was declared in Australia on 14 March 2008.

1.5 Australia's policy towards equine influenza

The AUSVETPLAN EI Strategy (AHA 2007b) summarises Australia's policy in response to an incursion of EI. In brief, the document describes EI as an OIE-listed disease that

has the potential for very rapid spread; the potential to cause illness and/or deaths in young foals and debilitated or old horses; and the potential to cause loss of performance. The document further describes EI as an important concern in the international movement of horses. This is due to the disease's potential for causing serious economic loss within the Australian equine industry through morbidity and mortality expected in naive populations, the constraints placed on the movements of animals for an unknown period, and the ongoing costs of any vaccination program.

The preferred approach is to control and then eradicate EI through:

1. quarantine and movement controls of equines, their products and other potentially contaminated items to prevent spread of infection
2. decontamination of facilities, equipment and other items to eliminate the spread of the disease agent from infected animals and premises
3. tracing and surveillance to determine the source and extent of infection
4. an awareness campaign to encourage cooperation by industry and the community
5. vaccination of horses (only with approval and under certain circumstances).

Importantly, successful implementation of the policy was and is considered to be strongly dependent on industry cooperation and compliance with control and eradication measures. For this reason, equine influenza is classified as a category 4 disease in the EADRA. Category 4 diseases are those for which costs are funded 20% by government and 80% by industry ensuring close cooperation between industry and government.

1.6 Australia's effective and open animal health system

Throughout the epidemic Australia maintained an open and transparent animal health system. The OIE was notified of the outbreak immediately (within 12 hours) upon confirmation of the disease, with frequent follow up reports. Trading partners were also notified independently and close cooperation between trading partners and Australia allowed tracing of exported horses.

The eradication of the EI epidemic demonstrated that Australia has an effective animal health system that can identify and eradicate emergency animal diseases. Australia is one of the few countries in the world that has eradicated EI. This also supports the seriousness with which Australia regards the biosecurity system and demonstrates the well developed disease control capability and regulatory processes in Australia.

1.7 Changes to quarantine procedures

As a result of the EI outbreak, quarantine requirements for imported horses have been strengthened. This includes implementing the Australian Government's response to recommendations from an independent inquiry held to examine the circumstances that contributed to the EI outbreak (www.daff.gov.au/about/publications). The Australian government accepted all 38 of the recommendations from the Equine Influenza Inquiry. These include *inter alia* inspection and review of pre-export quarantine (PEQ) facilities and procedures, additional testing for EI during PEQ and vaccination of horses with updated strains of EIV. The procedures during post-arrival quarantine (PAQ) have been significantly strengthened and include upgraded facilities at the quarantine stations, updated work procedures, inspection and review of facilities and procedures and testing of horses for EI virus during PAQ.

2. Disease control campaign

2.1 Introduction and context

Late in the evening on 24 August 2007, the New South Wales Chief Veterinary Officer advised the Australian Chief Veterinary Officer of equine influenza (EI) in horses with respiratory symptoms at Centennial Park Equestrian Centre, Randwick, Sydney, following confirmatory testing by PCR. The following morning an emergency disease response was instigated in NSW and a meeting of Australia's Consultative Committee on Emergency Animal Diseases (CCEAD) was held. CCEAD noted the developments in NSW, confirmed the presence of EI in Australia and recommended the immediate implementation of a 72-hour national horse standstill. By 26 August, CCEAD were

advised of two confirmed and two suspect infected premises in NSW and a suspect infected premises at Warwick, Queensland associated with horse movement from NSW. Over the next week, cases were confirmed in a range of locations in NSW and Qld, most of them linked to horse events that had been held prior to the finding of EI.

The CCEAD confirmation of the presence of an exotic disease listed as subject to arrangements prescribed in the EADRA and AUSVETPLAN was reported to the National Management Group (NMG). At its meeting on 27 August, the NMG endorsed the NSW EI Emergency Animal Disease Response Plan (EADRP), and agreed to invoke cost-sharing for a national EI response, based on EADRA principles and funding formulae, from 25 August 2007. This activated a number of pre-agreed actions aimed at controlling the disease. These actions included cost-sharing and reporting arrangements, the declaration of restricted and control areas around infected premises, tracing of horse movements and establishment of Local Disease Control Headquarters, State Disease Control Headquarters and a National Disease Control Centre. Extensive public awareness and communications programs were also initiated and contingency arrangements for the supply and registration of vaccine for emergency use commenced.

The objective of the national EI response was to contain the disease with a view to eradication. The field response was guided by the EI AUSVETPLAN. The key components of the response strategy were movement controls, bio-security measures, risk-based zoning and, subsequently, vaccination including vaccination buffer zones to contain the outbreak. Over the outbreak, a series of technical working groups were established by the CCEAD and the NMG to address issues around vaccination, scenario cost analysis, contingency planning, conditions for horse movements and proof of freedom.

As horse events were cancelled, movement restrictions applied and zones put in place, a key concern of governments and industry was to ensure that containment and eradication activities were tempered by and addressed socio-economic

considerations. This concern was addressed through providing government support programs and through the adoption of risk-based measures to permit the limited movement of some horses within and between zones, and for limited race meetings and sales activities to take place.

Although vaccination was not initially used, it became apparent that additional measures were required to contain the outbreak. On 17 September 2007, there was national agreement to implement a vaccination strategy to assist the containment response. A canary pox recombinant vaccine (ProteqFlu, Merial) was used as it provided rapid immunity, allowed for the use of tests that permitted differentiation between vaccinated and naturally infected horses, and this vaccine had proven effective when used previously in South Africa. The initial focus of vaccination was on containment. Subsequently, wider use was approved to both mitigate the consequences of equine influenza for sectors and groups in infected areas, and to provide insurance against possible outbreaks in targeted sectors or groups of horses in unaffected areas (thus facilitating greater movement and economic activity in those areas).

Over the period from August to December 2008, infection remained contained to areas within NSW and Qld, despite a large number of infected properties being found. Relatively little increase in the total infected area of Australia occurred after September 2007. Rather, most subsequent cases were infection of un-infected premises in already infected areas. The number of reports of new infections fell through October and November 2007 and outlying areas began to be cleared of infection and reclassified to lower risk status levels. The last newly infected premises displayed clinical sings 9 December 2007 in NSW and 25 December 2007 in Qld. Overall, the Australian EI outbreak involved an estimated 10 651 infected premises with approximately 80 000 horses infected. Approximately 136 000 horses were vaccinated.

By early 2008, the emphasis of the program shifted to clearing previously infected areas, reclassifying zones to lower risk status levels and freeing-up of horse

movements in response to the improving disease situation. The progression of previously-infected areas of NSW and Qld was based on nationally-agreed criteria and conditions including: the elapsed time since last case reported; resolution of all infected, suspect and dangerous contact premises; and surveillance (both targeted and random sampling).

