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Purpose of study: Botulinum neurotoxin serotype E (BoNT/E) outbreaks in the Great Lakes region cause large annual avian mortality events with an estimated 17,000 bird deaths reported in 2007 alone. It is proposed that environmental conditions following blooms of the native algae *Cladophora* may promote growth of *C. botulinum* within Great Lakes sediments and subsequent BoNT/E production. BoNT/E may then be mobilized from the lake bed through food chains consisting of exotic species. We set out to develop a sensitive *in vitro* assay for diagnosis of avian BoNT intoxication and analysis of drivers that contribute to these outbreaks.

Methods used: The BoTest™ Matrix E BoNT/E detection assay combines immuno-precipitation with high-affinity endopeptidase activity detection by Förster Resonance Energy Transfer to rapidly quantify BoNT/E activity in avian blood and other samples with detection limits comparable to the mouse bioassay.

Summary of results: Based upon the analysis of archived blood samples (n=87) collected from bird carcasses during avian mortality investigations, BoTest™ Matrix E detected picomolar quantities of BoNT/E following a two–hour incubation and femtomolar quantities of BoNT/E following extended incubation (24 hours), with 100% diagnostic specificity and 91% diagnostic sensitivity. Further, BoNT activity, as determined by the BoTest™ Matrix E assay, correlated linearly with total BoNT/E protein concentration contained within avian blood samples, as measured by sandwich ELISA (slope = 1.2). The BoTest™ Matrix E assay also detected BoNT/E in spiked mussels, fish and seaweed, suggesting its utility for analysis of food web components.

Conclusions: Sensitive *in vitro* assays for the diagnosis of botulinum intoxication in birds and for the identification of BoNT/E in food web components will facilitate understanding of environmental toxin mobilization pathways and will provide useful tools for the conservation of bird species in the Great Lakes region.

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