

# **Case Studies: Public Health Laboratories Respond to Threats A Case of Four Wheeling Botulism in Minnesota**

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Infectious Disease Laboratory Section**



**Analysis. Answers. Action.**

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# Organism

- *Clostridium botulinum*
  - Gram positive
  - Obligate anaerobic bacillus
  - Spores
    - Ubiquitous
    - Resistant to heat, light, drying and radiation





# Neurotoxins

- Seven different types: A through G
  - All cause flaccid paralysis
  - Only a few nanograms can cause illness
  - Different types affect different species

<b>Neurotoxin</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>
<b>Human</b>	X	X			X	X	
<b>Horses</b>		X	X				
<b>Cattle</b>		X	X	X			
<b>Sheep</b>			X				
<b>Dogs</b>			X	X			
<b>Avian</b>			X		X		
<b>Mink &amp; Ferret</b>	X		X		X		



# Human Disease

- Three forms
  - Infants <1 (most common form in US)
  - Foodborne (most common from home-canned foods)
  - Wound (spore enters wound in skin)
- All forms fatal and a medical emergency
- Incubation period: 12-36 hours



# Adult Clinical Signs

- Nausea, vomiting, diarrhea
- Double vision
- Difficulty speaking or swallowing
- Descending weakness or paralysis
  - Shoulders to arms to thighs to calves
- Symmetrical flaccid paralysis
- Respiratory muscle paralysis



# Infant Clinical Signs

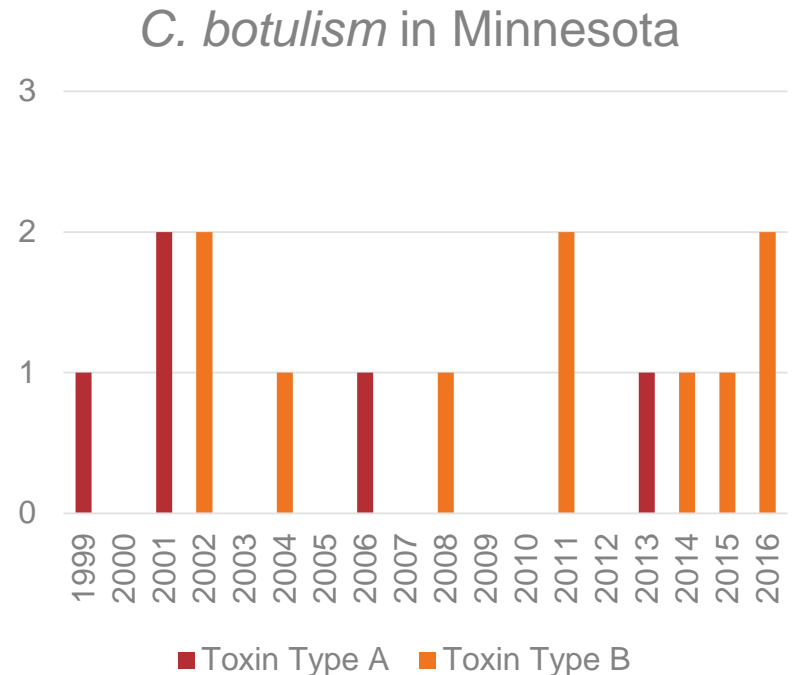
- Constipation
- Lethargy
- Poor feeding
- Weak cry
- Bulbar palsies
- Failure to thrive



