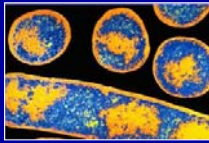


BOTULINUM TOXIN AS A BIOLOGICAL WEAPON



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Slides courtesy of Anthony Carbone, MD



OBJECTIVES

- Overview of Botulinum toxin
- Microbiology
- Pathogenesis
- Clinical manifestations
- Epidemiology
- Management: Diagnosis and Treatment
- Prevention and public health measures
- Research needs and future considerations



Botulism overview

- Disease – botulism
- Agent – botulinum toxin
- Source – *Clostridium botulinum*
- “Botulus” = “sausage” in Latin



Botulinum toxin

- Most poisonous substance known
- 1 gram lethal to 1 million
- Readily available worldwide
- Easily destroyed, difficult to disseminate
- Food-borne outbreaks in humans
- Intentional uses as a bioweapon
- First microbial toxin licensed for therapy



History

- Rare in ancient times? (pre 19th century)
- Phenomenon of food preservation
- 1793 – Botulism discovered – “wurstgift”
- 1895 – *Clostridium botulinum* isolated
- 1919 – Canned olives from CA
- 1973 – Canned vichysoisse soup
 - Led to federal regulations of canned food



Why botulinum toxin? Traits of a good bioweapon

- Desirable BW traits
 - Massive casualties
 - Induce prolonged illness
 - Resource intensive
 - Specialized care needed
 - Inadequate detection
 - Communicable
 - Incubation period
 - Non-specific symptoms
 - Mimic endemic infectious disease
- Botulinum toxin
 - Most toxic substance
 - Symptoms last for weeks
 - Mechanical ventilation and enteral feeding
 - ICU care often needed
 - Slow detection
 - Lengthy differential list
 - Symptom onset 18-72 hour
 - Denatures in air/heat/UV light
 - Non-communicable



History

- Weapons programs
 - 1930's - Japanese biological warfare Unit 731
 - Agent X – WW I and II era US and Germany
 - Soviet Union
 - 1990-? Iraq, 19,000L*
 - Others? N. Korea, Iran, Syria
- Bioweapon attempts via aerosol release
 - 1990-1995 Aum Shinrikyo (3 events?)
 - Multiple sites in downtown Tokyo, Japan
 - US military bases in Japan



Aum Shinrikyo 1990s



Reuters/No/Archive Photos
Shoko Asahara



Tokyo 1990



Tokyo 1993



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Tokyo 1995



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The Dalles Oregon 1984



(CNN)

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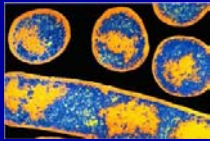
C. Botulinum microbiology

- Gram+, spore-forming, obligate anaerobe
- Soil and Marine habitats
- Classification system
 - Types A, B, C₁, C₂, D, E, F, G*
 - Based on antigenic specificities
- Produces botulinum toxin



Microbiology

- Botulinum toxins
 - Simple di-chain polypeptide
 - 100-kd "heavy" chain
 - 50-kd "light" chain
 - Bioactive component
 - Zn⁺⁺ containing endopeptidase
 - Lethal dose*
 - 0.09-0.15 µg – intravenous/intramuscular
 - 0.70-0.90 µg – inhalational
 - 70 µg - orally



Pathogenesis

- Classification/Route of exposure
 - Food-borne botulism
 - Ingest pre-formed toxin in contaminated food
 - Wound botulism
 - Toxin produced in contaminated wounds
 - Intestinal (infant) botulism
 - Intestinal colonization, toxin production in lumen
 - Infants: Ingestion of spores, honey?
 - Adults: hx of GI disease, surgery, antibiotic treatment
 - Another route -- inhalation

Pathogenesis

- Inhalational botulism
 - 1962 – 3 German lab workers
 - Demonstrated in primate studies
 - Weaponized in missiles and artillery shells
 - Japan bioterrorism attempts



Pathogenesis

- Entry via mucosal surface or wound
- Vascular transport
- Peripheral cholinergic nerve terminals
 - Neuromuscular junctions
 - Postganglionic parasympathetic terminals
 - Peripheral ganglia
 - Irreversible binding

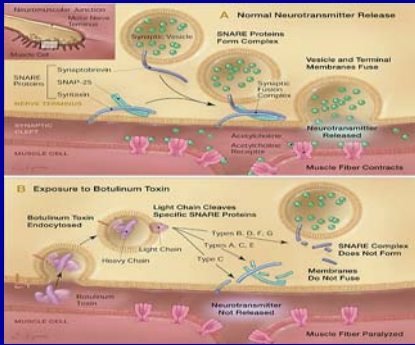


Pathogenesis

- Steps in Neurotoxin activity
 - Toxin binds to presynaptic nerve cells
 - Endocytotic internalization of toxin
 - Translocation to cytosol
 - Enzymatic cleavage of SNARE protein
 - Prevents formation of synaptic fusion complex
 - Acetylcholine blocked
- Identical neurologic signs
- Cure follows sprouting of new nerve terminals!*



