West Nile and Kunjin virus in Australia

Fact sheet

Introductory statement

West Nile virus (WNV) is an arthropod transmitted virus (arbovirus) with a global distribution on all continents except for Antarctica. Mosquitoes of the genus *Culex* are the primary vector of WNV and avian hosts are the main reservoir. Since 1999, WNV has become a significant emerging zoonotic disease, most notably in the United States of America (USA), causing severe neurological disease and death in many animal species, especially humans, horses and birds (Brault 2009). The exact mechanisms for the emergence of WNV are unclear but likely involve increased global travel, climatic and ecological factors, and novel viral genotypes.

There are four lineages of WNV, with the most pathogenic strains (e.g. USA strains NY99 and WN02) belonging to lineage 1 (Brault 2009). The Australian Kunjin virus also belongs to lineage 1 but is considered a distinct viral subtype (Brault 2009). As only one endemic genotype is present in Australia, the virus was likely introduced to Australia on a single occasion, most likely during early ship travel (May et al. 2011).

Prior exposure to Kunjin virus and closely related flaviviruses are hypothesised to provide protection against infection by WNV or other related viruses, as seen in animal transmission experiments in American house finches (*Carpodacus mexicanus*) infected with St Louis encephalitis virus (Brault 2009).

The focus of this fact sheet is on aspects related to emergent WNV and Australian wildlife, and the unique issues created by the presence of an endemic subtype, Kunjin virus in Australia.

Aetiology

West Nile virus (WNV), genus *Flavivirus*, family *Flaviviridae*. Other notable viruses in this genus include Japanese encephalitis, Murray Valley encephalitis, St Louis encephalitis and Dengue fever viruses.

**Grouping (non-taxonomic):** Arbovirus (*Arthropod borne virus*, see separate factsheet on Arboviruses for more details).

All of the major outbreaks of human encephalitis have been associated with lineage 1 viruses.
Natural hosts

Primary host: Birds
Other hosts: Humans and other primates; equids such as horses and donkeys; artiodactyls such as cattle, pigs, llama and deer; carnivores such as dogs, cats, seals and cetaceans; bats; rabbits; rodents; elephants; rhinoceroses, crocodiles and alligators.

World distribution

WNV is widely distributed through Africa, Asia, the Middle East and Europe, with only infrequent reports of human outbreaks until 1996. Three outbreaks occurred in Romania (1996), Russia (1999) and Israel (2000) with severe neurological disease. The reasons for this emergence are unclear. WNV was probably introduced to the USA in or before 1999 via infected mosquitoes in aeroplanes arriving in New York from the Middle East as the recently emergent USA strains (NY99 and WN02) are closely related to strains previously found in Israel (May et al. 2011).

There were almost 10,000 human cases in 2003 with a westerly spread from New York State (see http://www.cdc.gov/westnile/statsMaps/cumMapsData.html); around this time equine cases also began appearing in the USA, peaking at 15,257 in 2002, before declining over the next ten years, until 2012 when case numbers rose again. See http://usdasearch.usda.gov/search?utf8=%3F&affiliate=usda&query=equine+west+nile&commit=Search

Kunjin virus is considered endemic to Australia but has also been isolated in Sarawak, Malaysia (Ching et al. 1970).

Occurrences in Australia

Kunjin virus is the only WNV subtype that is present in Australia. It was first isolated from mosquitoes in north Queensland in 1960 and has since been found in every state. Kunjin is endemic to northern Australia but encroaches southward when heavy rains facilitate an increase in water bird (amplifying host) and mosquito (vector) density. Until recently, Kunjin virus was tied to only a few cases of non-fatal encephalitis in humans and horses; however, in 2011 an outbreak of neurological disease occurred in horses in south-eastern Australia with more than 1000 equine cases and 10-15% mortality (Frost et al. 2012). On average, Kunjin virus infected 1.4 humans annually over the period 2010-2014 within Australia.

Historically, disease caused by Kunjin virus in Australia has been relatively mild. However, a large outbreak of Kunjin virus encephalitis occurred in horses in 2011. Studies showed that this disease was caused by a more neuroinvasive strain of Kunjin virus. This increased virulence was the result of several amino acid substitutions. However, the amino acid substitution that allows the virus to cause disease in birds did not occur. Hence, no increases in avian mortalities were observed (Frost et al. 2012; Roche et al. 2013).

