

# Hitting the Trifecta:

Botulism in New York State

Christina Egan

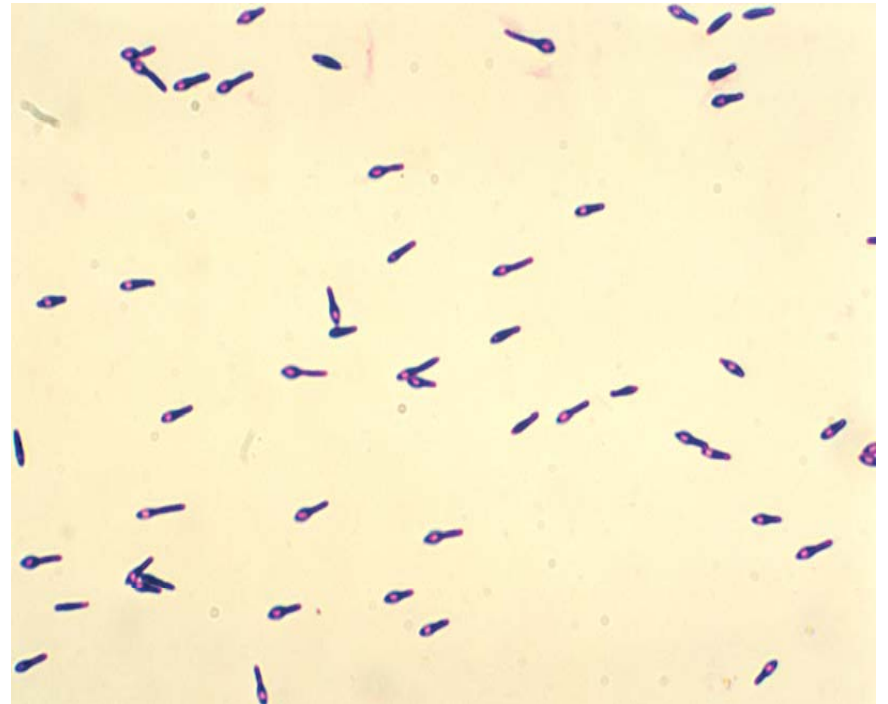
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# Outline

- ▣ Tools used for Botulism Testing
- ▣ 3 botulism events in 2012 in NYS
  - ▣ Wound botulism
  - ▣ Infant botulism
  - ▣ Food-borne botulism