Pathogenesis



JAMA. 2001; 285: 1059-1070

Clinical manifestations

- Identical neurologic symptoms manifest (all toxins subtypes)
- Incubation 18-36 hours
- **Symmetric descending flaccid paralysis**
- **Diplopia/Blurred vision**
- **Dysarthria/Dysphonia**
- **Dysphagia / Dry mouth**
- Dizziness
- Ptosis
- Nausea/vomiting/abdominal pain

Clinical Manifestations

- What is absent?
 - Afebrile!
 - Clear sensorium
 - Sensation intact
- Pupillary reflexes diminished
- Gag reflex diminished
- Deep tendon reflexes diminished
- Constipation/urinary retention

Epidemiology

- <200 cases annually in U.S.
- Food-borne botulism
 - Average 9 outbreaks/year, 2.5 cases/outbreak
 - Median of 24 cases annually
- Largest 20th century food-borne outbreak
 - Michigan 1977
 - 59 cases from home-preserved jalapeno peppers served at a restaurant



Epidemiology – Characteristics of Botulism outbreak

- Incubation period
 - Food-borne – 12-72 hours (2 hours-8 days)
 - Inhalational - uncertain
- All ages and genders equally susceptible
- Agent and vehicles
 - Colorless, odorless, and tasteless (?)
 - Vegetables, especially "low-acid" (higher pH)
 - Beans, peppers, carrots, corn
 - Non-preserved foods



Epidemiology

- Distribution and % of cases
 - Type A (~54%) Western U.S.A.
 - Type B (~15%) Eastern US Predominance
 - Type E (~27%) Pacific Northwest, AK, Great Lakes (marine)
- Mortality from food-borne botulism
 - 1950-59 25%
 - 1990-96 6%



Epidemiology

Bioterrorism considerations

- Factors suggesting deliberate release
 - Multiple cases of acute descending paralysis
 - Botulism with unusual toxin type
 - Type C, D, F or G
 - Type E from non-aquatic source
 - Lack of common dietary exposure
 - Common geographic factor
 - Multiple outbreaks with no common source
 - Lack of GI symptoms in food-borne outbreak



Diagnosis

- Clinical diagnosis
 - Specialized lab testing required
 - Routine lab testing unremarkable
- Public health emergency
- Coordinated efforts



Diagnosis

- Samples to collect
 - Serum
 - Gastric contents or vomitus
 - Stool or sterile water enema
 - Wound tissue
 - Suspected food
- Lab must be notified and pre-approved
- Confirmation 1-4 days, Culture 7-10 days



Differential Diagnosis

- Myasthenia Gravis
- Guillain-Barré Syndrome
- Stroke
- Intoxication
- Lambert-Eaton Syndrome
- Tick paralysis
- Poliomyelitis
- CNS infection
- Viral Syndrome
- CNS tumor
- Psychiatric illness
- Inflammatory myopathy
- Diabetic complications
- Hypothyroidism
- Overexertion
- Hyperemesis gravidarum
- Streptococcal pharyngitis
- Laryngeal trauma



Treatment and management

- Supportive care
 - Mechanical ventilation (20-60%!)
 - Enteral tube feeding or parenteral nutrition
 - Treatment of secondary infections
 - AVOID aminoglycosides and clindamycin
 - Reverse trendelenburg position with head support? (infants)



Treatment

- Passive immunization – Equine antitoxin
 - Divalent (AB), Monovalent (E)
 - Binds and inactivates circulating toxin
 - Minimize nerve damage (but no reversal)
 - Administer ASAP
 - Review dosing with public health officials
- Heptavalent antitoxin (ABCDEFG)
- Investigational (US Army)



Prevention and Public Health

- Prophylaxis
 - Immediate immunity
 - Endogenous immunity
 - Pentavalent (ABCDE) botulinum toxoid
- Oral/inhaled vaccine?
- Standard precautions
 - No isolation required



Prevention and Public Health

- Decontamination
 - Remove suspected contaminated food
 - Toxin destroyed by heat (85°C for 5 minutes)
 - Aerosolized botulinum toxin
 - 2 days for inactivation
 - Re-aerosolization of spores possible
 - Protective clothing
- Post-exposure
 - Wash clothing and skin
 - 0.1% hypochlorite bleach



Future considerations

- Minimize threat
- Diagnosis and Detection
 - Toxin typing
 - PCR, Enzyme assays
 - Aerosol detection (ELISA)
- Treatment considerations
- Immunization vs. therapeutic susceptibility



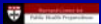
Botulism Summary

- Caused by botulinum toxin
 - Most toxic substance by weight known
- Previously weaponized/Attempted use
- Classic symptoms/Clinical diagnosis
 - Symmetric, descending, flaccid paralysis
 - Afebrile
 - Clear sensorium
- REPORT to Public Health Authorities
- Antitoxin needed ASAP
- Supportive care may take weeks



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