Australia declared its provisional freedom from EI on 14 March 2008 following the completion of comprehensive disease control and surveillance programs in both states. This was a key interim step for Australia in regaining its EI free status. The declaration of provisional freedom was based on: movement conditions to prevent spread of the disease had remained in place; more than 60 days had passed since the last premise was infected; all previously infected, suspect and dangerous contact premises had been resolved; and all nationally agreed proof of freedom testing requirements in previously infected areas were completed. Surveillance, under nationally-agreed guidelines, involved extensive testing of more than 34 000 horses under targeted and random sampling programs. No evidence that EI was still circulating in the horse population was found.

Each component of the disease control program is described in more detail below.

2.2 Movement restrictions

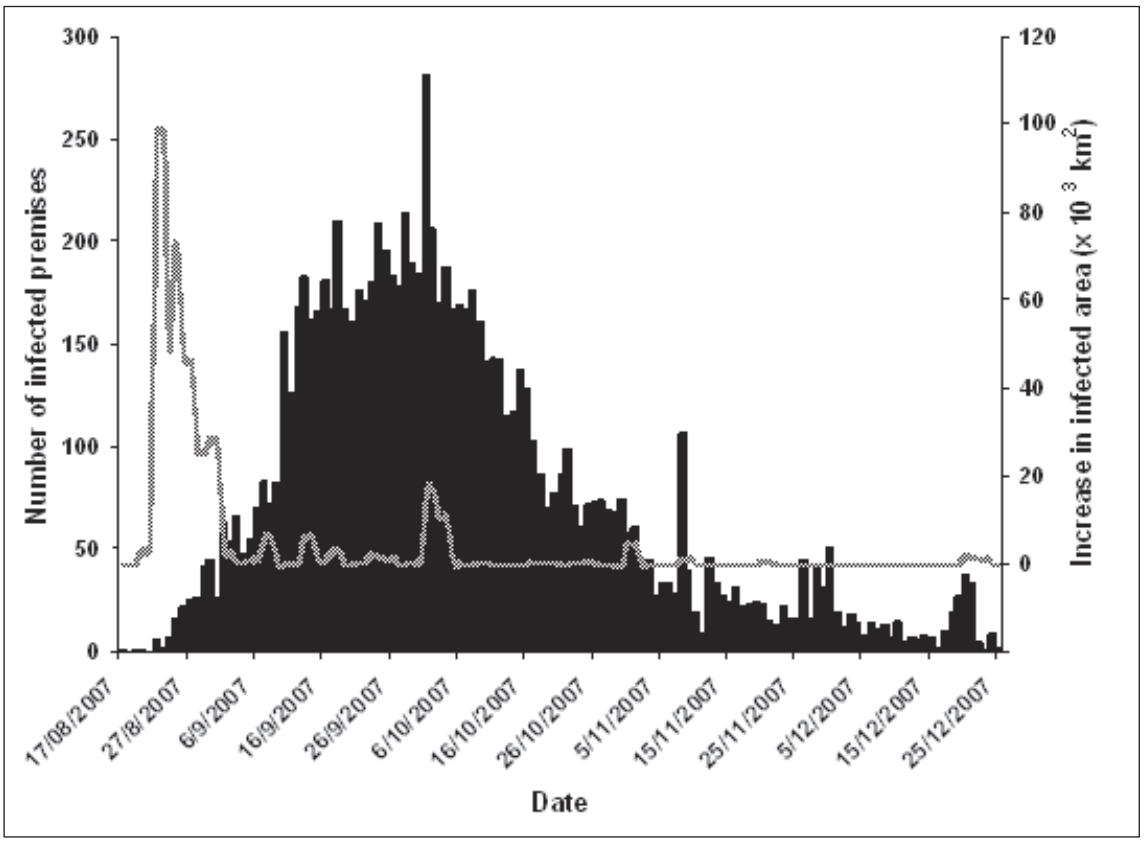
Following the diagnosis of EI late on 24 August, CCEAD met on 25 August 2007 and agreed to impose a national ban on horse movements. This prevented further dispersion of EI by horse movements or congregations at events. The national ban was maintained until 29 August 2007 when uninfected states and territories gradually began to ease movement restrictions within their own borders. However, uninfected states continued to prohibit horse events and the import of horses from NSW and Qld. Bans on the movement of horses from infected states were gradually eased after 17 October 2007. At this time the CCEAD agreed that movements of horses from NSW and Qld to unaffected states would not impact on those states' disease-free status, provided that stringent movement protocols were followed. Within NSW and Qld, complete movement bans were maintained until a risk-based

zoning system was implemented on 21 September 2007. After this time, bans on horse movements and aggregations were lifted in low risk areas. Bans were also lifted *within* the circumscribed *special restricted area* in NSW, an area with high density populations of horses and an already high incidence of disease ("purple" zone — see below).

Figure 5 shows the epidemic curve for the EI outbreak. It shows that the initial spread of infection across the landscape largely occurred in the first 10 days of the epidemic. After the movement standstill was instigated there was little expansion in the size of the infected area of Australia. Vaccination began on the 29th of September with the first round of vaccinations largely complete in late November. Although there was little increase in the size of the infected areas following the introduction of movement restrictions in late August, the number of infected premises continued to increase, peaking in October. These cases were largely associated with in-filling of existing infected areas associated with 'local spread'.

Figure 5: The epidemic curve for the equine influenza outbreak in Australia (Cowled, unpublished data).

The vertical columns represent the number of new infections each day by estimated onset of clinical signs, whilst the polygon represents the increase in the total area of infected land calculated every two days. Movement restrictions were instigated on the 25th of August 2007 following diagnosis of the index case late on the 24th of August. Emergency vaccination began on 29 September and most horses had received their first vaccination by November.



2.3 Public awareness and communication

Industry involvement in decision making was important since it was anticipated that a large portion of the costs of the response was to be accrued by industry (through the EADRA). Industry was represented on the CCEAD, as well as providing liaison officers at disease control centres and response staff with equine industry experience and contacts. Members also provided a valued industry perspective on strategic and tactical matters, ensuring an optimal response.

Additionally, industry involvement enabled a more coordinated response, increased the likelihood of public compliance with disease control measures and enabled access to industry infrastructure and resources. For example, the equine racing industry's equine health system became part of the emergency response. Industry fora such as mailing lists and meetings also acted as valuable media for communicating outbreak information. Furthermore, industry participation was essential in distributing government funded assistance packages.

2.4 Implementation of the risk-based zoning system

A zoning system based on evaluation of the risk that EI was present in an area was introduced on 21 September 2007. Zoning assisted the horse industry to resume its normal activities by allowing movements of horses in low risk areas of NSW and Qld. The zoning system also facilitated management of the outbreak by prioritising resource allocation and by preventing horse movements within high risk areas. Information about restrictions and controls applying in the different zones was communicated using colour coding. Declared zones (based on the risk of infection with EI) are listed below:

1. *Special Restricted Area — Purple zone*. A defined part of an infected area with high horse densities and where active spread of infection was considered inevitable was declared as a special restricted area (purple zone) in NSW. The movement of horses was allowed inside this zone, but not to other zones. The rationale was to facilitate normal activities while allowing EI to spread in areas where it was considered inevitable.
2. *Restricted Area*[2] *— Red zone*. Areas of infected states that contained active EI infection were classified as red zones.
3. *Control Area*[3] *— Amber zone*. An amber zone was an area within infected state assessed as low risk of EI but which was adjacent to infected area(s). The amber zone surrounded infected areas to ensure that there was a separation of at least 10km between any green zone and an infected area.
4. *Protected Area — Green zone*. Green zones were areas within infected states that were assessed as free from EI. These were physically separated from the infected areas by a buffer (control area).

