
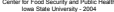
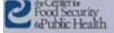
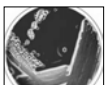




Slide 1	<p>Epsilon Toxin of <i>Clostridium perfringens</i> B and D</p> 	
Slide 2	<p><b>Overview</b></p> <ul style="list-style-type: none"> <li>• Organism</li> <li>• History</li> <li>• Epidemiology</li> <li>• Transmission</li> <li>• Disease in Humans</li> <li>• Disease in Animals</li> <li>• Prevention and Control</li> </ul> 	<p>In today's presentation we will cover information regarding the epsilon toxin of <i>Clostridium perfringens</i> Type B and D and its epidemiology. We will also talk about the history of the toxin, how it is transmitted, species that it affects (including humans), and clinical seen. Finally, we will address prevention and control measures.</p>
Slide 3	<p><b>Agent</b></p> 	
Slide 4	<p><b>The Agent</b></p> <ul style="list-style-type: none"> <li>• <i>Clostridium perfringens</i> <ul style="list-style-type: none"> <li>– Gram-positive bacteria <ul style="list-style-type: none"> <li>• Anaerobic rod</li> </ul> </li> <li>– Found in soil, decaying matter and intestinal tract of mammals</li> </ul> </li> <li>• 5 types (A-E) <ul style="list-style-type: none"> <li>– Types B and D produce the epsilon toxin</li> </ul> </li> </ul>  	<p><i>Clostridium perfringens</i> is a Gram-positive, spore-forming anaerobic rod which is found in soil, decaying matter and the intestinal tracts of many animals. There are five types of <i>C. perfringens</i> (A through E). <i>C. perfringens</i> types B and D produce the epsilon toxin. Image: <i>C. perfringens</i> on a blood agar plate. Double zones of beta hemolysis (breakdown of red blood cells) surrounding the bacteria indicate toxin production. Photo courtesy of <a href="http://ag.arizona.edu/pubs/general/resrpt1998/clostridium.html">http://ag.arizona.edu/pubs/general/resrpt1998/clostridium.html</a></p>
Slide 5	<p><b>Epsilon Toxin</b></p> <ul style="list-style-type: none"> <li>• Produced as an inactive protoxin</li> <li>• Activated by trypsin <ul style="list-style-type: none"> <li>– Removes a 13-residue N-terminal peptide</li> </ul> </li> <li>• Increases intestinal permeability</li> <li>• Increases vascular permeability <ul style="list-style-type: none"> <li>– Vascular damage and edema in brain, heart, lung and kidneys</li> </ul> </li> </ul> 	<p>The epsilon toxin is produced as an inactive protoxin. It becomes activated when trypsin removes a 13-residue N-terminal peptide. The toxin is known to increase intestinal permeability, and can also cause liver damage, elevate blood pressure and cause an increase in vascular permeability. This can lead to vascular damage and edema in many organs including brain, heart, lung and kidneys. The mode of action of the epsilon toxin is presently unknown, but recent work has indicated that the toxin remains on the outside of the cell, acting from the cell surface and causing an efflux of intracellular K<sup>+</sup>. This damages the cell membrane and eventually leads to cell death.</p>

## History



## History

- Iraq produced 90 gallons of *C. perfringens*
- 1945: Japan
  - Used shrapnel bomb containing *C. perfringens* on ten Chinese victims
  - Slow death from gas gangrene due to infection from bacteria

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Iraq produced 90 gallons of *C. perfringens* (type unknown to us) as part of their biological weapons program. In 1945, Japan used a shrapnel bomb containing *C. perfringens* bacteria on ten Chinese victims who were tied to posts. The wounds inflicted infection and they all died a slow death from gas gangrene. Experiments such as these were carried out in Japan from 1937 to 1945 as part of Japan's ambitious biological warfare program directed by Japanese General Ishii. Historical information about Epsilon Toxin of *C. perfringens* in biological warfare or bioterrorism is lacking.