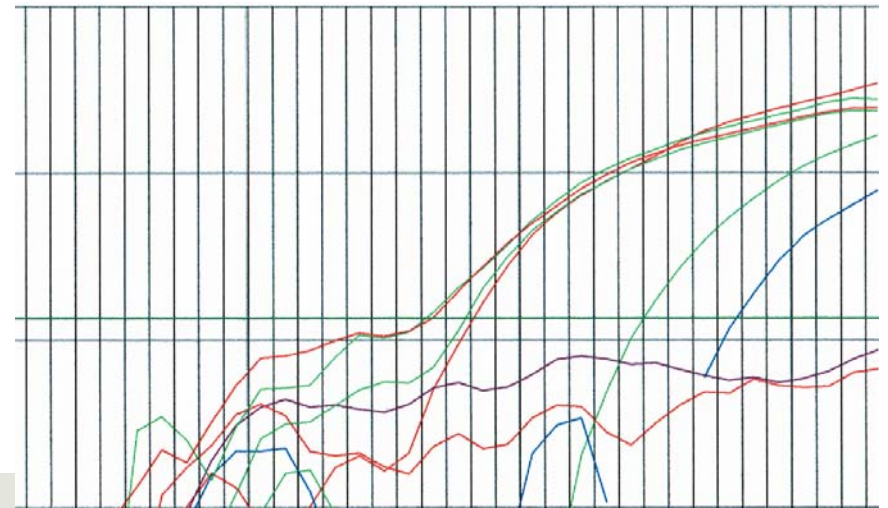


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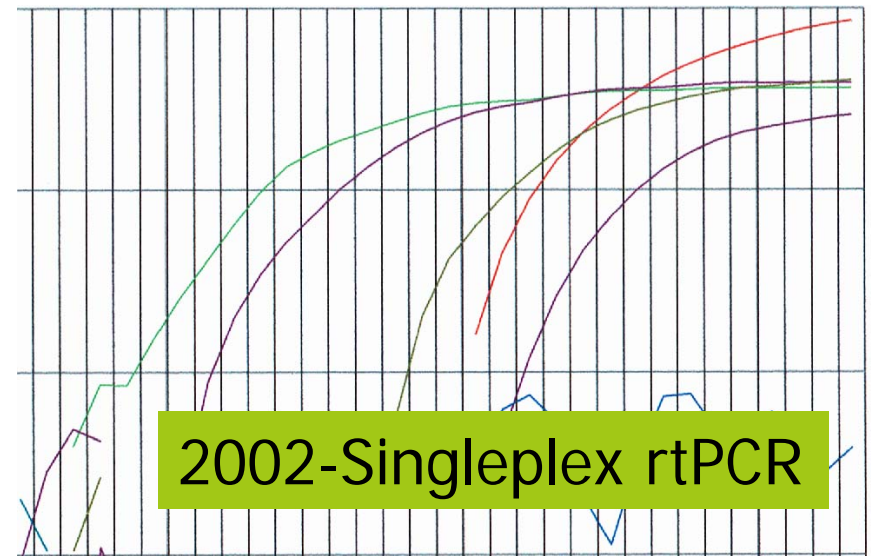
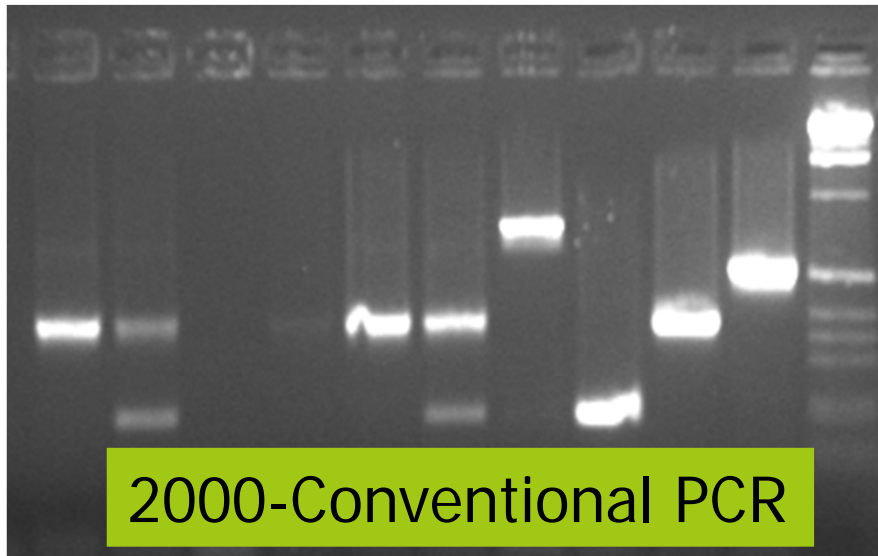
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# Real-time PCR as a Screening Assay for the Detection of *Clostridium botulinum* in Patient Specimens

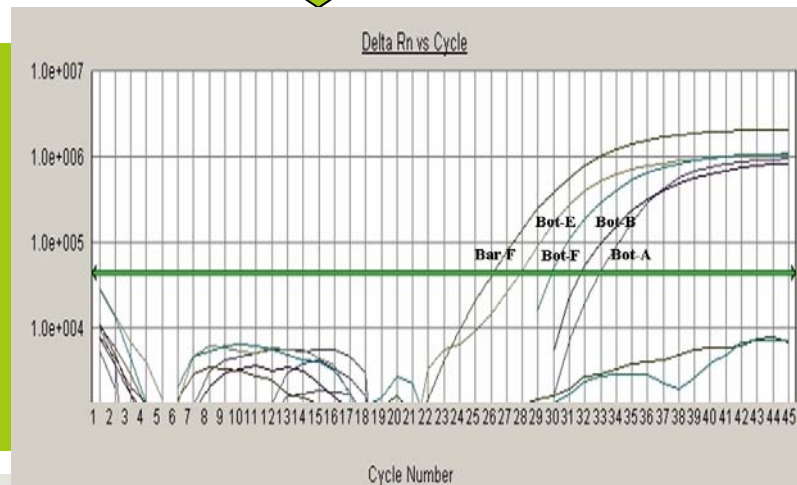
- Challenges in classical identification
  - Very small sample volumes
  - Gold standard: Mouse Bioassay
    - Difficult, time consuming, requires large samples or isolated organism
  - Turn around times are critical for public health
  - Validated a multiplex *C. botulinum* real-time PCR assay and approved for use by CLEP