[2] A relatively small declared area around an infected premises that is subject to intense surveillance and movement controls (AHA 2008).

[3] A declared area in which the conditions applying are of lesser intensity than those in a restricted area (the limits of a control area and the conditions applying to it can be varied during an outbreak according to need) (AHA 2008).

5. *Protected Area*[4] — *White zone*. Areas free of EI (historically or following reclassification of previously infected areas). Initially only uninfected states were declared as white zones, but later, substantial parts of NSW and Qld were reclassified to white after EI had been eradicated.

Zones boundaries were based on natural or artificial features that would restrict spread of infection. For example, the boundaries of zones were drawn through areas of low horse density associated with natural features precluding horse premises (such as national parks). Zones were regularly updated during the course of the outbreak based on surveillance results. Figure 6 shows zone progressions over the course of the epidemic.

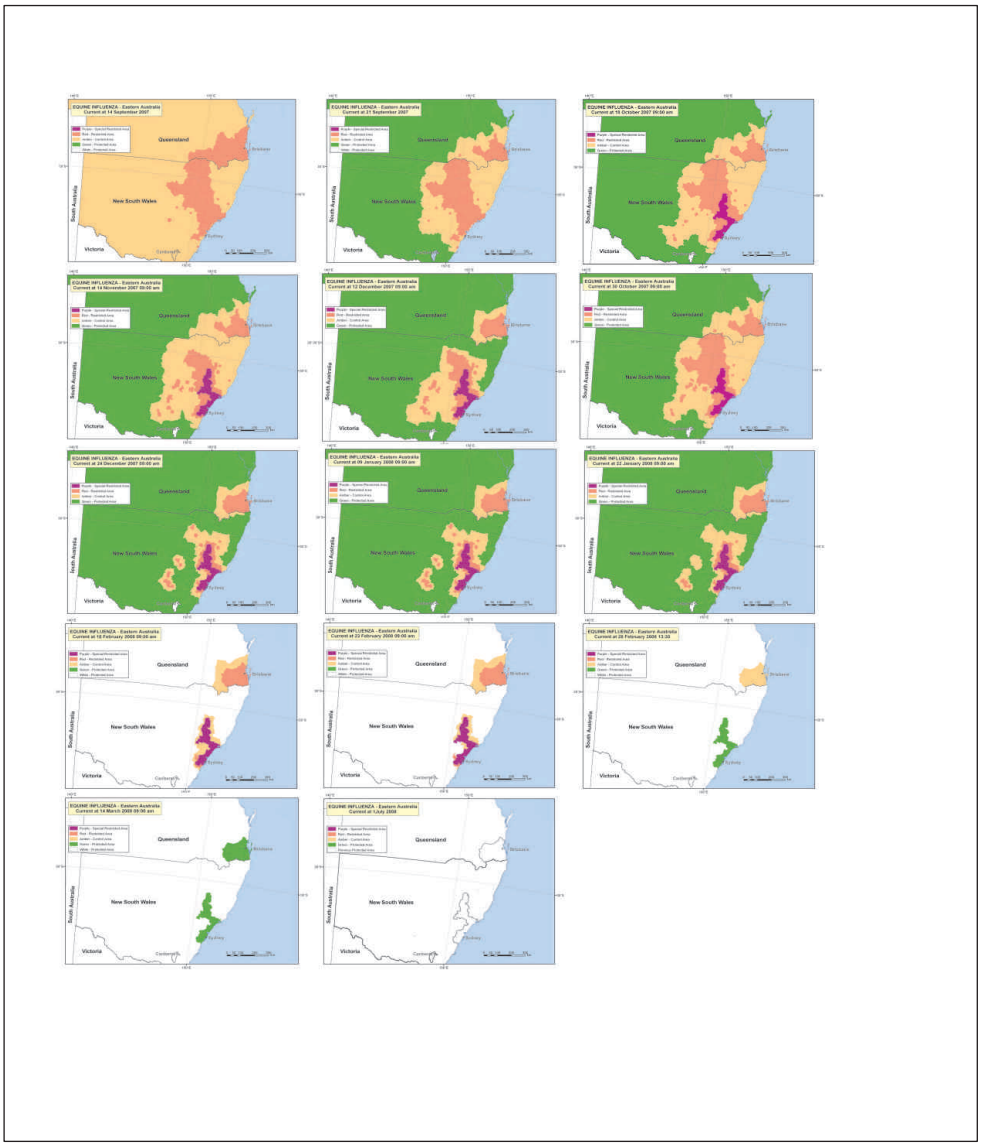
Buffer zones were created for the purpose of containing lateral spread from infected areas. Buffers took the form of natural barriers, horse-free areas, or immune (vaccinated) horse populations or any combination of these and were located in amber or red zones.

2.5 Laboratory capability and diagnosis

Laboratory support for EI control and subsequent proof of freedom testing was provided by the Australian Animal Health Laboratory Network. This network comprises the Australian Animal Health Laboratory (AAHL), all state and territory government laboratories and university and private veterinary laboratories, with official testing being conducted by relevant government laboratories.

[4] An area free of EI where few restrictions such as movement restrictions applied after the initial phase of the epidemic.

Figure 6: The progression of EI zones in Australia over the course of the epidemic.



While there have been more than 100,000 individual laboratory tests performed, the majority of diagnostic and surveillance samples were collected from the two infected states (NSW and Qld). The Elizabeth Macarthur Agriculture Institute in NSW and the Biosecurity Sciences Laboratory in Qld were responsible for initial diagnosis and investigation. AAHL provided the confirmation of diagnoses made in other laboratories. AAHL was also involved in the investigation of potential new foci of infection in NSW and Qld and suspect cases in non-infected states as required.

2.5.1 Samples for diagnosis

Samples used for initial and confirmatory diagnosis included mainly sera (clotted blood) for serology and nasal swabs (in transport medium) for virology. Samples from surveillance and suspect cases were initially tested by the respective state or territory government laboratories. Many positive or indeterminate cases were sent to AAHL for confirmatory testing. Samples from any quarantine station were sent directly to AAHL for testing.

2.5.2 Diagnostic methods

Both serology and virology have been used for initial and confirmatory diagnosis throughout the EI epidemic. For the purpose of differentiating infected from vaccinated animals (DIVA), both management-based and laboratory strategies were used.

2.5.3 Serology

The competitive enzyme-linked immunosorbent assay (cELISA), which was originally developed for avian influenza surveillance (Heine *et al.* 2007), was modified by AAHL for EI specific antibody detection during the epidemic. The modified test adopted a recombinant viral nucleoprotein (NP) antigen rather than a whole virus antigen prepared through egg culture, which improved the diagnostic sensitivity (approximately 96%) and specificity (approximately 97%). Because the canarypox vaccine used for disease management will not induce any NP specific antibodies in vaccinated horses, this feature has also allowed the cELISA to be used as a major laboratory method for DIVA purposes in the epidemic.