Epidemiology

Transmission

Bird-mosquito-bird transmission cycles – mainly Culex spp. mosquitoes. In Australia Cx. annulirostris is the primary vector though several other species are also competent vectors e.g. Cx. quinquefasciatus and Cx. gelidus (Jansen et al. 2008). Crows, magspies (Family Corvidae), house sparrows (Passer domesticus), house finches and other passerines develop the highest concentrations of virus in the blood and have the longest duration of viraemia (Phalen and Dahlhausen 2004). In Australia, wading birds (in particular the Nankeen
night heron, *Nycticorax caledonicus*) are considered key natural reservoirs for Kunjin virus (Prow 2013). Birds remain viraemic (and infectious) for two to seven days after initial WNV exposure. Shedding of WNV via oral and cloacal secretions begins one to three days after infection and may continue for up to 14 days (Nemeth 2012). WNV may persist in the skin after the cessation of viraemia, allowing mosquitoes to become infected for a longer (as yet undetermined) period of time. Mammals are dead-end hosts as they do not develop a sufficient viraemia to allow mosquito infection.

A recent serological study showed 12.7% of 675 European rabbits (*Oryctolagus cuniculus*) sampled across Australia in 2011-2012 were serologically positive to Kunjin virus (% positive by state: NT = 11.7, Qld = 37.3, NSW = 8.8, Vic = 23.4, SA = 3.7 WA = 10%, Tas = 5.3; Prow et al. 2014) and may represent a possible reservoir host. A survey of crocodiles in the NT found 12 of 49 apparently healthy animals to be seropositive for Kunjin virus (Ladds 2009).

Non-vector transmission via ingestion of infected mosquitoes, infected prey animals and contaminated water has also been demonstrated (Phalen and Dahlhausen 2004). Transmission of the virus in humans via blood transfusions, organ transplants, breastfeeding and in-utero is rare but possible. Accidental laboratory infections of humans have also occurred (Petersen et al. 2013).

The 2011 outbreak of Kunjin in south-eastern Australia followed a period of extensive flooding that ultimately resulted in a sixfold increase in mosquito numbers (Knope et al. 2014). WNV has been isolated from over-wintering mosquitoes and can also be transmitted from infected mosquitoes to their desiccation resistant eggs, suggesting this is the most likely way the virus survives over winter (Phalen and Dahlhausen 2004; McLean and Ubico 2007). In tropical and subtropical climates WNV transmission may occur year round, whereas temperate regions experience seasonal transmission e.g. June to October in the USA and Canada (Nemeth 2012).

**Morbidity rate**

**Humans:** 25% of infected humans develop West Nile fever. Of these, 1 in 150-250 develop neuroinvasive disease, increasing to 1 in 50 in people aged over 65 (Petersen et al. 2013).

**Horses:** Experimental studies suggest that about 10% of infected horses develop clinical illness (Travis 2008).

**Mortality rate**

**Humans:** Case fatality rate among patients with neuroinvasive disease is approximately 10%. However, this is age dependent, ranging from 0.8% for patients under 40 to 17% in patients over 70 (Petersen et al. 2013). There have been no recorded human mortalities due to Kunjin virus infection (Gray et al. 2011).

**Horses:** 20-40% of WNV cases result in death or euthanasia (Travis 2008). The 2011 arboviral outbreak resulted in 982 cases and a 9% fatality rate in Australian horses (Roche et al. 2013).

**Birds:** Mortality rate is low, except for the more virulent strain of WNV that was introduced into the USA in 1999. This strain has caused large numbers of bird deaths in the USA, Canada and Mexico, mostly among members of the *Corvidae*. Experimental infections with WNV caused mortalities in ring-billed gulls, blue jays, black-billed magpies, American crows, fish crows, common grackles, house finches, and house sparrows in the USA (Komar et al. 2003). In Australia, wild-caught little ravens (*Corvus mellori*) experimentally infected with Kunjin virus suffered zero mortality (n= 15; Bingham et al. 2010) and there is no information to suggest that Kunjin virus is pathogenic in Australian birds.
**Other species:** While WNV can infect a wide range of species, clinical disease and mortality is generally low. Deaths have been recorded in a dog, a wolf (*Canis lupus*), alpacas (*Lama pacos*), reindeer (*Rangifer tarandus*), a Barbary macaque (*Macaca sylvanus*), a harbor seal (*Phoca vitulina*), a killer whale (*Orcinus orca*), eastern fox squirrels (*Sciurus niger*), grey squirrels (*Sciurus carolinensis*), a rabbit (*Oryctolagus cuniculus*), an eastern chipmunk (*Tamias striatus*) and a crocodile monitor (*Varanus salvadorii*) (Travis 2008; St. Leger et al. 2011). In one report 300 of 9,000 farmed American alligators (*Alligator mississippiensis*) died over a period of two months due to infection with WNV virus (Jacobson et al. 2005); in another, more than 1000 infected alligators died over two years (Miller et al. 2003).