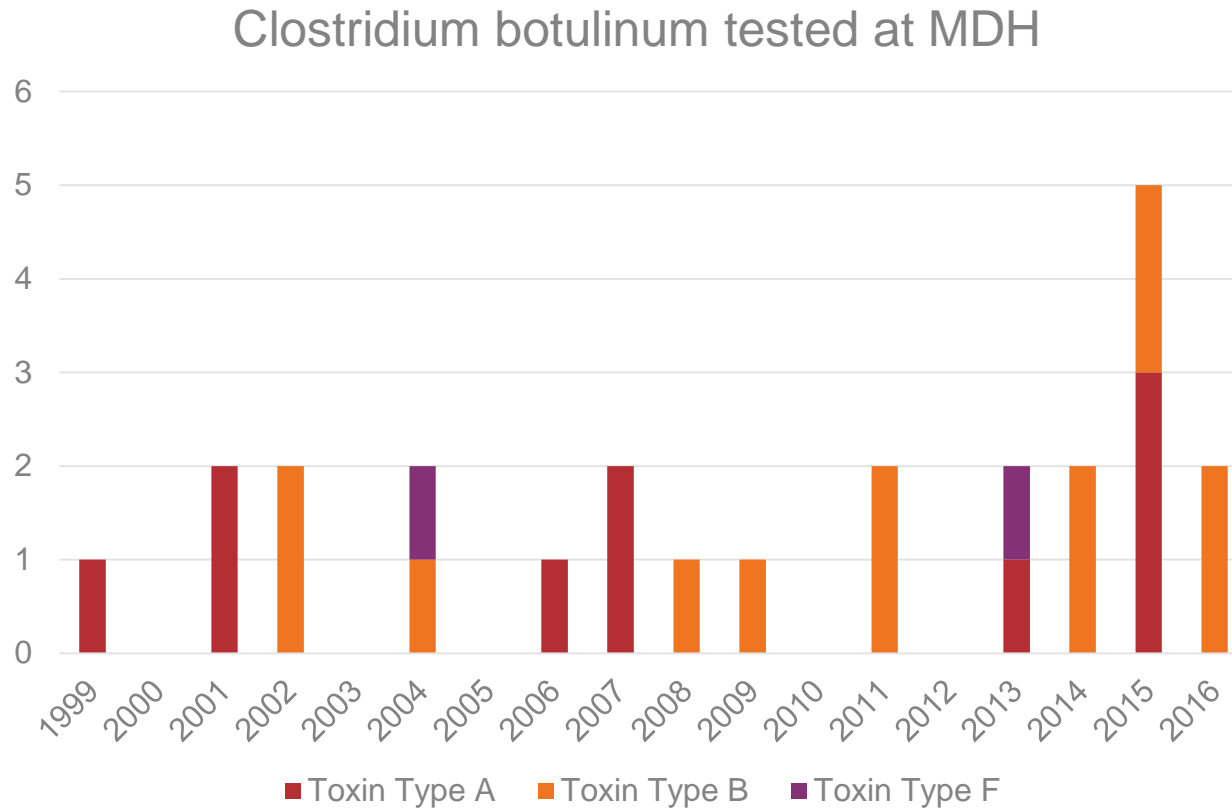
# Botulism in Minnesota

1999-2016

- 15 cases infant botulism
  - 10 toxin type B
  - 3 toxin type A
- 2 cases foodborne botulism (type A)
- 1 cases of wound botulism (type A)



# Clostridium botulinum testing at MDH



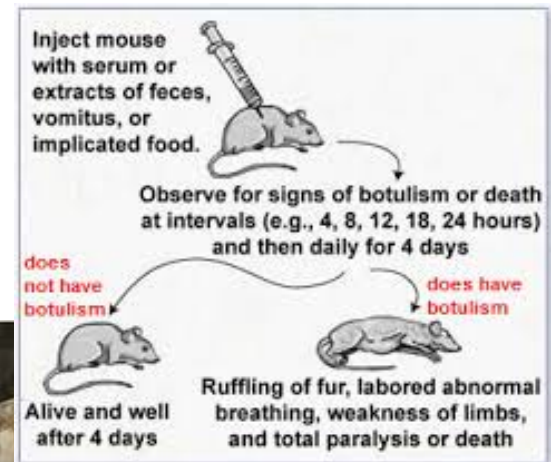
NOTE: Since 2013, all toxin A has been A silent B by PCR





# LRN testing of botulism in Minnesota

- PCR
  - Toxin Type A, B, C, D, E, F and G
- ELISA (food and environmental only)
  - Toxin Type A, B, E and F
- Mouse Bioassay
  - Toxin Type A, B, and F
- Future: MALDI



# LRN Testing

- Complicated
  - Not all labs can do MBA
  - Limited sample types
  - ELISA assay only good for two weeks
  - PCR testing
- Algorithm
- Fussy!