The OIE recommended haemagglutination inhibition (HI) test, targeting antibodies to a panel of haemagglutinin (HA) antigens from overseas H3N8 isolates such as Newmarket/95, Moulton/98 and/or Prague/56, was also used for diagnosis and surveillance in the epidemic. For proof-of-freedom testing, the HA antigen from the Sydney/07 isolate was used. The test was also applied for indeterminate cELISA results and confirming vaccination status as required.

Single radial haemolysis, which generally offers a more differentiating power than an HI test, may be used for measuring antibody titres post vaccination. Agar gel immunodiffusion test was also available. However, these tests were not considered for routine use in the epidemic due to their low diagnostic sensitivity and/or throughput level.

2.5.4 Virus detection

Virus isolation using embryonated chicken eggs and cells was employed as the gold standard to confirm EI infection status, especially for the index case and any new foci of infection. This test was only conducted at AAHL, where the required high biosecurity facilities are in place. Virus isolation, which often takes at least 2-3 weeks to complete for EI viruses, was not considered to be practical for initial diagnosis and investigation purpose. As such, a TaqMan real-time reverse transcriptase polymerase chain reaction (qPCR) for influenza A virus matrix genome, which was again modified from the existing protocol for avian influenza, was used. At AAHL, the conventional H3 specific PCR test was also employed.

A number of other virus detection methods were also considered. Although the antigen detection ELISA can correlate with viable virus and is available at AAHL, the test is generally not as sensitive as PCR test and was not officially used in the epidemic. In addition, sequencing and genetic analysis was used at AAHL to support diagnosis, epidemiological investigation and the selection process for the vaccine.

2.5.5 Quality assurance program

All government laboratories involved in the disease control and proof of freedom testing have been accredited for PCR and ELISA testing under the National Association of Testing Authorities (NATA). Before the EI outbreak, the two major diagnostic tools (cELISA and qPCR) for influenza A had been successfully transferred to all members within the laboratory network for avian influenza surveillance. In order to ensure the validity of these technologies for EI testing, there were several special meetings among key laboratory scientists to review and harmonise the

national laboratory approach during the epidemic, and relevant quality assurance programs including proficiency testing were also put in place.

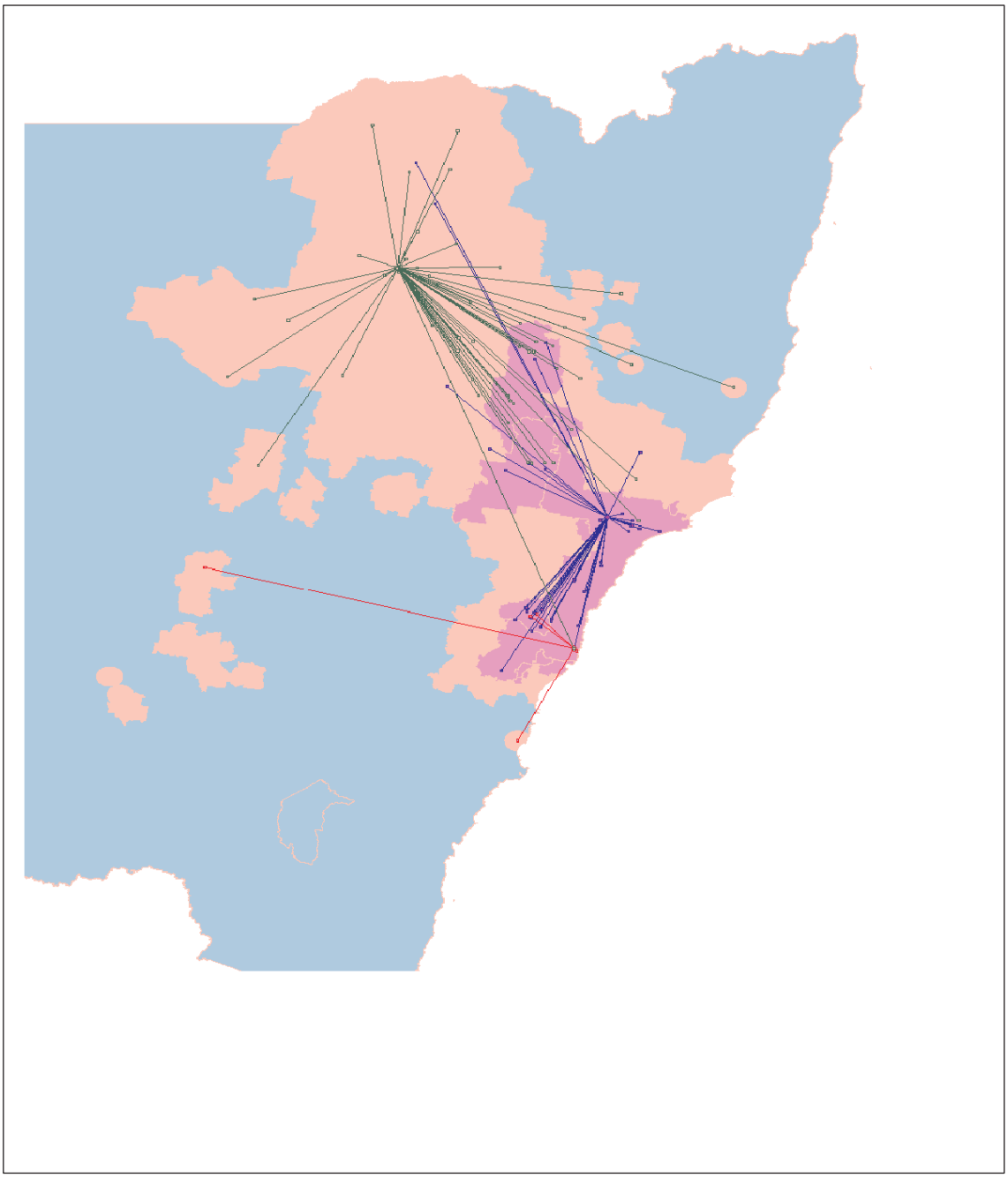
2.6 Disease tracing and surveillance in infected states

An extensive disease surveillance program in infected states gathered information using a variety of means during the epidemic. Following the first cases, state veterinary services conducted epidemiological tracing which involved the examination of horses and laboratory testing. See Figure 7 for an example of tracing conducted within NSW. However, it soon became evident that EI was widely disseminated and tracing could not be used as the only surveillance strategy. An extensive passive surveillance program (examination of clinical cases) was therefore rapidly developed to augment tracing. Over the course of the epidemic the surveillance system detected 10 651 premises infected with EI.

