Comparative Toxicity of Type E Botulinum Toxin in Several Lake Erie Fish Species

R.D. Moccia, A.Yule, I.K. Barker and J. Austin
University of Guelph and Health Canada
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THE PARADOX:

- How to explain the 'Fish' pathway of toxin ingestion in species like loons and mergansers which feed nearly exclusively on 'live' fish.
- Lack of apparent correlation between fish botulism epizootics and avian mortality patterns.

THE QUESTIONS:

- Are 'living' fish a reasonable transport mechanism by which BoNT (*Clostridium botulinum neurotoxin*) moves from point of origin to the bird?
- Is it plausible that live, but moribund, fish are selective prey species?
- Can 'live' fish harbour bird-lethal doses of BoNT?
- Do fish display a varying 'tolerance' to BoNT related to phylogeny, natural life history or primary feeding habitat? eg. benthic versus pelagic species
- Is there a potential human health hazard to people who consume BoNT laden fish?

Setting Out to Answer One Very Small Piece of the Botulism Ecology Puzzle

RESEARCH OBJECTIVES:

1. Develop fish botulism exposure model ("FBEM")
2. Test comparative sensitivity and temporal aspects of clinical intoxication for several candidate fish species
3. Develop Dose-Response Model
4. Determine Toxin titres in BoNT Mortalities

"FBEM"  Trout, goby, mudpuppy, perch …

GENERAL APPROACH:

- Produce and encapsulate BoNT-E at known Dosages (eg. 400 MLD)
- Intubate test fish
- Map the temporal aspects and clinical progression of intoxication
- Assay mortalities for BoNT titres
- Calculate expected toxin transport to bird

TOXIN PRODUCTION METHODS:

Method used to prepare the BoNT

- *C. botulinum* type E strain
- grown in tryptone-peptone-glucose yeast extract broth at 25˚C
- atmosphere of 10% H2, 80% N2, 10% CO2 for 4 days
- harvested by centrifuge
- concentration of type E neurotoxin assayed by mouse bioassay
- toxicity neutralized by monospecific type E antiserum: therefore, toxic effect was only due to type E neurotoxin (no other toxins in preparation)

CAPSULE PREPARATION:

- starch filler (60mg) was added to each gelatin capsule
- 100 ul of culture supernatant (containing 400MLD) was quickly added to gelatin capsule
- filled capsules immediately frozen

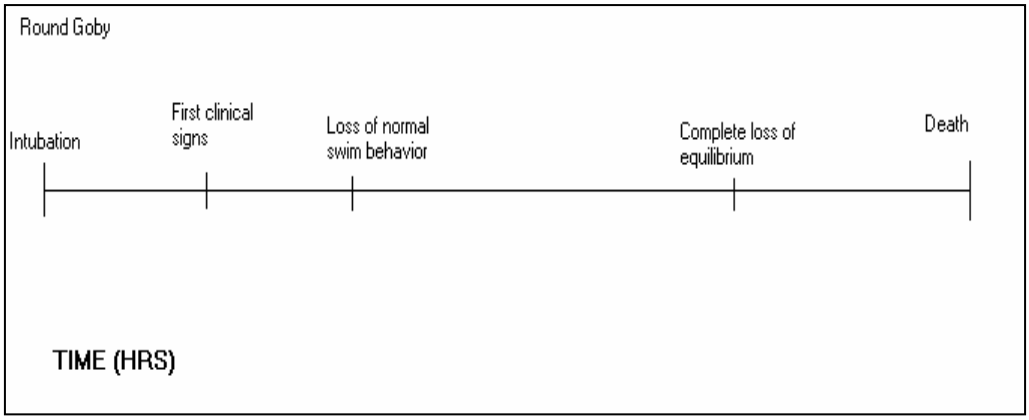
CONTROLS:

- possible effects of starch filler on toxicity was checked
- gelatin phosphate and buffer and starch filler mix had no effect

PRELIMINARY MORBIDITY SIGNS IN RAINBOW TROUT (400MLD)

TIME	COMMENT
0	- fish intubated
x	- time of capsule degeneration and toxin release
xx	- normal behavior
y	- restless, agitated, increased swimming behavior
yy	- first equilibrium loss (loss gradually increases), postural changes, righting ability gradually lost, progressive immobility
yyy or	- sporadic swimming (fish upside down and on side), no fin coordination righting ability, irregular 'breaching' behavior noted
z	- complete loss of any motor function
zz	- loss of respiratory reflex/death

Once Temporal Response Pattern Has Been Establish for the Various Species, Their Sensitivities and Onset of Clinical Signs Can Be Compared.



PRELIMINARY RESULTS-IMPLICATIONS TO BIRD PREDATORS:

1. LOSS OF EQUILIBRIUM: In a natural setting, fish showing equilibrium loss could represent "easy" prey for live-fish eating birds. Thus, such birds could be targeting intoxicated fish due to their abnormal behavior.

2. BREACHING BEHAVIOR: In a natural setting, fish showing breaching behavior would present an “easy” target for predators and maximize BoNT ingestion.

Alternate Species

Once the “FBEM” is established in the rainbow trout the following species will be tested:

- Round Goby
- Mudpuppy
- Yellow Perch
- Walleye
- others

EARLY THOUGHTS:

- Prolonged moribund state with high BoNT exposure
- Progressive clinical stages with respiratory reflex lost late in sequence
- Behavioural response may ‘target’ predators
- Live fish may be significant vector for toxin
- No idea yet of inter-species sensitivity