## Transmission



## Transmission: Humans

- Ingestion of *C. perfringens* A
  - Foodborne illness
  - Improperly prepared and handled foods
- Aerosolization of *C. perfringens* A
  - Expected to cause high morbidity and mortality
- Consequences of aerosolizing epsilon toxin not known at this time

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Little research concerning epsilon toxin exposure in humans has been conducted. *Clostridium perfringens* A (which does not produce the epsilon toxin) causes foodborne illness due to improperly prepared and handled foods, and aerosolization of *Clostridium perfringens* A could be potentially devastating with high morbidity and mortality. There is not enough evidence available to determine the consequences of aerosolization with the epsilon toxin.

## Transmission: Animals

- Normal intestinal inhabitant
- Fecal-oral transmission
- Ingestion of large quantity
  - Contaminated soil, water, feed
- Proliferation often caused by disruption of normal intestinal bacteria



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*C. perfringens* is a normal intestinal inhabitant. Transmission can occur by fecal-oral route, or by ingestion of a large quantity of the bacteria through contaminated soil, water or feed. Another mode of transmission occurs when an animal's normal intestinal flora is disrupted and *Clostridium* are allowed to proliferate. The picture is a gram-stain of rods of *Clostridium perfringens* in a horse. <http://ag.arizona.edu/pubs/general/resrpt1998/clostridium.html>

## Disease in Humans



### ***Clostridium perfringens* A**

- Incubation 8-22 hours
  - Intense abdominal pain
  - Diarrhea
  - Dehydration common side effect
- Most symptoms end by 24 hours
- Very young and very old most severely affected

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There is very little in the literature on human illness from epsilon toxin B and D so signs listed here are for *Clostridium perfringens* A, a common source of food borne illness. Symptoms develop 8-22 hours after ingestion. The typical presentation is intense abdominal pain and diarrhea. Symptoms generally regress within 24 hours but may persist longer in very young or very old people. Minor symptoms can persist for up to two weeks. Again, there is no data available of the effects of epsilon toxin in people.

### **Diagnosis and Treatment**

- Diagnosis
  - Specific immunoassays available
  - Diagnosis of human disease unproven
- No treatment for epsilon toxin
  - Penicillin used to treat *C. perfringens*
- Clindamycin and rifampin
  - May suppress epsilon toxin production
- Supportive
  - Fluid replacement
  - Monitor electrolytes

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Acute serum and tissue samples should be collected and quickly sent to a laboratory. Specific immunoassays are available but their usefulness in diagnosis of human disease is unproven. There is currently no known treatment for epsilon toxin. Penicillin is useful in the treatment of *C. perfringens* if given early. Image: test tubes in laboratory. Experimentally rifampin and clindamycin have shown efficacy in animal models against epsilon toxin. There are vaccinations available for the epsilon toxin in animals and toxoids are also used with success. There is no evidence of their effectiveness in humans. Supportive treatment including fluid replacement and monitoring electrolytes is recommended.

## Animals and Epsilon Toxin



### **Animals and Epsilon Toxin**

- Rapidly fatal enterotoxemia
- Produces edema
  - Edema in brain
    - Can induce death and necrosis of brain tissue



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Epsilon toxin is a potent toxin produced by *Clostridium perfringens* types B and D, which are responsible for a rapidly fatal enterotoxemia in animals. One of the main properties of epsilon toxin is the production of edema. Image: sheep.

### ***C. perfringens* D**

- Lamb enterotoxemia
  - Overeating disease
- Epsilon toxin
  - Systemic toxemia
  - CNS lesions, opisthotonus, convulsions, sudden death
  - Kidney lesions
    - Pulp kidney disease

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*Clostridium perfringens* type D causes lamb enterotoxaemia, also known as lamb overeating disease. The organism establishes in the gut and multiplies. The epsilon toxin produces a systemic toxemia. This leads to CNS lesions including opisthotonus, convulsions and sudden death. Kidney lesions are also commonly associated with this form of the disease.