Passive surveillance relied on the reporting of suspect cases by private and government veterinarians and members of the public. EI is a notifiable disease in Australia. Since the Australian horse population was naïve to EI and infected horses exhibited obvious clinical signs, passive surveillance was an effective tool for finding new cases. Passive surveillance was enhanced with extensive public and veterinary education campaigns focusing on clinical signs, high-risk geographic zones and procedures for reporting cases. Passive surveillance resulted in the identification of many infected premises in infected areas. Passive surveillance was also important in the green zones of NSW and Qld to provide confidence that disease had been contained within red and purple zones.

Targeted and random surveillance became increasingly important during the later stages of the outbreak to look for residual disease, for reclassifying areas and as a prerequisite for declaration of disease freedom. This surveillance component is discussed in section 3.

Figure 7: Infected premises traced from key locations in NSW. Figure courtesy of Evan Sergeant, NSW DPI/Austvet services.



Computerised information systems were used to manage disease information. Records about premises were entered into databases detailing infection status, horse population, laboratory results, comments on clinical signs and dates. The databases were linked with several software programs such as geographic information systems (GIS) and database management software. The results of surveillance analyses were promptly reported back through the Australian Animal

Health System and the public through state and national situation reports and public education campaigns. These channels facilitated communication about strategy decisions throughout the epidemic, such as the reclassification of zones and the implementation of new buffer zones.

2.7 Disease surveillance in non-infected jurisdictions

Surveillance in non-infected jurisdictions initially focused on horses that might have moved from infected areas before the outbreak was diagnosed and before implementation of the movement ban. All horses moved from NSW and Qld two weeks before the first diagnosis of EI in Australia were traced and their owners contacted. Horses that had travelled from high-risk zones were examined for clinical evidence of infection and were sampled and tested for EI with PCR and ELISA. Owners of traced horses from low-risk areas were given advice and government veterinarian contact details in the unlikely event that clinical signs were displayed. See Table 3 for details of the number of horses traced by each state.

Table 3: The number of horses traced from NSW and Qld to non-infected jurisdictions. No traced horses were infected with EI.

State/Territory	Number of horses traced
Northern Territory	16
South Australia	33
Tasmania	22
Victoria	62
Western Australia	36
Total	**169**

The second key component of surveillance in non-infected states was investigation of suspect cases. As in infected jurisdictions, local media campaigns were used to inform the public and veterinarians about clinical signs, the notifiable nature of the disease and correct reporting procedures using the EI telephone hotline. Subsequently, reported cases of equine respiratory disease and other more nondescript signs were investigated in every state of Australia. No evidence of EI was

found. See Table 4 for details of suspect case investigations in non-infected jurisdictions both during and after the epidemic.

Table 4: The number of suspect case investigations in non-infected jurisdictions.

Jurisdiction	Number of suspect cases during epidemic	Number of suspect cases: 14 March to December 2008
Northern Territory	4	0
South Australia	172	102
Tasmania	60	24
Victoria	116	85
Western Australia	66	115

2.8 Biosecurity

During the epidemic, biosecurity was important both in order to prevent long range movement of the virus and to reduce local spread (spread to neighbouring premises).

The potential for local spread was reduced by detailed public awareness programs emphasising biosecurity guidelines and through the distribution of equipment packs to horse owners and veterinarians. These guidelines provided specific information on topics such as equipment and vehicle decontamination, movement requirements, managing visitors, quarantine and isolation, fence security, reporting of suspect cases and specific veterinary education such as sampling and handling protocols. Despite these measures, local spread was a significant feature of the epidemic, especially in high-density peri-urban areas confirming the highly contagious nature of EI in naïve populations. Emergency vaccination was used in some areas to slow local spread.

As the epidemic waned, relaxation of movement restrictions was necessary to support continued functioning and to ensure the on-going variability of the horse industry. Potentially, this increased the risk of long range movements of EI. This risk was managed by applying different levels of biosecurity to identified risk categories

of horses. Susceptible horses (unvaccinated or with no natural immunity) were prohibited from moving in high-risk zones due to the unacceptable risk that these horses might be incubating disease. Movements of horses with vaccinated or natural immunity following infection were allowed within higher risk zones. The movement of immune horses from low risk to higher-risk zones was unrestricted. However, immune horses could only be moved from high-risk to low-risk zones under controlled conditions. Movements from infected premises, suspect premises or dangerous contact premises were not permitted. Additionally, vaccinated horses were required to undergo a pre-movement quarantine period (up to two weeks), two negative PCR laboratory tests three days apart, a veterinary examination and a thorough physical decontamination before being allowed to move. Upon arrival at the lower-risk zone, a further post-arrival isolation period of seven days was required. Horses recovered from natural infection had less strict biosecurity requirements for movement with shorter periods of pre- and post-arrival quarantine, and only a single ELISA test to demonstrate immunity.

2.9 Vaccination

The CCEAD recommended vaccination on 17 September 2007 as an additional tool to contain the spread of disease. No available commercial vaccines contained the strain of virus identified in the Australian outbreak and nor were any suitable vaccines identified that contained the specific components recommended by the OIE. However, a number of vaccines contained strains of virus which it was considered would be likely to produce acceptable immunity to the outbreak strain. Subsequently, a canary pox recombinant vectored vaccine (ProteqFlu, Merial) was chosen based on a number of criteria, including:

1. evidence that the vaccine would produce quicker and stronger immunity than inactivated vaccines and would reduce virus shedding within fourteen days of the initial dose
2. evidence that the vaccine had been used successfully during the 2003 equine influenza outbreak in South Africa

3. potential for application of laboratory techniques to enable 'differentiation of infected from vaccinated animals' (DIVA techniques). This was crucial to Australia's ability to provide adequate evidence of freedom from equine influenza.

The vaccine strategy focused on:

1. increasing the level of immunity within infected areas to deprive the disease of new hosts
2. protecting high-risk enterprises outside the infected areas as a contingency against the possibility of spread of the disease
3. assisting business continuity by providing some immunity to horses of high earning potential (racehorses and standardbreds) in uninfected states
4. Containing the spread of disease by creating buffer zones around infected areas. See Figure 8 for the location of the vaccination buffer areas in New South Wales and Queensland at the height of the outbreak.

More than 300 000 doses of vaccine were used during the epidemic. See Table 5 for the number of vaccines used by each Australian jurisdiction.

Table 5: Data on the number of horses vaccinated by state during the epidemic (source Animal Health Australia).

State/Territory	Estimated horses vaccinated	Vaccine doses used
Australian Capital Territory	500	1 200
New South Wales	68 000	160,487
Northern Territory	0	0
Queensland	55 000	112 191
South Australia	2 500	3843
Tasmania	100	388
Victoria	10 000	25 178
Western Australia	6	12
Total	**136 106**	**303 299**

Australia is confident that all vaccinated animals are easily identifiable and traceable for the purposes of proof of freedom. Strict controls on importation, storage and use

of the vaccine were imposed to prevent unauthorised use. Detailed record keeping and permanent animal identification using electronic microchips was performed. Laboratory DIVA techniques are also available to distinguish between EIV immunity derived from recombinant vectored vaccine and natural infection.

