

EXOTIC

Equine influenza

Fact sheet

Introductory statement

Equine influenza (EI), colloquially known as horse flu, is currently caused by the H3N8 subtype of the influenza A virus. EI virus is the leading viral cause of respiratory disease in horses. Other equines are susceptible, and particularly severe disease has been seen in donkeys. Several instances of horse to dog transmission have also been documented. Until 2007 Australia was free of EI, however a breach of quarantine led to an outbreak in eastern Australia. An aggressive and highly successful control and eradication program ended the outbreak within five months. Australia has now been recertified free of the virus.

If you suspect a case of EI you should immediately call the free **Emergency Animal Disease Watch Hotline (1800 675 888)**.

Aetiology

EI is caused by an influenza A virus (family *Orthomyxoviridae*). Influenza viruses are enveloped, negative-sense RNA viruses with segmented genomes categorized by serological responses to two surface proteins (haemagglutinin and neuraminidase, abbreviated H and N). To date, viruses known to cause EI belong to the H7N7 and H3N8 subtypes of influenza A. The H7N7 subtype caused the first recorded EI outbreaks but has not caused any recent outbreaks and is thought to be either extinct or circulating in low levels in wildlife. The H3N8 virus is the agent currently responsible for EI outbreaks worldwide.

Natural hosts

Birds, and particularly waterfowl, are the suspected natural reservoir for influenza A viruses and are the source for the virus recombinant subtypes that occasionally cause influenza pandemics in humans, swine, fowl, poultry, and horses (Taubenberger and Morens 2010). Asia is the centre for influenza A zoonotic cross-species transmission (including human influenza pandemic subtypes such as H1N1), but once these emerge from wild populations they can circulate among animal or human populations for decades. The annual recurrence of influenza in humans ("seasonal flu") is due to evolution of resistance by viruses, such that each year a new variant is present and circulates in human populations (antigenic drift). Against this background of

cyclical transmission of old subtypes are occasional outbreaks of new recombinant subtypes (antigenic shift) that probably result from co-infections in avian, swine or human hosts. These then infiltrate novel or susceptible populations and cause pandemics; H3N8 is an example of a new subtype that likely emerged from birds to infect horses. The same subtype can invade the same host species more than once; the emergence of a unique H3N8 variant caused an outbreak in Chinese horses in 1989, but it quickly subsided and the new strain disappeared (Guo et al. 1992). H3N8 can be transmitted among any equids such as domestic horses, feral horses (brumbies), and domestic and feral donkeys (Gilchrist and Sergeant 2011). There are no confirmed cases of EI in feral equids in Australia (Gilchrist and Sergeant 2011). Transmission from horses to dogs has been documented (Crawford et al. 2005; Yamanaka et al. 2009), including in Australia (Kirkland et al. 2010). There is no evidence of virus persistence in dogs or of dog to dog transmission, therefore dingoes and domestic or feral dogs should not play a role in persistence of EI virus in Australia (Kirkland et al. 2010).

World distribution

EI is broadly distributed worldwide, of those countries that carry out EI surveillance, only five (New Zealand, Ethiopia, Ecuador, Lithuania, Iceland) have never reported a case of EI.

Occurrences in Australia

Until recently, Australia was free from EI epidemics, however in late August of 2007 EI was first discovered infecting horses at Eastern Creek Animal Quarantine Station (ECAQS) near Sydney (Kirkland et al. 2011). Japan was the likely source of the infected horses as the strain isolated from Australian horses was identical to the strain causing a concurrent EI epidemic in Japan (Watson et al. 2011). It is unclear how the virus was able to escape quarantine, but an official inquiry documented some problems with biosecurity standards at ECAQS (Callinan 2008). Major foci for the subsequent outbreak were horse events held in Maitland, NSW, Narrabri, NSW, and Warwick, QLD (Moloney 2011). In the months of August and September of 2007 there were 169 new outbreaks of EI in NSW and 70 in Qld (OIE). The outbreak lasted five months and EI spread over 280 000 km² and infected more than 70 000 horses (Webster 2011). Aggressive containment and vaccination efforts were able to quickly and successfully curtail the spread of the epidemic and by late December 2007 there were no more clinical cases. By June 2008 Australia was again considered free from EI, and recertified free in December 2008 (Moloney 2011).

Epidemiology

EI virus is a highly contagious virus contracted by inhalation (Paillot et al. 2006) that can spread easily through the air and via horse-to-horse contact and fomites (Moloney 2011). Humans can spread EI infections between horses by direct contact or via fomite transmission. Horses, donkeys, mules and zebras are the only known hosts capable of shedding EI virus and infecting a new host. The incubation period for EI lasts 2-4 days, followed by a viral shedding period of 7-10 days. There is no carrier state and the virus survives less than 18 days in the environment (Gilchrist and Sergeant 2011). During the 2007 outbreak in Australia most transmission occurred within infected premises and between nearby infected premises (Moloney 2011). However, alarming examples of putative virus spread by airborne or fomite means across distances of as far as 13 km were suspected (Moloney 2011). Morbidity can be very high for horses kept in confined areas (as much as 100%), however, mortality is very rare and most frequently reported in young, susceptible foals (Cullinane and Newton 2013).

