Clostridium botulinum

Introduction

- **About 900's:**
  - **Certain foods caused typical poisoning.**
  - **Emperor Leo VI of Byzantium forbade the manufacture of blood sausages.**

Introduction

- **1793: An outbreak caused by blood sausages was described in Wildbad, Germany**

Introduction

1820

- **Kerner collected data on 230 cases of typical poisoning.**
- **Disease became known as "Kerner's Disease."**

Introduction

1897

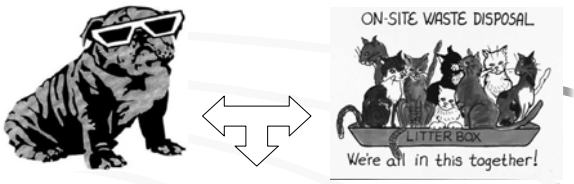
- **van Ermengem isolated an anaerobic bacterium from cured raw ham that had caused "Kerner's Disease" in 23 people and killed 3.**

Other Findings

Extract from ham & a culture of m.o.

killed a number of different experimental animals with the same signs as the disease in humans

Other Findings



proved to be fairly resistant

Introduction

- **van Ermengem called the organism *Bacillus botulinus* after botulus.**
  - **Latin for sausage**
- **Later named *C. botulinum***

Introduction

- **The *C. botulinum* strain isolated by van Ermengem was later designated type B.**
- **The name of the disease was changed from Kerner's Disease to botulism.**

Back to Germany

1904

Landman investigated botulism caused by <u>canned</u>, white beans.

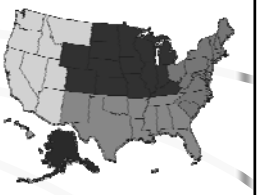
Landman Findings *Cont.*.

1904

- **The signs and symptoms were typical for botulism.**
- **The antitoxin Landman produced did not cross-react with van Ermengem's strain.**
- **Landman had discovered *C. botulinum* type A.**

How about the US?

- **Between 1918-1922:**
  - **297 cases and 185 deaths**
  - **mainly in California**



Introduction

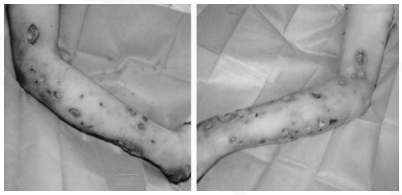
- **1922: *C. botulinum* type C was isolated**
  - **caused paralysis in chickens and cattle**
- **1929: *C. botulinum* type D was isolated**
  - **from cattle that died from paralysis**

Introduction

- **1936: *C. botulinum* type E was isolated**
  - **from smoked fish that caused botulism in the US and Russia**

Introduction

- **1951: Wound botulism was described for the first time.**



Introduction

- **1960: *C. botulinum* type F was isolated in Denmark**
  - **from liver paste that caused human botulism**

Introduction

- **1970: *C. botulinum* type G was isolated in Argentina**
  - **from soil**
  - **no reported cases of poisoning with this type in man or animals**
- **1976: Infant botulism was recognized.**

Introduction

- **1985: Hall et al. found that a strain of *C. barati* produced type F botulinal toxin.**
- **1986: Aureli et al. and McCroskey et al. isolated strains of *C. butyricum* that produced type E botulinal toxin.**
- **1973–1996: CDC documented 724 cases of verified foodborne botulism in American adults; mainly associated with home-canned vegetables.**

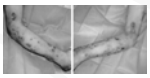
Illness & Causative Agent

- Botulism is a serious paralytic illness.
- It is caused by a nerve toxin that is produced by the bacterium.
- It is a rare illness.
- It is much feared.

Categories of Human Botulism

1. **Foodborne botulism**
2. **Infant botulism**
3. **Wound botulism**
4. **Unclassified**






Categories of Human Botulism

- **Foodborne botulism**
  - **This type of food poisoning is caused by the ingestion of foods containing the potent neurotoxin.**
  - **The neurotoxin is formed in the food during growth of *C. botulinum*.**

Categories of Human Botulism

- **Infant botulism**
  - **It was first recognized in 1976.**
  - **This type of poisoning affects infants under the age of 12 months.**
  - **It is caused by the ingestion of *C. botulinum* spores.**

Categories of Human Botulism

- **Infant botulism**
  - **The spores germinate & multiply, colonizing the intestinal tracts of infants, and produce neurotoxin.**
  - **The neurotoxin travels through the bloodstream to the central nervous system and causes flaccid paralysis.**

Categories of Human Botulism

- **Infant botulism**
  - **Infant botulism has been reported in 41 states nationwide.**
  - **The incidence is 1 case per 100,000 live births.**
  - **Case fatality rate is below 4%.**