2.10 Summary

The EI eradication campaign was a coordinated national response involving cooperation between government and industry at many different levels. EI was eradicated from Australia through a combination of movement restrictions, zoning, enhanced biosecurity and vaccination. These measures contained the outbreak to a defined and relatively small part of Australia. Horse populations within infected areas rapidly became immune to EI through natural infection or through vaccination[5]. Comprehensive surveillance, along with an excellent laboratory response, permitted risk-based reclassification of zones to ensure that any outlying infection was rapidly contained. The last cases occurred in late December 2007. In 2008 the emphasis of the program shifted to clearing previously infected areas, relaxing the control of horse movements in response to the improving disease situation, reclassifying zones to lower-risk levels and testing for proof of freedom (see Section 3 below).

3. Proof of freedom testing

3.1 Introduction

The EI epidemic was successfully contained to only 3% of the Australian land area using a combination of movement restrictions, zoning, vaccination and enhanced biosecurity measures. The majority of Australia thus retained its EI free status, which allowed eradication and surveillance resources to be directed towards the high risk areas.

[5] Vaccination, while providing good protection against clinical disease, does not necessarily prevent infection. Vaccinated horses that become infected excrete less virus for shorter periods than naïve horses.

3.2 Zoning

The OIE recognises the importance of zoning[6] for disease control and trade (OIE 2008). According to the OIE Terrestrial Animal Health Code (OIE 2008), the EI status of a zone can be determined based on the following criteria:

1. the outcome of a risk assessment identifying all potential factors for EI occurrence and their historic perspective;
2. whether EI is notifiable in the whole country, whether an on-going EI awareness programme is in place, and whether all notified suspect occurrences of EI are subjected to field and, where applicable, laboratory investigations;
3. if appropriate surveillance is in place to demonstrate the presence of infection in the absence of clinical signs in horses.

According to these criteria, a large zone of Australia (97% of the land area) retained its EI free status throughout the outbreak. This was achieved through the containment of infection to distinct areas of NSW and Qld using the methods described in section two.

Within the two infected states, subpopulations of infected horses were zoned from disease-free subpopulations using natural and artificial geographical barriers. Throughout the outbreak, these boundaries were regularly adjusted based on surveillance, tracing and other epidemiologically-based risk factor information. Four states (Victoria, Tasmania, South Australia, Western Australia) and two territories (the Australian Capital Territory and the Northern Territory) together with large portions of NSW and Qld had escaped infection and formed a disease-free zone (see the green and white areas of inset in Figure 8).

[6] Zoning is a procedure implemented by a country with a view to defining subpopulations of distinct health status within its territory for the purpose of disease control and/or international trade. Zoning applies to an animal subpopulation defined primarily on a geographical basis (using natural, artificial or legal boundaries). Spatial considerations and good management play important roles in the application of zoning (OIE 2008).

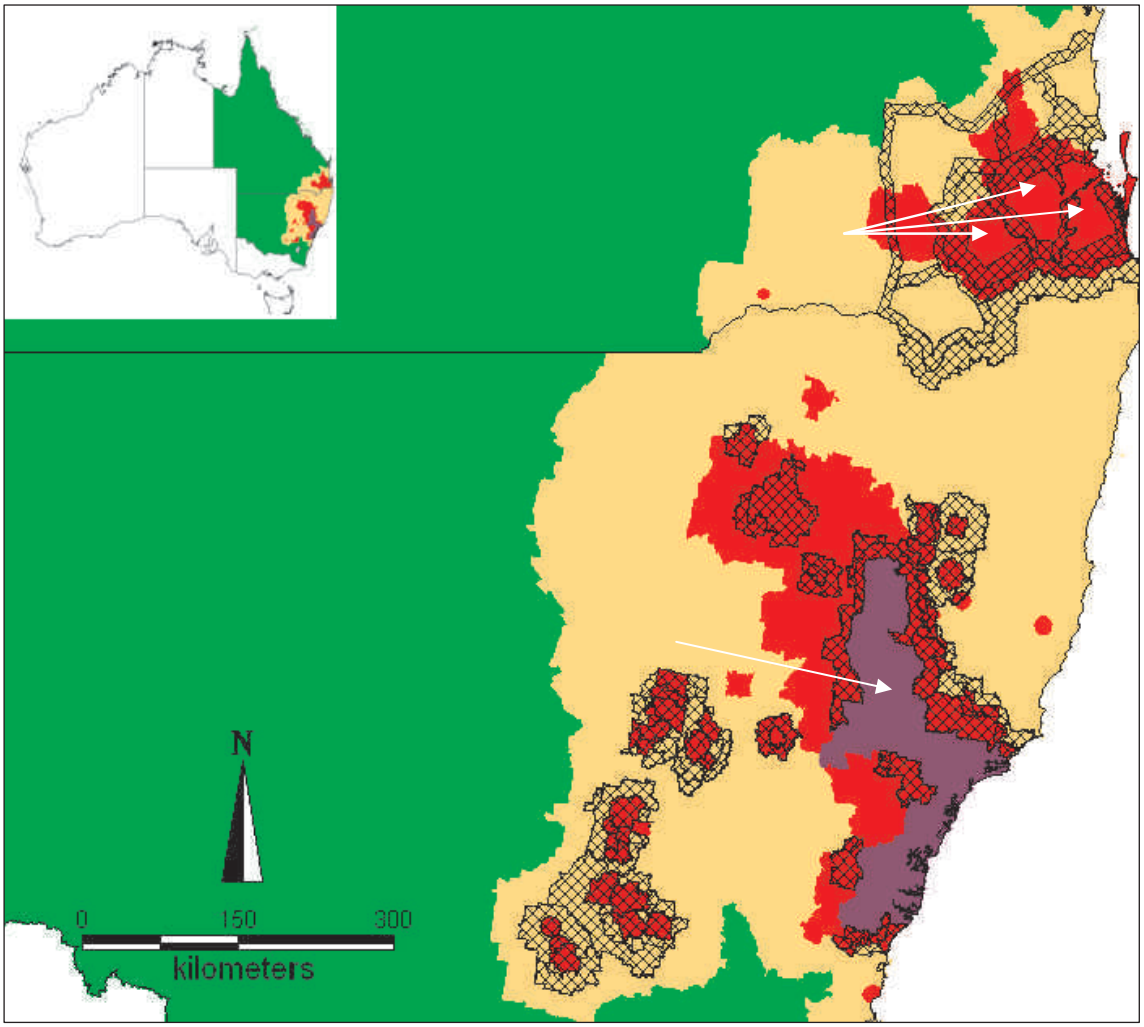
The disease-free zone subsequently only required a temporary movement ban, restriction on movements from infected areas, surveillance and tracing of imported horses from NSW and Qld, and investigation of suspect cases to ensure continuing freedom from infection. An extensive public education campaign and the competency of the state veterinary services meant that passive surveillance was effective in the non-infected zone; with all suspect case investigations ruling out EI (see Table 4). Tracings from infected states were comprehensively followed up and all tested negative (see Table 3).