Clinical signs

A highly infectious fever (up to 42°C), nasal discharge, and a dry cough are the most common and reliable signs of EI infection in horses (Cullinane and Newton 2013). A horse cough sounds remarkably similar to a human cough. Other signs may include depression, anorexia, myalgia, limb oedema, and enlarged lymph nodes on the jaw (Cullinane and Newton 2013). Secondary bacterial infection frequently accompanies EI infection, and may result in prolonged fever, nasal discharge and coughing, as well as pneumonia or pleuritis.

Diagnosis

During the 2007 Australian outbreak, RT-PCR amplification and sequencing of viral RNA from nasal discharge were used to diagnose the presence of virus in infected horses with high-speed (within hours) precision and reliability. In addition, bELISA were used to detect EI antibodies from serum samples (Moloney 2011). As these methods proved very useful tools during the successful control of the 2007 outbreak, it is unlikely that older, or additional, diagnostic tools are necessary. Virus isolation may still be desired (for strain comparisons) and can be conducted using hen's eggs or cell cultures (Cullinane and Newton 2013).

Clinical pathology

Haematology from early stages of infection may reveal mild or moderate non-specific anaemia, leukopenia, monocytosis, and lymphopenia. Moderate leukocytosis may occur within 3-7 days of infection, and fibrinogen counts may become elevated, in the event of secondary bacterial infection. Protein serum amyloid content tracks the progress of infection closely and is correlated with the severity of infection. Blood electrolytes and proteins will appear normal or will change due to symptoms, such as anorexia.

Pathology

Gross: diffuse consolidation of all lung lobes (Buergelt and Del Piero 2014; Patterson-Kane et al. 2008).

Microscopic: Bronchiolar and alveolar necrosis, hyaline membrane formations in alveoli (in acute phase), Type 2 pneumocyte hyperplasia (in chronic phase), hyperplasia and squamous metaplasia of airway epithelium, neutrophils and monocytes in alveolar spaces (Buergelt and Del Piero 2014; Patterson-Kane et al. 2008).

Differential diagnoses

The same symptoms (interstitial pneumonia) caused by EI virus can also be caused by infection with equine viral arteritis virus, equine herpesviruses, *Rhodococcus equi*, *Klebsiella pneumoniae*, *Streptococcus* sp., *Pneumocystis jiroveci*. Additionally, poisoning with the plants crofton weed (*Ageratina adenophora*) and perilla mint, the plant component (pyrrolizidine alkaloids), or the weed killer paraquat, can generate the same symptoms as EI (Buergelt and Del Piero 2014).

Laboratory diagnostic specimens

Swabs of nasal discharge should be placed in virus transport medium to detect presence of viral RNA and for virus isolation. Specimens should be collected from acute cases since virus replication is quickly brought under control by the host immune response. Serum is used for detection of antibodies. Swabs and serum should either be tested immediately or stored at -80°C, however, freezing at higher temperatures for short

periods of time can still provide valuable diagnoses, and antibodies are much more heat stable and can be treated with fewer precautions.

Laboratory procedures

Virus isolation provides information on EI virus subtype, strain, and genetic relationships to other EI viruses. This can be performed by inoculating and incubating nasopharyngeal swabs in embryonated chicken eggs or Madin-Darby Canine Kidney Cells. Isolated virus can then be tested against antisera for haemagglutinin and neuraminidase characterization (H and N). ELISA are available for testing viral nucleoprotein from nasopharyngeal swabs (Myers and Wilson 2006) and for anti-EI antibodies in serum (Read et al. 2011). These assays are based on monoclonal antibody reactivity (visualized and quantified using fluorescent tracers) to either viral envelope proteins or anti-virus antibodies. They are 96 well plates with reagents and instructions, and require < 300 µL of serum. 50-100 µL of nasal swab fluid are needed to amplify and identify viral RNA using RT-PCR. Automated and customized versions of this assay were used as a diagnostic tool during the 2007 outbreak in Australia (Foord et al., 2009; Read et al. 2011). These assays use virus-specific primers and a thermocycler to break apart and reconstitute (and multiply) viral RNA fragments. These fragments can then be sequenced for comparison to gene sequence libraries available on GenBank.

Treatment

Rest is essential for recovery from EI. Horses that are not allowed to rest for several weeks may develop complications even after the infection appears to be over. Antibiotics can be used if secondary bacterial infections are suspected. Antiviral drugs are currently only used experimentally.