### ***C. perfringens* D**

- Cattle and sheep
  - Neural manifestations
- Goats
  - Diarrhea common
- Mortality
  - Highest in lambs
  - Calves and goats non fatal subacute and chronic disease



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*C. perfringens* D in cattle and sheep often manifests as a neural disease. Presentation of goats infected with *C. perfringens* D is often that of diarrhea. Mortality is very high among lambs but is generally less severe in calves and goats causing a non-fatal subacute disease.

### ***C. perfringens* B**

- Lamb dysentery
  - Newborn lambs (less than 3 weeks)
    - 95% mortality
- Enterotoxemia
  - Neonatal calves
  - Neonatal foals
  - Mortality is high but not as severe as in lambs



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*C. perfringens* B causes disease in several species and is especially harmful to newborns. It causes lamb dysentery and hemorrhagic enteritis in neonatal calves and foals. The two toxins associated with type B may be additive or synergistic (beta and epsilon toxin). Lamb dysentery is an acute disease of lambs younger than 3 weeks. Many may die before signs are seen, but some newborns refuse to nurse and become listless. They often have blood tinged diarrhea and die within a few days. Mortality rates are 95%. In calves, there is acute diarrhea, abdominal pain, convulsions, and opisthotonos. Death will often occur within a few hours. In less severe cases animals survive for a few days and recovery is possible. Foals experience acute dysentery, toxemia, and rapid death.

### **Diagnosis and Treatment**

- Diagnosis
  - ELISA
    - Can detect epsilon toxin with biological fluids
    - Intestinal, peritoneal, pericardial
  - Toxin-antitoxin neutralization test
  - PCR
- Treatment
  - Penicillin
    - Effective if given early
  - Vaccination
  - Toxoids

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Diagnosis of animal infection with epsilon toxin due to infection with *C. perfringens* B and D is by ELISA, toxin-antitoxin neutralization testing and PCR. The treatment of choice for infection with *C. perfringens* is penicillin. It is effective if given early. Vaccinations are available for prophylaxis of infection with epsilon toxin. If there is not time for immunity to be achieved with vaccination, effective prophylactic measures can be achieved with toxoid treatment. Specific treatment for *Clostridium perfringens* B is usually ineffective because of the severity of the disease. Hyperimmune serum may be beneficial, and orally administered antibiotics may also be useful. The disease is best controlled by vaccination of the pregnant dam during the last third of pregnancy. Ewe immunization probably is the most satisfactory method of control for infection with *C. perfringens* D as well.

### **Prevention and Control**



## Prevention and Control

- Disinfect with soap and water
- Health care workers
  - Should follow standard safety precautions
- Vaccine available for animals
- Follow FDA/USDA guidelines for proper handling of food

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Disinfect exposed areas with soap and water. Health care workers should follow proper safety precautions. There are several vaccinations available for animals for prevention of disease with epsilon toxin. *C. perfringens* B is best controlled by vaccination of the pregnant dam during the last third of pregnancy. Ewe immunization probably is the most satisfactory method of control for infection with *C. perfringens* D as well and enterotoxemia caused by *C. perfringens* D in feedlot lambs can be controlled by reducing the amount of concentrate in the diet. To prevent food poisoning in humans, due to *Clostridium perfringens* A, leftover cooked meat should be refrigerated promptly and reheated thoroughly (internal temperature, 75° C) before serving. (Further instructions on safe food handling and preparations can be achieved by searching FDA or USDA sites about food safety and food handling).

## Epsilon Toxin as a Biological Weapon

- Toxin can be manufactured by fermentation of *C. perfringens*
- Chemical synthesis is impractical
- Aerosolization capabilities
  - Insufficient information available at this time
  - Acute pulmonary illness

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Epsilon toxin can be manufactured by fermentation of *C. perfringens*, but chemical synthesis is impractical. Aerosolization capabilities of epsilon toxin are unknown at this time; aerosolization with *C. perfringens* alpha toxin would be expected to cause an acute pulmonary illness.

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