**Incubation period**

**Humans:** Usually 2-14 days but can be as long as 21 days (Petersen et al. 2013)

**Birds:** 7-10 days

**Other species:** Unidentified.

**Clinical signs**

Affected humans may develop three clinical syndromes (in order of increasing rarity):

**West Nile fever** – fever, headache, tiredness and body aches, occasional rash on the trunk and swollen lymph glands. Symptoms may last from a few days to several weeks.

**West Nile encephalitis / meningitis / neuroinvasive disease** – headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness and paralysis. Neurological effects may be permanent.

**Acute flaccid paralysis or West Nile poliomyelitis** – asymmetric limb weakness or paralysis without sensory loss and sometimes with preceding pain. Fever, headache or other WNV associated symptoms may be absent. Respiratory muscle paralysis and respiratory failure can sometimes occur. 80% of paralysis cases occur in conjunction with encephalitis/meningitis.

Infection with Kunjin virus in humans tends to produce a mild disease consisting of lymphadenopathy, fever, lethargy, rash and muscle weakness. Confirmed cases of Kunjin are rare, with 13 cases reported between 2004 and 2011 (two from Vic., six from Qld, two from WA and three from the NT). The NT cases were part of a recent review of ten NT patients with Kunjin virus infection. All patients had a fever, three presented with encephalitis, three with meningitis, three with arthralgia, myalgia or rash and one with fever alone (Gray et al. 2011).

**Clinical signs in horses** include ataxia, incoordination, wide stance in forelimbs, weakness, altered temperament, mild fever (inconsistent), blindness, muscle trembling, seizures, facial paralysis, dullness, recumbency and death (Travis 2008; Tee et al. 2012).

**Clinical signs in birds** include anorexia, weakness, depression, weight loss, neurological signs (ataxia, tremors, disorientation, circling, impaired vision, torticollis and paresis), recumbency and sudden death. Feather abnormalities can occur in owls, eagles and *Falconiformes*. Experimentally infected American crows died after four to eight days (McLean and Ubico 2007; Travis 2008; Nemeth 2012). However, experimentally infected Australian little ravens displayed only mild signs of lethargy and reduced food consumption but recovered fully by ten days post infection (Bingham et al. 2010).
**Clinical signs in alligators** include anorexia, lethargy, tremors, swimming on their side, spinning in the water and opisthotonus (Jacobson et al. 2005).

Of particular relevance to Australian fauna, a captive crocodile monitor (*Varanus salvadori*; endemic to New Guinea) exhibited neurological signs of WNV infection in the USA (Travis 2008).

**Diagnosis**

Diagnosis is made based on history, clinical presentation and confirmatory laboratory testing. Flavivirus infection should be considered in wild bird mortality if involving a large proportion of the flock (together with differential diagnoses listed below). In these circumstances and in suspicious cases in horses and humans in particular, the challenge lies in establishing if detected WNV is an exotic / more pathogenic strain or the enzootic Kunjin subtype.

**Clinical pathology**

Humans with severe disease may have changes in cerebrospinal fluid (CSF) with lymphocytes usually predominating, elevated protein and normal glucose; occasional hyponatraemia, occasional leucocytosis, lymphocytopenia or anaemia, with possible normal CT or MRI presentations (Petersen et al. 2013).

Raptors infected with WNV in North America developed serum antibodies five to seven days post infection. These levels increased four-fold or more within the first month after infection and persisted for at least four years (Nemeth 2012).

**Pathology**

In humans, histologic findings of WNV encephalitis include a predominantly lymphocytic perivascular inflammation, microglial nodules, necrosis and loss of neurones. The deep grey nuclei, brainstem and spinal cord are most affected (Hayes et al. 2005).

Horses have evidence of a multifocal lymphocytic polioencephalomyelitis, multifocal glial nodules, occasional neuronophagia and perivascular haemorrhage (Cantile et al. 2001).

Gross lesions in birds may be absent but reports include splenomegaly, myocardial pallor, cerebral congestion and subdural haematomas in cases overseas. Histologically, lesions were variable and included finding such as haemorrhage in the brain, especially the cerebellum, perivascular lymphocytic cuffing, Purkinje cell necrosis, myocarditis, lymphoplasmacytic enterocolitis, splenic necrosis, hepatic necrosis, pancreatitis, interstitial nephritis and endophthalmitis (Steele et al. 2000; Nemeth 2012).