3.3 Surveillance to demonstrate proof of freedom in previously infected areas

The OIE Terrestrial Animal Health Code (OIE 2008) states that if an outbreak of clinical equine influenza occurs in a previously free country, zone or compartment, disease free status can be regained 12 months after the last clinical case. However, surveillance for evidence of infection should be carried out during that 12-month period at a level sufficient to provide at least a 95% level of confidence of detecting infection if it is present at a prevalence rate exceeding 1%. Following eradication of EI from infected areas of Australia, an extensive surveillance program (which exceeded OIE requirements) concentrated on demonstrating freedom from infection in those areas.

This surveillance took a staged approach with the first stage focusing on demonstrating eradication of EI in isolated disease clusters remote from the major zones of infection. The second stage concentrated on surveillance to demonstrate eradication of disease from the heavily infected areas of NSW (purple zone) and South East Qld (red zone). A third stage involved confirmatory surveillance to demonstrate disease had not infected feral horse populations. Figure 8 outlines the infected area in Australia (red and purple zones) and other management zones.

Figure 8: The peak area of infection during the Australian Equine Influenza epidemic.
Green, amber, red and purple zones are represented by their respective colours while hatched areas represent buffer zones. The red and purple zones were structured to contain all infected premises in Australia. Arrows mark the heavily infected NSW purple and south east Qld red zones.
The surveillance program occurred in two stages. Firstly, clusters of disease remote from the NSW Purple and SE Qld red zone were eradicated and surveillance to demonstrate freedom conducted. These clusters were mostly in the central western portions of NSW and are obvious in the figure as isolated red zones. Secondly, eradication and then surveillance occurred in the heavily infected NSW purple and SE Qld red zones, which together contained more than 90% of infected premises.



Surveillance for proving disease freedom in previously infected, remote clusters focussed on determining the basic population data and immunity levels (both natural and vaccine-induced) within regions and ensuring that all infected, suspect and dangerous contact cases had been resolved. A minimum period of 60 days after the

last identified case was required before an area could be considered for reclassification. In NSW clusters involving a small number of infected premises, sero-surveillance was used on previously infected premises to demonstrate that infection had passed (immunity was present). Investigation of neighbouring properties (PCR testing) was also conducted to ensure that no lateral spread of infection had occurred. In addition, an extensive random survey of horse premises in the area was undertaken to ensure a 95% level of confidence that disease would be detected if its prevalence exceeded 1%. In areas where extensive vaccination occurred, more extensive surveillance results were available due to routine sampling of horses (for PCR and ELISA) at vaccination. In addition, a random survey of horse premises in the area was also performed to ensure a 95% level of confidence that disease would be detected if its prevalence exceeded 1%.

After all remote clusters were demonstrated to be free of disease, surveillance then focussed on the purple zone of NSW and the red zone of south east Qld. Because infection had been widespread in these areas (these zones contained more than 90% of all infected premises), more extensive surveillance was done in order to establish confidence that eradication of disease had been achieved. The program used a range of approaches:

1. *A low probability of EI still being present in the zones was established*

Surveillance started after it became apparent that disease had likely waned in the purple and SE Qld red zones. That is, surveillance started 30 days after the last reported case and after all epidemiologically significant premises were resolved (traces, suspect premises, infected premises). In addition, a succession of negative surveillance results were accumulated from targeted surveillance at events, vaccination, veterinary practices and from suspect case investigations performed before targeted and random surveillance began.

2. *Zone were divided into separate areas*

The red zone in Qld and the purple zones were divided into nine separate areas for surveillance based on geography and risk profile (3 in Qld and 6 in NSW). In the case

of the purple zone, each of the areas chosen for surveillance for freedom were progressively quarantined from the rest of the purple zone as surveillance occurred in that zone. Movement restrictions not previously present (in the purple zone) were introduced to prevent re-introduction of disease into each surveyed zone.

3. Targeted and passive surveillance was conducted

High-risk groups of horses were preferentially sampled as part of targeted surveillance and investigation of suspect cases was continued. See Table 6.

Table 6: The surveillance conducted to demonstrate proof of freedom in the Qld red zone and NSW purple zone.

Surveillance using PRC tests was conducted from 22 December 2007 until Australia's provisional declaration of freedom on 14 March 2008. Results are presented as the number of premises unless marked otherwise.

Surveillance method	NSW	Qld
Random surveillance	1 911	951
Targeted surveillance	> 4 500	3663 (horses)
Suspect case investigation	132	346

4. Random surveillance of the population was undertaken to provide assurance that EI was not present

Stratified random sampling was then undertaken by area across the purple and SE Qld red zones to provide 95% confidence that less than 1% of premises could be infected in each of the areas. More than 300 premises were selected randomly in each of the 9 areas and all horses, up to a maximum of 200 (in NSW) and 30 (in Qld), were sampled (nasal swabs) for PCR and serological testing on each selected property. A total of 16 874 horses were tested from 2862 properties in the two states. No evidence of active EI infection was found. For each area this level of testing provided >95% confidence that less than 1% of properties could be infected assuming just one infected animal was present on the property (Table 7). To complete the surveillance programs in these areas, a total of approximately 34 000 horses have undergone targeted, random or passive surveillance for EI using PCR swabs.

One positive PCR (from 4243 tests) was recorded in Qld in a horse destined for export. Extensive investigation was conducted with further samples taken from the horse in question, as well as all other horses on the premises. PCR results from the re-sampled horses were all negative. The investigation showed that the horse had received a killed EI vaccine (an export requirement) at the same time that nasal swabs were taken. Circumstantial evidence suggests that the positive PCR resulted from contamination with the killed EI vaccine. Studies later conducted by the Qld animal health laboratory with the killed EI vaccine used, demonstrated that the vaccine reacts strongly in the PCR test even at 10^{-16} dilution. The horse was subsequently released for export.

During the random PCR survey of the purple zones in NSW, 24 weak-positive PCR results were reported (from 12 631 tests). These were consistent with residual (non-infectious) viral material present in the nasal passages of previously infected horses. However, to demonstrate that no residual infection was present, the properties of origin for these 24 cases and associated dangerous contact premises were further investigated; 547 additional PCR tests of in contact, high risk horses were undertaken on horses from 62 properties, all with negative results. Targeted surveillance of the neighbours of the last infected premises demonstrated that no neighbours were infected with virus.

Table 7: Random PCR surveillance in previously infected zones

Area	No. of properties sampled	No. of horses tested	Estimated confidence
NSW			
1	316	1646	95.80%
2	314	4209	95.71%
3	315	1487	95.75%
4	321	1827	96.00%
5	324	1712	96.12%
6	321	1750	96.00%
Sub-total	**1911**	**12631**	
Qld			
1	331	1386	96.33%
2	309	1397	95.43%

3	311	1460	95.52%
Sub-total	**951**	**4243**	
Total	**2862**	**16874**	

Feral horse populations near infected areas are small and widely dispersed and risk assessments revealed that these populations are unlikely to sustain EI if they ever became infected. Despite this, risk management procedures were implemented to prevent the introduction of infection to feral horses. This included vaccination of domestic horses in premises close to feral horses, and ensuring physical separation between domestic and wild horses. To detect any EI in feral horse populations in the unlikely event of spread from domestic populations, individuals from four populations of feral horses in NSW and Qld were sampled and tested for EI. No infection was detected. Another population was observed for clinical signs of EI, with none being detected during the epidemic. Targeted surveillance of horse premises near feral horse populations revealed no EI on these premises.

