

Nidoviruses in freshwater turtles Fact Sheet June 2024

Key points

- Bellinger River virus, a *Serpentovirus* in the order Nidovirus, was discovered following a large mortality event in 2015 in the only wild population of the freshwater Bellinger River snapping turtle (*Myuchelys georgesi*).
- A subsequent outbreak of the virus affected several species of captive freshwater turtle in Qld.
- The source of the virus is not known.
- Over 400 wild turtles died during the disease outbreak in 2015, but no further disease related to the virus has been reported in the Bellingen River system since then.
- A captive breeding program has been established to safeguard the Bellinger River snapping turtle, which is now listed as critically endangered.

Aetiology

Bellinger River virus (BRV) falls in the species *Berisnavirus,* subgenus *Snaturtovirus,* genus *Pregotovirus,* subfamily *Serpentovirinae,* family *Tobaniviridae,* suborder Tornidovirineae, in the viral order Nidovirales. These are positive strand RNA viruses. Bellinger River virus appears most closely related to Serptenoviruses which have been found to cause respiratory disease in pythons and lizards^[1] (see also WHA Fact Sheet "*Nidoviral respiratory disease in Australian lizards*").

Other unrelated nidoviruses have been found in turtle species in the Northern Hemisphere.

One Health implications

Wildlife and the environment: the Bellinger River snapping turtle (BRST) was considered rare and inhabited a restricted habitat before 2015^[2]. Surveys showed that a very large proportion of the adult BRST population died during the disease outbreak, resulting in a severe threat of extinction of the species, which is now listed as critically endangered ^[1-3]. The threat to other freshwater turtle species is not known.

Domestic animals and humans: there is no indication that these viruses cause disease in domestic animals or humans.

Occurrence in Australia and world distribution

The first report of BRV was in February 2015, in a wild population of BRST, in the Bellinger River system in northern NSW. The outbreak of the virus caused the death of over 430 wild turtles, over a period of around 7 weeks^[1]. No disease or death was reported in any other species in the Bellinger

River system at that time, including the Murray River turtle (*Emydura macquarii*) and eastern longneck turtle (*Chelodina longicollis*), which both occupy the same rivers. There have been no further clinical cases of BRV in the wild since 2015. Apparently healthy young and adult BRST were collected from the river system, outside the area affected by the virus at the time, and brought into captivity to safeguard the species.

In 2019, BRV was detected in a collection of captive Australian freshwater turtles in Qld. The affected turtles (species other than *M. georgesi*) showed signs of disease and death similar to those seen in the 2015 event. Both BRV and another novel nidovirus were identified in sick and apparently healthy animals. The source of the virus has not been confirmed ^[4].

An unrelated nidovirus has been discovered in a pond slider turtle (*Trachemys scripta*) from a farm in Denmark ^[5]. A nidovirus in the family *Arteriviridae* was identified in Chinese softshell turtles (*Pelodiscus sinensis*), during an outbreak of severe haemorrhagic disease affecting multiple body organs ^[6]. Partial sequence of another nidovirus from the family *Arteriviridae* was detected in the gut, liver and lungs of an apparently healthy Chinese broad-headed pond turtle (*Mauremys megalocephala*) ^[7]. None of these viruses have been reported in Australia.

Epidemiology

The natural route of transmission of nidoviruses in reptiles is not well understood. Several transmission routes are considered possible, including faecal-oral, via fomite and via aerosols ^[8]. Pathogenic nidovirus in pythons has been experimentally transmitted via oral and upper respiratory tract exposure, and virus was subsequently detected in oral secretions and faeces of infected animals ^[9].

In the 2015 BRV outbreak, adults appeared to be predominantly affected, with few, if any, juveniles affected. There were very few adults found alive in surveys following the end of the disease outbreak ^[2]. Animals <u>without</u> signs of disease that were tested during the outbreak showed no, or very low levels, of the virus ^[1].

The nature of the 2015 disease outbreak in the Bellingen River indicates that the virus was introduced into a naïve population, but the source of the virus, and how it came to enter the population, is not known ^[1]. Other factors may have contributed to the 2015 disease outbreak. In the leadup to the outbreak, environmental conditions were hot and dry and river levels were extremely low, with a river flood event occurring in the week immediately prior to the start of the outbreak. In addition, there had been a general warming of the environment over the past 50 years due to climate change ^[2].

It is not known why adult turtles seemed to be preferentially affected compared to juveniles, however the diet of the two age classes is different in the wild, and it has been suggested that adult turtles may have been in poor body condition due to lack of food resources, before the disease outbreak began. Turtles that were collected for the insurance population, upstream of the disease outbreak, appeared to have low body condition scores when they were first captured ^[2]. Other studies have refuted this claim ^[10] It is not considered likely that the BRST population were

immunocompromised as a result of low genetic variability, as this would not explain the lack of effect of the virus on juvenile turtles ^[2].