Categories of Human Botulism

- **Infant botulism**
  - **In California the incidence from 1985 to 1995 was 7.1 cases per 100,000 live births.**
  - **Estimated medical cost/case at $85,000 (total cost = $31 million).**

Categories of Human Botulism

- **Infant botulism**

- **Honey is one vehicle that has been associated with infant botulism by a number of laboratory and epidemiological studies.**

Categories of Human Botulism

- **Infant botulism**

- **Honey is now thought to account for no more than 5% of cases.**
- **California cases may come from spores on wind-blown dust.**

Categories of Human Botulism

- **Wound botulism**
  - **This illness results from the pathogen itself infecting a wound.**
  - **Foods are not the vehicle of transmission.**
  - **The microorganism produces the neurotoxin which is transmitted to other parts of the body via the blood.**
  - **Rare form of illness**

Categories of Human Botulism

- **Unclassified**
  - **Resembles infant botulism, but affects adults.**
  - ***C. botulinum* colonizes the intestinal tract of adults and produces the toxin in vivo.**
  - **Thought to occur after antibiotic treatment depleted the indigenous intestinal flora.**

Recorded Botulism Cases in the US: 1973–1996

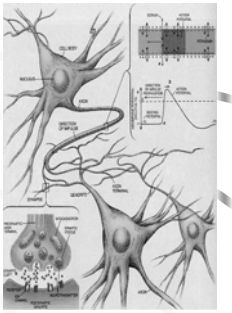
Botulism type	Range/yr	Total (all years)
Food	8–86	**724**
Infant	**0-99**	**1444**
Wound	**0-25**	**103**
Unclassified	**Not avail.**	**39**

Classification of *C. botulinum*

- **There are seven types of *C. botulinum***
  - **A, B, C, D, E, F, and G**
  - **based on the serological specificity of the neurotoxin produced**

C. botulinum Toxins

- *C. botulinum* produces eight toxins (A, B, C_1, C_2, D, E, F and G).
- All are <u>neurotoxins</u> except C_2.



Classification of *C. botulinum*

- **Types A, B, E, and, very rarely, F are associated with human botulism (foodborne, wound and infant types).**
- **Types C and D affect animals.**
- **Type G has not been linked to illness up to this date.**

C. botulinum Toxins

- Some strains produce pairs of toxins
- These are designated subtypes
  - The capital letter identifies the type of toxin in greater amount
  - The lower case letter identifies the type of toxin produced in lesser amount

C. botulinum Toxins

- An example:
  - strain isolated from a case of infant botulism was classified as subtype

Distribution of Serotypes in Human Botulism in the US

Type	Cases (%)	Deaths (%)
A	38	52
B	38	12
E	9.7	10
F	0	0
Unknown	13	0

C. botulinum groups

- Another classification of *C. botulinum* strains is based on physiological differences.
  - ➢ growth temperature
  - ➢ pH
  - ➢ water activity
  - ➢ sodium chloride concentration

C. botulinum Groups

- *C. botulinum* strains are divided into four groups.
  - ➢ group I*: proteolytic and produce neurotoxins type A, B, and F.
  - ➢ group II*: nonproteolytic and produce neurotoxins type B, E, and F.

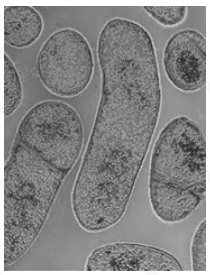
*the most commonly involved in human illness.

C. botulinum Groups

- *C. botulinum* strains are divided into four groups.
  - ➢ group III: variably nonproteolytic or proteolytic and produce neurotoxins type C and D.
  - ➢ group IV: proteolytic and produce neurotoxin type G.

Characteristics of *C. botulinum*

- Gram positive
- Sporeformer
- Anaerobic
- Rods
- Produce a potent neurotoxin



[A. Dowsett/Science Photo Library]

Characteristics of *C. botulinum*

- pH values for growth
  - ➢ Types A and proteolytic B (Gp I), pH **4.6**-8.5
  - ➢ Minimum pH for E (Gp II) is:
    - ❖ 6.2 at 5°C, and
    - ❖ 5.4 at 30°C

Characteristics of *C. botulinum*

- Limiting water activity
  - ➢ Type A 0.95
  - ➢ Type B 0.94
  - ➢ Type E 0.97

Characteristics of *C. botulinum*

- Limiting salt concentration for growth
  - ➢10.7–12% NaCl
  - ➢Non-proteolytic most sensitive

Characteristics of *C. botulinum*

- Growth temperature
  - ➢Type A and proteolytic B (Gp I) 10–50°C
  - ➢E and non-proteolytic B and F (Gp II) 3.3–45°C
  - ➢Spores are highly resistant to freezing

Characteristics of *C. botulinum*

- Redox potential
  - ➢Optimum growth occurs at Eh of –350 mV
  - ➢E is the least anaerobic, 0–100 mV

Characteristics of *C. botulinum*

- Heat resistance defined
  - ➢Decimal reduction time (D value; 90% kill)
  - ➢Time required to reduce the microbial population by 1 log cycle.