Prevention and control

EI vaccines are available and widely used in countries where the disease is endemic. The most commonly commercially available vaccines are based on inactivated virus or virus subunits, which are effective for producing an antibody response to live EI and provide most horses with protection. Horses arriving in Australia are required to be vaccinated and quarantined. Current vaccines require frequent (annual) boosters. However, new adjuvants (chemicals added to vaccines to increase their effectiveness) and new vaccines based upon live attenuated virus are being developed that more effectively mimic the natural immune (humoral and cell-mediated) response to infection. There is concern that H3N8 has evolved sufficiently that cross-reactivity of the vaccine to the two major strains (a North American and Eurasian strain) is not guaranteed. Vaccines may be needed for both major types to ensure protection. The vaccine adopted for controlling the 2007 outbreak (ProteqFlu) is a recombinant live virus vaccine combining fragments of two H3N8 strains with harmless canarypox live viruses.

The potential for EI to spread into feral equids is a serious concern. Vaccination of domestic equids and physical separation between domestic and feral equids are important steps in preventing future EI outbreaks from spreading into the feral equid population – such an outbreak would be extremely difficult to manage.

Surveillance and management

The 2007 EI outbreak in Australia should be considered a model for how to control and manage EI outbreaks. In fact, serious study of the methods used to control this outbreak could prove useful for controlling any dangerous disease. It is astonishing that this highly contagious virus was contained and ultimately eradicated

in a country with large, disconnected rural areas, millions of domestic horses, and the world's largest population of feral horses. Several factors and strategies appeared key to this success: 1) Rapid development of fast, precise diagnostic laboratory procedures (RT-PCR). 2) Movement control measures recommended by government (the "standstill") were communicated effectively by multiple media outlets. 3) Despite causing serious social strain, these were immediately and faithfully adopted by private horse owners within and outside the affected area within 3 days of the first detection of the virus in Australia. 4) Despite the fact that no large stores of vaccine were available in country, an effective vaccine was chosen, approved, and imported quickly enough and in large enough quantities for vaccine and surveillance teams to create buffers around the affected areas. 6) Surveillance and vaccination efforts also prevented spread of EI to feral horses; anecdotal and available survey information about the location of brumby herds were quickly compiled and used to designate high risk areas, and vaccination buffers were created near these locations. 5) There was enough political and economic will to favour an eradication policy, which led to a collaborative atmosphere among scientists, the public, and government, to aggressively control the spread of the disease. Even though this outbreak was rapidly curtailed, effort should be directed at preventing another quarantine breach.

Statistics

Wildlife disease surveillance in Australia is coordinated by the Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au.

The National Wildlife Health Information System contains no records of EI in feral horses in Australia.

Research

Most current EI research is focused on outbreak case studies, interest in cross-species transmission (horses to dogs), vaccine development research, and immunological studies. Interest in all influenza A viruses that infect multiple hosts (birds and mammals) is ongoing due to concern about human pandemics.

Human health implications

H3N8 is not considered a concern for causing diseases in humans. However, H3N8 can infect humans in a controlled laboratory setting, and several putative historical (e.g. pre-1900) equine and human influenza pandemics co-occurred (Morens and Taubenberger 2010). It is speculated that in the past horses may have played a more prominent role in influenza A viral evolution due to their previous prevalence and close proximity to people (Morens and Taubenberger 2010). Influenza A viruses are notoriously mysterious and unpredictable, and historical accounts of human flu pandemics that were preceded or followed by equine pandemics suggest the possibility that EI could contribute genetic material to human influenza virus. This could ultimately trigger a human pandemic, in a similar fashion as the 2009 H1N1 swine flu pandemic. Additionally, avian, swine, and equine flu viruses all appear to occasionally infect individual human hosts, though the virus does not then spread to additional humans.

Conclusions

EI virus is the leading viral cause of respiratory disease in horses and can cause mortality in foals. Australia was free of EI until 2007 when there was an outbreak in eastern Australia. An aggressive and highly successful control and eradication program ended the outbreak within five months and Australia has now been recertified free of the virus. Strict quarantine procedures and vaccination programs will be imperative to prevent future outbreaks of EI in Australia.

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- The latest information on EI is available from the World Organisation for Animal Health (OIE) website: <http://www.oie.int/our-scientific-expertise/specific-information-and-recommendations/equine-influenza/>
- The Disease Strategy for the control and eradication of EI, version 3.1, 2011 is available as part of the AUSVETPLAN series and is located at: <http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/EFLU-13-FINAL11May11.pdf>
- Wildlife considerations as part of response are addressed in the WARS AUSVETPLAN: <http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/WARS3.3-18-FINAL21Jun11.pdf>

Acknowledgements

The original Wildlife Health Australia EI fact sheet was drafted by Jill Swan and updated by R. Woods and then Peter Holz. This latest version represents a substantial re-write by Sean P. Graham and Crystal Kelehear.

Updated: 06 April, 2015.

To provide feedback on this fact sheet

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