An intensive field survey was undertaken 6 months after the end of the initial outbreak and this found high levels of BRV (by qRT-PCR) in 29% of apparently healthy wild BRST and in two Murray River turtles from the Bellingen River system ^[1], indicating that it is possible for animals to carry the virus without expressing clinical signs. Ongoing surveys of the Bellinger River system, free-living BRST and sympatric turtle species after the disease outbreak to November 2020 found low levels of virus in turtles but no signs of disease. Prevalence of virus-positive turtles declined from 2015 to 2020 ^[11].

Clinical signs

During the 2015 outbreak, Bellinger River snapping turtles were mostly found dead or moribund in poor body condition, apparently blind ^[12] with bilateral eye inflammation and conjunctivitis . Some animals had tan foci on the skin of the ventral thighs or hind limb weakness ^[1]. Many animals also had a slight clear nasal discharge ^[2]. In contrast to other reptiles infected with nidoviruses, respiratory disease was not the dominant syndrome ^[8].

Animals affected in the 2019 captive outbreak showed similar signs to those described for wild turtles ^[4].

Pathology

Post-mortem findings included animals in poor body condition. Histopathology revealed periorbital, splenic and nephric inflammation and necrosis, with the most significant pathological changes in the kidneys. There was fibrinonecrotising splenitis and nephritis with multisystemic fibrinoid vasculopathy. Virus was detected in glandular epithelial cells, the lacrimal gland, in degenerate or necrotic renal tubule epithelial cells and in areas of vasculitis. Virus was also detected in lesions in the urinary bladder, scattered granulocytes in the oedematous urothelium and occasionally in the myocardial ^[1]. There was also pathology associated with secondary bacterial infections ^[2].

Diagnosis

RT-PCR, electron microscopy, virus isolation in cell culture, immunohistochemistry, and *in situ* hybridisation may be used to aid diagnosis. Swabs from live animals for PCR can be collected from oral cavity, cloaca and eyes, with conjunctival swabs being the most sensitive ^[11]. Virus can be detected in both serum and plasma ^[1]. There is currently no serological test available for the detection of nidovirus-specific antibodies in reptiles ^[8]. A wide range of tissues should be collected at post-mortem examination for both PCR and histopathology ^[11].

Treatment, prevention and control

Treatment of affected captive animals is symptomatic, including supportive care. During the 2015 outbreak, moribund turtles were euthanased because of poor survival rates, and to lower biosecurity risks ^[2].

Supportive treatment of wild turtles is generally not possible unless they are taken into care. Control of the disease in the wild is difficult and the unknown source of the virus makes prevention challenging. Mitigation of any impacts that may be contributing to the susceptibility of the wild turtles to viral infection should be attempted. General principles of biosecurity, including quarantine and testing of newly arrived or translocated animals, should be applied to all situations where animals are moved by human means. See

<u>https://wildlifehealthaustralia.com.au/Portals/0/ResourceCentre/BiosecurityMgmt/National Wildli</u> <u>fe_Biosecurity_Guidelines.pdf</u> for more information.

A captive breeding program has been under way since 2015, to help ensure the future of the BRST. A small number of turtles have been reintroduced to the Bellingen River system from captivity ^[10].

Research

There is a need for transmission trials to further explore the epidemiology of this disease, however this is hampered by the critically endangered status of the host ^[10]. Further information is needed on the source of the virus, how it came to enter both the wild and captive populations, and factors that drive development of disease in the host. A serological test for antibodies to this virus would greatly assist epidemiological studies ^[8].

Surveillance and management

Wildlife Health Australia administers Australia's general wildlife health surveillance system, in partnership with government and non-government agencies. Wildlife health data is collected into a national database, the electronic Wildlife Health Information System (eWHIS). Information is reported by a variety of sources including government agencies, zoo based wildlife hospitals, sentinel veterinary clinics, universities, wildlife rehabilitators, and a range of other organisations and individuals. Targeted surveillance data is also collected by WHA. See the WHA website for more information <u>https://wildlifehealthaustralia.com.au/Our-Work/Surveillance</u> and <u>https://wildlifehealthaustralia.com.au/Our-Work/Surveillance/eWHIS-Wildlife-Health-Information-System</u>.

We encourage those with laboratory confirmed cases of this condition in native Australian to submit this information to the national system for consideration for inclusion in the national database. Please contact us at <u>admin@wildlifehealthaustralia.com.au</u>.

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Wildlife Health Australia recognises the Traditional Custodians of Country throughout Australia. We respectfully acknowledge Aboriginal and Torres Strait Islander peoples' continuing connection to land, sea, wildlife and community. We pay our respects to them and their cultures, and to their Elders past and present.

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