Affected American alligators have a moderate heterophilic to lymphoplasmacytic meningoencephalomyelitis, necrotizing hepatitis, splenic necrosis, pancreatic necrosis, myocardial degeneration and necrosis, interstitial pneumonia, stomatitis and glossitis (Jacobson et al. 2005).

**Differential diagnoses**

Diseases of humans and other animals which cause serious neurological signs with or without fever should be considered as differentials to WNV infection e.g. rabies and other viral encephalitides including other arboviral infections, bacterial meningitis, brain abscesses, tetanus, botulism, hepatic encephalopathy, other
toxins or poisonings and vitamin E or A deficiency. Additional differentials in horses include equine encephalomyelitis, and in birds, avian encephalomyelitis and avian paramyxoviruses.

Laboratory diagnostic specimens

If the clinical picture and history suggest WNV infection, serum should be collected within 8-14 days of onset of illness or CSF collected within 8 days of onset of illness and sent frozen or refrigerated to a reference laboratory. In Australia, samples from suspect animal cases should be submitted to CSIRO Australian Animal Health Laboratory or the relevant state/territory reference laboratory for definitive laboratory diagnosis. Samples for exclusion of WNV should include full tissue sets for virus isolation and detection (fresh and formalin-fixed). Brain and spinal cord are the preferred tissues for virus isolation from horses. In birds, kidney, heart, brain and intestine should be submitted. Blood and tissue samples from early clinical cases are more likely to yield viable virus. There are differences in sample collection and preservation for birds compared with mammals and those wishing to exclude WNV infection in Australian birds should contact the diagnostic laboratory prior to sample collection for specific guidelines.

Laboratory procedures

Laboratory tests which may be used on serum and CSF are: MAC-ELISA to detect IgM, neutralisation assays, PCR and virus culture. The latter two together with immunohistochemical analysis may also be performed on tissue from autopsies/necropsies.

A greater than four-fold increase in virus specific neutralizing antibody titre detected by the plaque reduction neutralization test between two serum samples collected two to three weeks apart usually confirms acute WNV infection (Hayes et al. 2005; Petersen et al. 2013). One study found that the sensitivity of PCR in 28 patients with serologically confirmed neuroinvasive disease was 57% in CSF and 14% in serum (Hayes et al. 2005).

Treatment

No specific treatment exists. Most animals and humans recover spontaneously from WNV infection and develop lifelong immunity (Brault 2009). Supportive treatment of severe cases would be indicated. In-vitro trials with antivirals have been promising but several clinical trials have yet to be concluded (Petersen et al. 2013). As non-bird species are regarded as incidental hosts (and unable to perpetuate WNV infections), culling of infected animals is not indicated as a control measure.

One study into the treatment of WNV infection using alpha interferon has been listed by the CDC as meeting its scientific criteria (See: http://www.nyhq.org/doc/Page.asp?PageID=DOC000030) and other clinical trials are ongoing.

1 These viral identification tests take longer than the antibody tests but will be required to distinguish between Kunjin virus and other WNV strains to determine significance of positive WNV antibody results.
**Prevention and control**

A vaccine is not available for humans and will probably not be developed as it is unlikely to be cost-effective, unless disease incidence increases substantially (Zohrabian et al. 2006).

There are four commercially available equine vaccines. These have also been tested in birds with variable results. Thirty-one out of 37 eastern loggerhead shrikes (*Lanius ludovicianus migrans*), and 58.3% of a range of raptor and corvid species developed neutralizing antibodies. However, none of the 16 Chilean flamingos (*Phoenicopterus chilensis*) and 10 red-tailed hawks (*Buteo jamaicensis*) that were vaccinated developed detectable antibodies (Nusbaum et al. 2003; Bertelsen et al. 2004; Johnson 2005; Nemeth 2012; Petersen et al. 2013). Kunjin virus was used to vaccinate eight American crows. Fourteen days later all crows had seroconverted. When challenged with a lethal dose of WNV there was no morbidity or mortality in contrast to a group of unvaccinated control crows all of which died within five days of infection (Hall and Khromykh 2004).

Reducing mosquito bites has been the only effective method of reducing transmission. Broad-scale animal movement controls or environmental vector control have not proven effective.

