**Clostridium botulinum**

**Introduction**

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

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

- 1820
  - Kerner collected data on 230 cases of typical poisoning.
  - Disease became known as "Kerner's Disease."

- 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 canned, 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

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

• 1951: Wound botulism was described for the first time.

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

• 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.

• 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
   - 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*.

2. 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.

3. Wound botulism

4. Unclassified

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.

- 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).

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

• 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.

• 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

• 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.


<table>
<thead>
<tr>
<th>Botulism type</th>
<th>Range/yr</th>
<th>Total (all years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>8–86</td>
<td>724</td>
</tr>
<tr>
<td>Infant</td>
<td>0–99</td>
<td>1444</td>
</tr>
<tr>
<td>Wound</td>
<td>0–25</td>
<td>103</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Not avail.</td>
<td>39</td>
</tr>
</tbody>
</table>
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, C₁, C₂, D, E, F and G).
- All are neurotoxins except C₂.

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Cases (%)</th>
<th>Deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>38</td>
<td>52</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>E</td>
<td>9.7</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>
**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

**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

- 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)

  - 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 \textit{C. botulinum}
\begin{itemize}
\item Radiation resistance
\item To cause $10^{12}$ fold reduction
\item 47 – 54 kGy for type A spores
\item 10 – 11 kGy for type B spores
\item 7 – 9 kGy for type E spores
\item 12 kGy for type F spores
\item 48 kGy is the accepted dose for sterilization of food spores
\end{itemize}

Mechanism of Toxin
\begin{itemize}
\item Neurotoxin binds to neurons
\item internalized
\item prevents release of acetyl choline (neurotransmitter)
\end{itemize}

Nature of Food Botulism
\begin{itemize}
\item Intoxication
\item Onset is about 18 – 36 hrs after ingestion of the food containing the neurotoxin.
\item Symptoms vary from a mild to severe illness.
\end{itemize}

Clinical Symptoms
\begin{itemize}
\item Symptoms include:
\item nausea and vomiting
\end{itemize}

Clinical Symptoms
\begin{itemize}
\item Symptoms include:
\item neurological signs
\item blurred or double vision
\item difficulty in speaking or swallowing
\item fatigue
\item lack of muscle coordination, and
\item difficulties in breathing
\end{itemize}

Nature of Illness
\begin{itemize}
\item Other symptoms include:
\item gastrointestinal problems
\item cramps
\item abdominal pain
\item diarrhea, or
\item constipation
\end{itemize}
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.

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**BOTOX®**

*Botulinum Toxin Type A*  
Purified Neurotoxin Complex  
**THE STRENGTH OF EXPERIENCE**