# Case A



- 12 YO Male
- August 9 ATV accident
  - Surgically repaired shoulder and scalp
  - Discharged August 11
- August 16
  - Difficulty swallowing
  - Weak and altered speech
  - Taken to ER –normal chest and head CT

# Case A

- August 17
  - Transferred to Children’s in Minneapolis
  - “looked septic”
  - Pus in wound on shoulder
  - Intubated
  - Reactive left pupil, but right pupil was difficult to assess because eye was swollen shut
  - Suspect ptosis in that eye

# Case A

- Physician thinks of botulism, but defiantly not the classic presentation
- Patient did NOT have paralysis but did have progressive weakness
- Shoulder grew *Enterobacter*
- 8/21 CDC consult on case and Anti-toxin released

# Summary of MDH testing of Case A

- Received 6 samples/isolates
  - Serum (low volume) only enough for MBA
    - MBA inconclusive (one B mouse didn't die)
  - Second Serum
    - MBA positive for Toxin Type A
  - Isolated colony (called submitter to have them send isolate to MDH)
    - PCR positive for Toxin Type A silent B
    - Confirmed as Toxin Type A by ELISA

# Case B

- 5 month old boy
- 1/8/16 onset of mild, nondescript symptoms
- 1/12/16 seen at pediatrician for:
  - Lethargy
  - Muted cry
  - Constipation
  - suspect URI- given supportive care and prune juice

# Case B

- 1/15/16 seen by pediatrician for Well Child visit
  - Hoarse cry
  - Mild congestion
  - CBC normal
- Pediatrician visit
  - Continued lethargy and constipation
  - Infant “seems floppier
  - Congestion and hoarse voice resolved
  - Referred to Neuro
    - Neuro suspects botulism
    - Sends back to primary for stool culture



# Summary of MDH testing of Case B

- MDH receives stool 2/13
  - Toxin Type B detected by MBA, PCR and ELISA
- California does NOT release BabyBIG
- 17<sup>th</sup> case of outpatient infant botulism documented

# Select Agent Reporting

- Form 4 for Identification
  - Toxin
  - Isolate
- Timing of reporting
- Amended reports



# Select Agent Documentation



## REPORTING THE IDENTIFICATION OF A SELECT AGENT OR TOXIN FROM A CLINICAL/DIAGNOSTIC SPECIMEN (APHIS/CDC FORM 4A)

FORM APPROVED  
OMB NO. 0579-0213  
OMB NO. 0520-0076  
EXP DATE 10/31/2010

### INSTRUCTIONS

Detailed instructions are available at <http://www.selectagents.gov/form4.html>. Answer all items completely and type or print in black ink. This report must be signed and submitted to either APHIS or CDC:

Animal and Plant Health Inspection Service  
Agriculture Select Agent Services  
4700 River Road Unit 2, Mailstop 22, Cubicle 1A07  
Riverdale, MD 20737  
FAX: (301) 734-3652  
E-mail: [AnSAS@aphis.usda.gov](mailto:AnSAS@aphis.usda.gov)

Centers for Disease Control and Prevention  
Division of Select Agents and Toxins  
1600 Clifton Road NE, Mailstop A-45  
Atlanta, GA 30329  
FAX: (404) 471-8469  
E-mail: [CDCForm4@cdc.gov](mailto:CDCForm4@cdc.gov)

Accession Number:

(For Program Use ONLY)