# Evolution of *C. botulinum* PCR



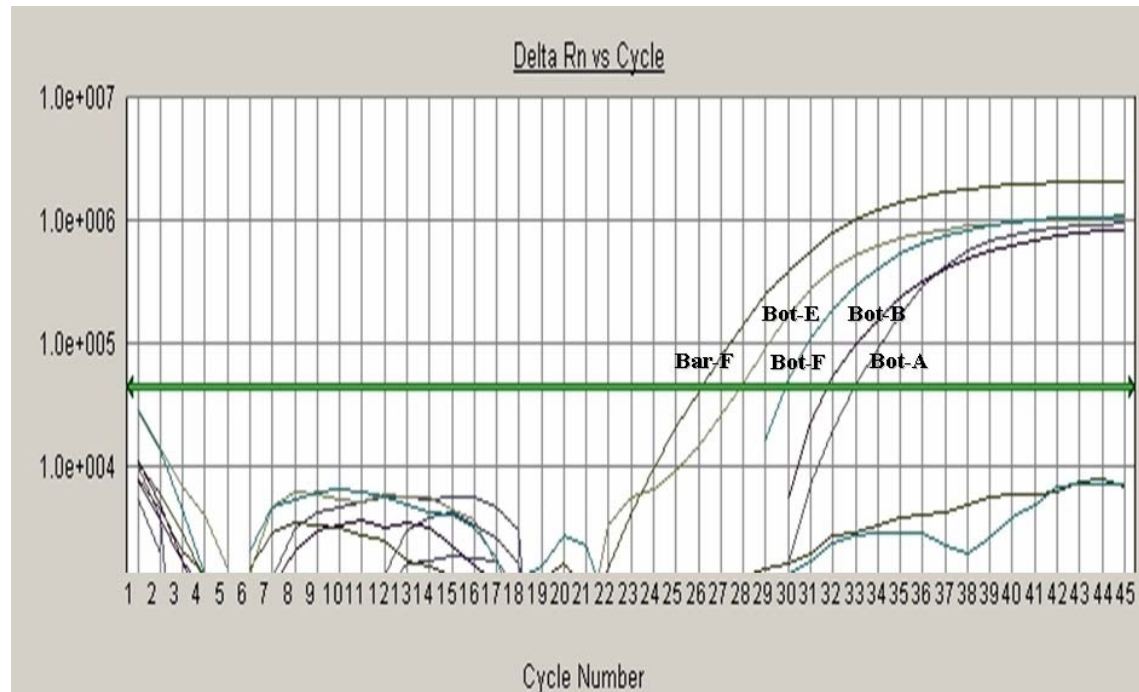
2005-Multiplex rtPCR  
*C. botulinum* toxin  
2007- Addition of *C. baratii* toxin type F



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# Assay Validation- *C. botulinum* toxin DNA



- Current format consists of 2 multiplex real-time PCR assays
  - 1<sup>st</sup>- Detects toxin type A,B, and F
  - 2<sup>nd</sup> Detects toxin type E and *C. baratii* toxin type F
- Validated using automated extraction with off-board lysis
- Detects <100 gene copies per target

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# Botulism in NY

## Human Clinical Botulinum PCR

Year	Cases	Samples	Positive samples
2003	2	2	2
2004	5	9	1
2005	3	4	0
2006	1	2	2
2007	10	40	8
2008	5	5	2
2009	4	5	1
2010	1	2	2
2011	7	8	4
2012	11	57	17

2012:

Infant botulism

Food-borne botulism

Wound botulism

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# Infant botulism

## Albany County – 2 month old female

- Onset 6/17
- Exclusively breast fed
- Parents avid gardeners
  - Mother allowed child to suck on soil-covered fingers for comfort
- *C. botulinum* Type A and B toxin genes detected in stool; *C. botulinum* type A toxin isolated

# Botulism Case#2

## Initial Case Investigation

### Past medical history

- Obesity, diabetes, hypertension, gastric bypass surgery in 1/2009
- 3/2012: Hospitalized for intra-abdominal abscess and sepsis
- 4/7: Re-admitted with 1 day history of dizziness, tongue-numbness
- 4/8-9: Progressive weakness, affecting cranial nerve functions; including, facial weakness, mouth numbness, ocular impairment, shallow breathing, loss of reflexes, along with vomiting, stomach cramps, and subsequent stomach perforation
- 4/11: Total facial paralysis; only able to move fingers/toes
  - Hospital working to rule-out other differential diagnoses
  - Patient specimens shipped overnight to WC for botulism testing

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# Foodborne, Wound or Adult Intestinal Botulism?