Following the completion of this extensive surveillance, Australia declared provisional freedom from EI on the 14 March 2008. Since then, passive and targeted surveillance has been in place and suspect cases have all ruled out EI. Table 8 lists suspect case investigations that occurred after provisional freedom was declared. There is a high level of confidence that the continuing public education campaign and competent animal health surveillance network in Australia would have detected any cases of EI after 14 March 2008.

Table 8: Suspect case investigations and targeted surveillance in NSW and Qld since provisional freedom from EI was declared 14 March 2008 (till December 2008).

Jurisdiction	Suspect case investigations	Targeted surveillance
NSW	85	Nil
Qld	95	1433

4. Ongoing assurance of freedom (mixing, movements and suspect cases)

After achieving provisional freedom on 14 March 2008, horses were allowed to move freely in Australia, and events, shows and other activities recommenced. Some limited measures continued to apply in previously infected areas including travelling horse statements, event registration and biosecurity measures at events. The remaining measures were lifted on 30 June 2008.

As mixing of recovered and vaccinated horses with naïve horses occurred, the broader horse population effectively served as a sentinel system for EI in Australia. No EI has been detected in any of these naïve populations, despite continued investigation of suspect cases. The absence of any evidence on infection in these horse populations further supports Australia's case for freedom from EI.

The following sections (4.1–4.3) present data on the movements and mixing of horses, and on suspect cases investigations of EI that have occurred following the lifting of restrictions.

4.1 Inter-jurisdictional horse movements

This section presents indicative data to illustrate the scale of domestic horse movements within Australia and demonstrates that extensive movements of horses between the jurisdictions have occurred.

The best data available comes from Western Australia (WA), which is located approximately 2000 km from the formerly infected parts of NSW and Qld. WA regulates the movement of susceptible species (including horses) to WA from the rest of Australia to prevent the introduction of liver fluke. There were 1142 horses moved into WA from other areas of Australia between June and December 2008. Large numbers (567) of these were from the formerly infected states of NSW and Qld. See Table 9.

Table 9: Movements of horses from Australia into Western Australia between June and December 2008.

Jurisdiction of origin	Number of horse imports to WA
Australian Capital territory	3
New South Wales	408
Northern Territory	1
Queensland	159
South Australia	112
Tasmania	2
Victoria	424
Western Australia	33 (returning horses less than 30 days out of state)

The most extensive movements of horses occur between jurisdictions along the more closely settled eastern seaboard of Australia (Queensland, Victoria and NSW). The scale of the movements that occur in this part of Australia are demonstrated by registered movements that occur between Victoria and NSW. Between January and June 2008 Victoria closely regulated the movement of horses from the neighbouring, formerly infected state of NSW[7]. There were 7700 permits to move horses from NSW issued. The consignment size varied, but it was estimated that some 25,000 horses moved from NSW into Victoria (and back to NSW again) during the period.

4.2 Horse events

No restrictions on horse events were imposed after June 2008. Figures are available for the number of thoroughbred and standardbred races that occurred in Australia between June and December 2008 (Sources: Australian Racing Board 2008: Harness Racing Australia 2008) — see Table 10. On the eastern seaboard of Australia horses frequently move between states to race. Additionally, some states recorded the number of events for other horse sectors. It was also possible to utilise industry advertisements to tally events by state. These figures allow an estimate of horse events per state, although these data under-estimate the total number of events held.

[7] Note that before 14 March, movements of horses from NSW to Vic were restricted and tightly controlled.

Table 10: Selected events by jurisdiction held between June and December 2008.

State/Territory	Thoroughbred race meetings	Standardbred race meetings	Other horse events
Australian Capital Territory	9	Reported in NSW	2
New South Wales	318	226	3720
Northern Territory	45	N.A.	9
Queensland	227	136	259
South Australia	82	74	100
Tasmania	41	36	12
Victoria	268	222	736
Western Australia	130	131	28

4.3 Suspect case investigations

Suspect case investigations of exotic diseases in Australia can be categorised to several different levels according to the concern that disease is present. Each level has an increasing level of response, ranging from level one (a simple field investigation) to level 7 (an eradication program) — see Table 11. All investigations of suspect EI since June 2008 have progressed to level 2 (epidemiological and state based laboratory investigation with PCR and ELISA testing) or in some instances level 3 (additional investigation by the AAHL).

Table 11: Categories of exotic disease investigations.

Category of investigation	Description
1	Field investigation by government officer
2	Investigation by state/territory government animal health laboratory
3	Specimens sent to AAHL
4	Specimens sent to overseas reference laboratory
5	Regulatory action taken (quarantine or police)
6	Alert or standby
7	Eradication

The AAHL testing regime includes the following:

1. PCR testing

A Taqman PCR (qPCR) (for matrix Flu A and H3) is used for all samples. If the test is negative, EI is excluded. In the event of a positive, the sample is reported as suspect and virus isolation is used for confirmation. An indeterminate result leads to a conventional gel based PCR (for H3) for clarification.

2. Serology

The cELISA and HI are used to provide serological information important to determine the historical infection status of a horse. This can also help differentiate infected from vaccinated animals.

Since 14 March 2008, there have been 506 suspect cases of EI investigated in Australia. No evidence of EI infection has been found. Tables 4 and 7 list the suspect case investigations that have occurred by jurisdiction in Australia since 14 March 2008.

5. Summary and conclusion

In 2007 Australia experienced a large outbreak of EI associated with the escape of virus from imported horses in a quarantine station. As part of the process of maintaining Australia's EI free status quarantine requirements for imported horses have been strengthened. This includes implementing the Australian Government's response to recommendations from an independent inquiry held to examine the circumstances that contributed to the outbreak of EI.

The EI epidemic was confined to a zone of just 3% of Australian land area. In the uninfected zone, passive surveillance and tracing of horses from affected areas demonstrated that historical freedom from EI was not threatened. In the infected area, a strong disease control program successfully eradicated EI by first concentrating on clusters remote to the main epidemic, and then on the heavily infected NSW purple and south east Qld red zones. Surveillance programs in the infected zone took a flexible and localised approach and used targeted, passive and stratified random sampling. Approximately 34 000 PCR tests on individual horses were negative with only 25 false positives.

A total of 16 874 horses from 2862 properties were tested during the random surveillance programs in NSW and Qld with no evidence of active EI infection found. For each of the nine areas surveyed, the level of testing provided >95% confidence that less than 1% of properties could be infected assuming just one infected animal was present on a property.

Despite extensive mixing of naïve populations of horses with formerly infected horses, a comprehensive passive surveillance program and ad hoc targeted surveillance has not detected EI since December 2007. Australia thus declares freedom from EI according to the provisions in the OIE terrestrial animal health code.

6. Writing Group

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