Submit completed form only once by either e-mail, fax, or mail

SECTION A – REFERENCE LABORATORY INFORMATION					
1. Name of individual completing Sections A and B: First: _____ MI: _____ Last: _____		2. E-mail address: _____		3. Telephone #: _____	
4. <input type="checkbox"/> Registered Entity (APHIS or CDC Registration #: _____) <input type="checkbox"/> Clinical or Diagnostic Laboratory (non-registered entity (NRE)) (NRE # (provided by APHIS or CDC): _____)			9. Entity name: _____		
5. Responsible Official or Laboratory Supervisor name (if same as field 1 then skip to field 9): First: _____ MI: _____ Last: _____			10. Address (NOT a post office address): _____ _____		
6. E-mail address: _____		7. Telephone #: _____	8. Fax #: _____	11. City: _____	12. State: _____
13. Zip Code: _____					
SECTION B – SELECT AGENT OR TOXIN IDENTIFIED FROM CLINICAL/DIAGNOSTIC SPECIMEN(S)					
1. Select Agent or Toxin Identified: _____			2. Date identified: _____		
3. Case/patient/sample ID #(s): _____		4. # of samples received: _____	5. Sample type received: _____	6. Case/patient origin (zip code): _____	
7. Type of test performed (e.g., PCR, mouse bioassay, ELISA): _____					
8. Dispositions of select agent or toxin by entity listed in Block A9 (complete all that apply): <input type="checkbox"/> Transferred (Provide entity name and date of transfer. Entity: _____ Date: _____) <input type="checkbox"/> Destroyed (Provide destruction method and date. Method: _____ Date: _____) <input type="checkbox"/> Retained (Provide name of Principal Investigator retaining sample. Name: _____)					
9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin? <input type="checkbox"/> No <input type="checkbox"/> Yes (If Yes, you are required under 7 CFR Part 331.19, 9 CFR Part 121.19, and 42 CFR Part 73.19 to complete and submit an APHIS/CDC Form 3)					
10. Do you anticipate receiving additional samples/specimens for this case/patient that originate from the initial case (e.g., patient, environmental sample)? <input type="checkbox"/> No <input type="checkbox"/> Yes (If Yes, please refer to the guidance instructions at <a href="http://www.selectagents.gov">www.selectagents.gov</a> for further directions.)					
11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> N/A NOTE: Please request completed and signed Sections C & D from each facility that was in possession of the specimen(s).					
12. Sample Provider Entity Name: _____					
13. Sample Provider Point of Contact: First: _____ MI: _____ Last: _____		14. Sample Provider E-mail Address: _____		15. Sample Provider Contact Number: _____	
16. Comments / Notes: _____ _____ _____					

I hereby certify that the information contained in Sections A and B of this form is true and correct to the best of my knowledge. I understand that if I knowingly provide a false statement on any part of this form, or its attachments, I may be subject to criminal fines and/or imprisonment. I further understand that violations of 7 CFR 331, 9 CFR 121, or 42 CFR 73 may result in civil or criminal penalties, including imprisonment.

Signature of Responsible Official/Laboratory Supervisor: \_\_\_\_\_ Date Signed: \_\_\_\_\_

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# Form 4 for Case A

- Complicated reporting

## 15. Comments / Notes:

A total of 3 samples were tested for botulinum neurotoxin by mouse bioassay.

1. 2015317440/H13647 (sample) was tested by mouse bioassay 8/21/15 but results were inconclusive. Disposed of on 8/26/15.
2. 2015317752/F70002 (sample) identified on 8/25/15 and disposed of 8/26/15
3. 2015317762/W31810 (isolate) identified 9/16/15 and disposed of 9/16/15.

3 additional isolates were sent by the submitter and tested by MDH for informational purposes. All were positive for botulinum neurotoxins by ELISA but were not confirmed by mouse bioassay. 2015318582/T43777, 2015318583/S76666, and 2015318584/S76662 were identified as isolates on 9/23/15 (see additional amended Form 4).



# On a Side Note

- *Francisella tularensis*
  - 2015
    - 4 animals
    - 4 humans
  - 2016
    - 3 animals
- *Brucella* species
  - 2015
    - 15 Brucella (4 MN residents and 7 from foreign clients)
  - 2016
    - 3 Brucella (all from foreign clients)

This means lots of Form 4's for the Select Agent program



QUESTIONS?

Thank  
You!!!



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