There are numerous pathways via which WNV could be introduced to Australia including migratory birds, wind-blown insects, and passage of infected mosquitoes, animals or humans via aircraft or boat. The most likely pathway has been identified as the introduction of infected mosquitoes to Australia within an aircraft. As such, insecticides are used within aircrafts to manage this risk (Jansen et al. 2013). A recent predictive model showed that the probability of North American WNV being introduced to Australia (and subsequently establishing) via an infected mosquito inside an aircraft is low (Hernández-Jover et al. 2013).

WNV is a nationally notifiable animal disease in Australia and is included on the list of Emergency Animal Diseases for Australia. National response policy to a virulent WNV outbreak is detailed in AUSVETPLAN Response Policy Briefs (Animal Health Australia 2013). If an outbreak of imported WNV is detected in Australia, the policy is to consider eradication by movement controls over the (imported) animals and vector abatement on the premises. However, eradication is considered unlikely to succeed if WNV becomes established in an enzootic cycle in Australia because competent hosts and vectors abound. Thereafter, efforts would be concentrated on vaccination of high-risk groups where possible, surveillance, public awareness campaigns and other vector avoidance measures (Animal Health Australia 2013).

**Surveillance and management**

Australia has surveillance programs utilising sentinel chickens and mosquito traps to detect Kunjin virus and climate monitoring to predict outbreaks. Annual reports on the sentinel chicken monitoring program can be found at [http://www.health.gov.au/internet/main/publishing.nsf/content/cda-arboanrep.htm](http://www.health.gov.au/internet/main/publishing.nsf/content/cda-arboanrep.htm) and wider surveillance efforts in “Animal Health in Australia” (Animal Health Australia 2015).

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](mailto:admin@wildlifehealthaustralia.com.au). Wildlife Health
Australia recommends consideration of WNV in evaluating wild bird mortality events in Australia and is interested in receiving the results of any diagnostic testing for WNV or Kunjin virus in Australian wild birds.


**Statistics**

As of April 2016, 84 ‘Events’ were identified, involving a range of bird species, that had tested negative to WNV in the national database. One brush-tail possum (*Trichosurus vulpecula*) was also tested in 2011 and found to be negative for WNV.

**Human health implications**

Facts sheets containing on WNV and Kunjin virus in humans in the Australian context are available from commonwealth and state departments of human health (e.g. https://www2.health.vic.gov.au/public-health/infectious-diseases/disease-information-advice/west-nile-virus-kunjin-virus). A survey of three indigenous populations in the NT in 1988 and 1989 found a positive seroprevalence to Kunjin virus of 30% for the 834 individuals tested (Gray et al. 2011). Clinical disease is rare with 31 cases reported between 2001 and 2011, mostly from northern Australia (Roche et al. 2013).

See also the CDC portal (see http://www.cdc.gov/ncidod/dvbid/westnile/index.htm).

**Research**

Research questions which apply to Australia and its wildlife include:

- What is the seroprevalence for Kunjin virus in native Australian fauna? The answer to this question will help better understand potential reservoir species.
- What are the risks of introduction of WNV (including the role and relative importance of migratory and other bird species)? How and when will we know if it is introduced? Would flying-foxes and possums act as reservoirs, as they do for other arboviruses? Would crocodiles be as adversely affected as alligators? Would monitor lizards (*Varanus* spp.) suffer morbidity or mortality?
- What are the disease risks for humans and wildlife? Why are some individuals of some species more severely affected?
- Why has WNV emerged in other parts of the world? Why has it not emerged in Australia? What protection, if any, do endemic arboviruses such as Kunjin virus provide? Are we doing enough to prevent a WNV incursion – if this is possible?
- How could we distinguish between Kunjin and other WNV infections more easily / quickly?

Ongoing Australian research includes studies on WNV vaccines and diagnostics at the University of Queensland and studies on host competence at CSIRO.

**Conclusions**

Kunjin virus, a strain of WNV, is endemic in Australia. During 2011 a more virulent strain of the virus caused an outbreak of neurological disease in horses, with significant mortality, in south-east Australia.
Despite ongoing studies in the USA and elsewhere, many questions remain around the emergence of WNV. Until more specific information is available, the risk to Australia and its wildlife (especially threatened species) from exotic strains of WNV remains unknown. Competent hosts and vectors for WNV are widely present in Australia and establishment of exotic strains of the virus is considered to be a strong possibility. Surveillance and preparedness for the emergence of virulent strains of WNV in Australia is recommended.

**References and other information**

Animal Health Australia (2013) Response policy briefs (Version 3.5). Primary Industries Ministerial Council, Canberra, ACT.


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**To provide feedback on this fact sheet**

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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