Characteristics of *C. botulinum*

- Heat resistance
  - ➢121.1°C (250°F): DRT = 0.20–0.21 min for the most resistant (A and proteolytic B)

Characteristics of *C. botulinum*

- Heat resistance
  - ➢121.1°C (250°F) / 3 min to achieve 10^{12}-fold reduction (standard for low acid canned foods), “bot cook”
  - ➢0.3–0.6 min causes 10^{6}-fold reduction and is standard for canned, cured meats

Characteristics of *C. botulinum*

- Radiation resistance

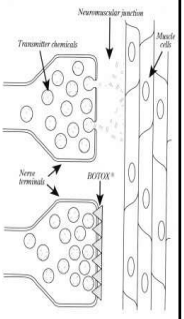
  To cause 10^{12} fold reduction
  - ➢47 – 54 kGy for type A spores
  - ➢10 – 11 kGy for type B spores
  - ➢7 – 9 kGy for type E spores
  - ➢12 kGy for type F spores
  - ➢48 kGy is the accepted dose for sterilization of food spores

Mechanism of Toxin

Neurotoxin ⇨ binds to neurons ⇨

internalized ⇨

prevents release of acetyl choline (neurotrasmitter)



Nature of Food Botulism

- Intoxication
- Onset is about 18 – 36 hrs after ingestion of the food containing the neurotoxin.
- Symptoms vary from a mild to severe illness.

Clinical Symptoms

- Symptoms include:
  - ➢nausea and vomiting

Clinical Symptoms

- Symptoms include:
  - ➢neurological signs
    - ▪ blurred or double vision
    - ▪ difficulty in speaking or swallowing
    - ▪ fatigue
    - ▪ lack of muscle coordination, and
    - ▪ difficulties in breathing

Nature of Illness

- Other symptoms include:
  - ➢gastrointestinal problems
    - ▪ cramps
    - ▪ abdominal pain
    - ▪ diarrhea, or
    - ▪ constipation

Pathogenic Dose

- Few nanograms of *C. botulinum* neurotoxin can cause illness.
- The neurotoxin produced is probably the most toxic compounds made by a biological system.
- About 1 oz. (28.4 g) of this toxin can kill 200 million people.
- Fortunately, the incidence of the illness is low.

Foods Implicated in Botulism

- Any food that can support the growth of this pathogen or allow the germination of its spores and eventually toxin production can be associated with this illness.
- Low acid foods (pH>4.6)

Foods Implicated in Botulism

- Home-canned or -preserved low-acid vegetables
  - asparagus, tomatoes, beans, mushrooms
  - peppers, corn, baked potato, chopped beets
  - garlic in soybean oil

Foods Implicated in Botulism

- North American Indian specialties
  - fish and fish eggs
  - seal flippers
- Other implicated foods include luncheon meats, ham, sausage, smoked and salted fish, and lobster.

C. botulinum Outbreak

- In 1994, in Oklahoma, a 47-year old man was hospitalized for symptoms of progressive dizziness, blurred vision, slurred speech difficulty swallowing, and nausea.
- Twenty-four hours earlier the patient had eaten some home canned green beans and beef and potato stew.

C. botulinum Outbreak

- Upon testing:
  - The green beans tested negative for the toxin
  - The stew tested positive for the toxin

C. botulinum Outbreak

- Apparently,
  - ➢the stew was cooked,
  - ➢covered tightly,
  - ➢left out for four days at room temperature, and
  - ➢then eaten without reheating.

Prevention

- Assurance of destruction or inhibition of *C. botulinum*.
- Keep foods out of the temperature danger zone (4.4 – 60°C or 40 – 140°F).
- Botulinum toxin is destroyed by heating at 80°C for 30 min or boiling or a few minutes. Thus re-heating foods properly can be a controlling factor.

Detection of Organism & Toxin

- Compendium of Methods for the Microbiological Examination of Foods.
- Enrichment of culture in cooked liver or cooked meat medium.
- Plating on blood agar or egg yolk agar, and incubating anaerobically.

Detection of Organism & Toxin

- Toxin can be detected and typed by mouse inoculation.

Assumption: Active toxin will kill mouse

- An ELISA method has been developed for detection of toxin.