Wildlife Health Australia

Diagnosis of avian botulism in Australia

Fact sheet

Introductory statement

Botulism is a paralytic disease that is caused by ingestion of a neurotoxin produced by the bacterium *Clostridium botulinum*. Avian botulism can occur in any bird species, but is most frequently seen in ducks, geese, swans, ibis, egrets and pelicans, and is often fatal. This Fact Sheet focuses on diagnostic tests available for avian botulism in Australia; see the WHA Fact Sheet "Botulism in Australian wild birds" for more general information on the disease. Further information can be sourced from Chapter 38 (Avian Botulism) of the US Department of Interior and US Geological Service - Field Manual of Wildlife Diseases: *General Field Procedures and Diseases of Birds (1999-001)* at www.usgs.gov/centers/nwhc/science/field-manual-wildlife-diseases and Chapter 26 "Botulism" in "Clostridial Diseases of Animals" (Le Maréchal et al. 2016b) .

Diagnosis

Diagnosing botulism can be challenging as there are no gross or microscopic lesions associated with this disease. A presumptive diagnosis is usually based on a combination of clinical signs, species affected, a lack of significant lesions upon necropsy (Rocke and Bollinger 2007), the prevailing environmental conditions (e.g. condition of water body where dead or sick animals are located), and knowledge of previous outbreaks in the area. The finding of maggots in the digestive tract may be suggestive of botulism intoxication (Le Maréchal et al. 2016b). Detection of toxin in gut contents or in maggots collected from dead birds further supports a diagnosis of botulism (Rocke and Bollinger 2007). *Clostridium botulinum* can be carried in the gut of healthy birds without clinical signs, but there is limited evidence that types C, D and C/D mosaic are part of the normal avian gut flora (Le Maréchal et al. 2016b; Palmer 2018). The presence of *C. botulinum* in the gut alone is not diagnostic for the disease and a definitive diagnosis requires the demonstration of toxin in serum or tissue and exclusion of other differential diagnoses.

Detection of toxin

Seven distinct types of botulinum toxin, designated by the letters A to G, have been identified. Waterfowl mortalities due to botulism are usually caused by type C toxin (or type C/D mosaic), although type E has

caused sporadic mortalities among fish-eating birds (such as gulls) mainly around the Great Lakes in North America (Rocke and Bollinger 2007; Le Maréchal et al. 2016b).

The detection of toxin can be difficult and varies with the course of the disease and quality of samples. Where possible, multiple blood samples should be collected from acutely affected birds. These should be interpreted in the context of results from gut samples collected from freshly dead birds. There appears to be a greater chance of detecting circulating toxin in blood from peracute cases (Stämpfli 2014). Blood sampling from a number of suspected botulism cases is recommended, as individually affected animals may have low levels of toxin circulating in their blood if the toxin is bound at the nerve cell membranes, which can lead to a false negative result (Rocke and Bollinger 2007). In autolyzed samples, the toxin may be too degraded to be detectable by traditional methods, however it should still be detectable by PCR and/ or cELISA (Palmer 2018).

Clostridium botulinum in the gut of healthy birds may spread and proliferate in other tissues after death. Toxin formation in carcasses post-mortem can lead to false positives results (de Witte 2006).

Tests available

Not all states and territories in Australia perform testing for botulinum toxin (BoNT). Tests include a capture enzyme-linked immunoassay (cELISA) (to identify type C and D toxins) and PCR (Le Maréchal et al. 2016b) . Mouse inoculation is no longer used in Australian government laboratories for botulism diagnosis.

The **cELISA** binds clostridial toxins using a plate coated with anti-clostridial toxin antibody and then detects the toxin with a second anti-clostridial toxin labelled antibody. Accuracy of the cELISA may be affected by the factors mentioned above. The detection level of the cELISA is reported to be very close to the Mouse Lethal Dose (MLD), however the ability of the cELISA to detect all botulism cases during an outbreak can vary significantly, and it is not known what factors most affect accuracy in a "real-life" setting. Accuracy of detection in serum samples in particular may be problematic as the presence of toxin in serum seems to be short-lived and not all serum samples from animals showing severe signs of botulism are positive (the same is reported to be true in human cases of botulism) (Palmer 2018).

The cELISA test is available at the Biosecurity Sciences Laboratory (BSL) in Brisbane, Qld[1] and at the Department of Primary Industries and Regional Development (DPIRD) Western Australia Diagnostic Laboratory Services (DDLS). The cELISA detects type C (and type D toxin) but not type E toxin.

The **PCR** is specific for type C (including type C/D mosaic) or D type toxin and detects RNA/DNA as surrogate toxin-associated markers (Le Maréchal et al. 2016a). The PCR test is available at the Department of Primary Industries and Regional Development (DPIRD) Western Australia, Diagnostic Laboratory Services (DDLS). Liver, blood and gut content are appropriate samples; liver samples may provide the most accurate results (Le Maréchal et al. 2016a).

If testing is required, your local diagnostic lab should be consulted prior to sample collection for advice on the most appropriate collection, storage and transport techniques (see for example www.business.qld.gov.au/industry/agriculture/land-management/health-pests-weeds-diseases/sample-testing/acceptance).

[1] Biosecurity Sciences Laboratory, Health and Food Science Precinct, 39 Kessels Rd,
Coopers Plains Qld 4108. Phone 07 3708 8762 email bslclo@daf.qld.gov.au (submission enquiries).

What to report

Botulism is listed by the OIE (World Animal Health Organisation) as a reportable disease in wildlife (www.oie.int). As some states/territories are unable to routinely utilise out-of-state testing, and in the absence of a definitive diagnosis, **suspected botulism can be reported based on clinical signs, a lack of significant lesions upon necropsy, exclusion of other differential diagnoses and any relevant environmental conditions**. Suspected cases of botulism can be reported to your local Department of Primary Industries or WHA Wildlife Coordinator (www.wildlifehealthaustralia.com.au).

References

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Acknowledgements

We are extremely grateful to the many people who had input into this fact sheet. Without their ongoing support production of these fact sheets would not be possible.

Updated: January 2019

To provide feedback on this fact sheet

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.

Disclaimer

reason of any person relying on information in this fact sheet. Persons should accordingly make and rely on their own assessments and enquiries to verify the accuracy of the information provided.

Wildlife Health Australia

Find out more at www.wildlifehealthaustralia.com.au
email admin@wildlifehealthaustralia.com.au
or call +61 2 9960 6333