BCDC and CEH Food Protection discuss case food history

- Consumption of store-bought canned goods, but nothing home-canned
- Consumption of tilapia and cod (Goya brand, eviscerated and salted)

LHD performs home visit identify food in the home

- No findings of homed canned items
- No identification of risky food prep/storage techniques
- Packaged cod, commercial jarred garlic, frozen sofrito collected for testing

WC receives specimens (gastric contents, whole blood, rectal swab, serum)

- Prelim positive results for the rectal swab by rtPCR
- Prelim positive results for gastric contents by rtPCR
- Positive specimens by mouse bioassay and culture

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# Botulism – New York City, March 2012

- 39 year old male
- Onset 2/27 – vomiting and diarrhea
- Hospitalized 3/3 – Myasthenia gravis crisis?
- Botulism suspected on 3/9
  - Heptavalent antitoxin (H-BAT) released from CDC pending laboratory testing
  - Rapid improvement post-H-BAT; discharged 3/13
- Botulism type B toxin (MBA) and toxin gene (PCR) detected in stool at NYC PHL and WC

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## Tofu suspected of giving botulism to two people in Queens

Botulism is a rare but potentially fatal foodborne illness

Comments (14)

BY KERRY WILLS / NEW YORK DAILY NEWS  
Saturday, March 31, 2012, 2:18 AM

Recommend 102 recommendations. Sign up to see what your friends recommend. Twitter 2

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Fresh, unrefrigerated bulk tofu from Flushing market may have gotten two people sick with botulism.

ULLI DEKORRETT SAUCES

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## 3 Weeks Later...

- 36 year old female
- Onset 3/26 – nausea, vomiting, diarrhea progressing to slurred speech
- Hospitalized 3/28 – blurry vision, difficulty swallowing, weakness in extremities
  - Same hospital as case 1
  - H-BAT released by CDC on 3/29; discharged 4/18
- Probable case
  - *C. botulinum* toxin A gene detected in stool (PCR) at WC

# Foodborne Botulism in NYC

- One confirmed case in preceding 20 years
- Two cases with onset dates less than 4 weeks apart
  - Both patients born in China; Mandarin-speaking
  - Emigrated to the U.S. within the past 2 years
  - Both reside in same Queens community; admitted to same hospital
- Common contaminated commercial product?
- Other shared exposure?

# Epidemiologic Investigation

## Case 1

- Home-fermented tofu
- Preserved Chinese duck eggs
- A pig-ear dish maintained at room temperature

## Case 2

- Home-fermented tofu
  - Co-worker
  - Self
- Cases denied knowing each other



Fermentation container used by case 1

# Environmental Investigation

- Fresh bulk tofu purchased at same Queens supermarket
  - Case 1: purchased January, 2012
  - Case 2: purchased after February 6
  - Same vendor supplied tofu to store at both of these times
    - Vendor subsequently closed business, left U.S.
    - No evaluation of facility
- At store, tofu kept in unrefrigerated tub; bare hand contact with product

# Laboratory testing of Food Samples

## □ Case 1

### □ Home-fermented tofu

□ pH=6

□ Positive by PCR for toxin B gene

□ Mouse bioassay positive for toxin type B

### □ Raw pig's ear

□ Negative by PCR for *C. botulinum* toxin genes

## □ Case 2

### □ Home-fermented tofu

□ pH=6

□ No toxin or toxin genes detected

## □ Bulk tofu collected from supermarket on 3/29

□ pH=5

□ No toxin genes detected

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# Investigation continues

- Isolated *C. botulinum* toxin type A from the first patient
- Isolated *C. botulinum* toxin type A from the implicated tofu
- Are they related?



# Use of Next Generation Sequencing for *C. botulinum* Typing



<http://www.fluidigm.com/sample-quantitation.html>

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# SNP Analysis of Tofu and Patient

- Analyzed 2.4 million base pairs
- Detected 4,173 SNPs between food and reference strain
- Detected 4,159 SNPs in patient that were shared by both patient and tofu.
- 14 SNPs appeared to be unique to Tofu
- Upon manual analysis, 14 SNPs were errors in software
- 2 strain appear to be identical!

# Conclusion

- In 2012, we had some very unique cases of botulism
- Need to have expertise in working with varied and complex samples
- NGS looks to be very promising as a typing method
- NGS may be very useful to help understand *C. botulinum* neurotoxin complex and other genomic determinants of toxin expression

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