PHR250, C7
4/10/06

Clostridium perfringens

Maha Hajmeer

History

1890s: Two scientists, F.W. Andrewes and E. Klein, associated eating foods contaminated with *Clostridium welchii* (now *C. perfringens*) with several foodborne outbreaks characterized with mild to severe diarrhea and abdominal pain.

1892: This agent was found in a variety of diseases such as gas gangrene (primarily caused by α-toxin), appendicitis, puerperal fever, and enteritis.

1933: Studies by Hobbs et al. showed that eating food contaminated with *C. perfringens* could lead to diarrheal food poisoning.

1939 to 1946: Several outbreaks observed:
In the United Kingdom during World War II where shortage of meat led to the practice of cooking meat for later consumption.

1943: The first warning of food poisoning due to anaerobic sporing bacilli including *C. perfringens* came from Knox and Macdonald. Children became ill after eating school meals containing heavily contaminated gravy that was made the previous day.

1945: McClung described the first proven outbreak of *C. perfringens* in the U.S. McClung examined four foodborne outbreaks associated with eating chicken steamed 24 hours before consumption.

1948: Severe necrotic gastroenteritis occurred in Germany. The vehicle for the illness was home canned rabbit, and the associated strain was *C. perfringens* type C.

1954: Experiments in USA with human volunteers were conducted.
- The strain fed was English strain of *C. perfringens*.

- Volunteers failed to develop any symptoms of disease.

- Public health significance was not fully accepted in USA until the 1960s.

- By the 1960s and 70s, enough information had accumulated to indicate that *C. perfringens* foodborne poisoning is caused by the release of the toxin during sporulation of the microorganism in the intestine of infected individuals who ate food heavily contaminated with *C. perfringens*.

- In the 1970s, much advancements were made in determining the presumptive mode of action of the enterotoxin. This was after toxin purification conducted by Hauschild and Hilsheimer (1971) and Stark and Duncan (1972).

Characteristics of *C. perfringens*
Gram positive, nonmotile, encapsulated rods with square ends. Anaerobe but more oxygen tolerant than *C. botulinum*. Produces acetone, butanol, ethanol, butyric acid, acetic acid, propionic acid lactic acid, carbon dioxide and hydrogen. Ferments sugars, starch and pectin.

Classification of *C. perfringens* is based on toxins produced

Toxins	alpha	beta	epsilon	iota
Type A	**+**	**0**	**0**	**0**
B	**+**	**+**	**+**	**0**
C	**+**	**+**	**0**	**0**
D	**+**	**0**	**+**	**0**
E	**+**	**0**	**0**	**+**

The alpha toxin is a phospholipase, the others are hemolysins or cause necrosis.

The different types cause a variety of diseases in animals, some of them very severe.

Types A and C, seldom D, produces enterotoxin; spores are formed readily in the intestinal tract and are the form in which the organism survives in the environment. Spores are seldom formed in food, sporulation requires a well-buffered medium rich in nutrients.

Type A is hemolytic (alpha/beta) or non-hemolytic; killing of 90% of spores takes 6 – 17 minutes at 100˚C for non-hemolytic strains and less than one minute for betahemolytic strains.

Best estimates of annual cases and deaths for foodborne diseases, USA.

	Cases	Percent	Deaths	Percent
C. perfringens	**248,520**	**1.8**	**7**	**0.4**
Total bacterial	**4,175,565**	**30.2**	**1,297**	**71.7**
Total foodborne	**13,814,924**	**100**	**1,809**	**100**

Prevalence of *C. perfringens* in foods

Pork	0 - 39%
Cooked pork	45%
Beef	22%
Chicken	0 - 54%
Seafood	2%

Clinical features of *C. perfringens*

Perfringens food poisoning is described by severe abdominal cramps and diarrhea; no pyrexia, shivering, headache, dehydration, or other symptoms of infection.

Dehydration and other complications can lead to death — few have been reported.

Perfringens foodborne illness caused by Type C strains is more serious and often fatal. This is also known as enteritis necroticans or pig-bel diseases. Fortunately it is rare.

Incubation time to illness is 8 to 24 hours.

Duration of illness is 12 to 24 hours. Symptoms may persist in some individuals for 1 to 2 weeks.

Large numbers of *C. perfringens* found in feces and food; significant amount of enterotoxin also found in feces.

Infective dose is high. Large numbers of vegetative cells (~10^8) need to be ingested to cause symptoms.

Mechanism of disease

C. perfringens produces toxin-mediated infection known as *C. perfringens* enteritis.

C. perfringens produces food poisoning without colonization.

C. perfringens grows in food → ingested cells begin to sporulate after passing stomach → enterotoxin produced in small intestines → symptoms.

C. perfringens food poisoning outbreaks tend to be large scale, large volumes of food produced, but there are few if any fatalities.

Enterotoxin production by *C. perfringens* is associated with sporulation. Sporulation is poor in most foods, which therefore contain little or no enterotoxin, but rapid in small intestines where

pH is right and well buffered.

Sporulation begins in the intestines in 3 hours, enterotoxin found in 10 – 12 hours when free spores are released.

Human illness is diagnosed by the symptoms of perfringens poisoning. It is confirmed by detecting the toxin in the feces of patients. Confirmation is also possible by finding the causative agent in implicated foods or feces of patients.

Heat shock, 70 – 100˚C, boosts germination and yields of enterotoxin. Therefore, if food such as meat containing spores is heated and left for some time at growth temperature the “primed” spores will produce rapidly growing vegetative cells that in turn will produce plenty of spores and enterotoxin in the small intestines — unless the food is boiled immediately before consumption, which will kill the vegetative cells in the food and preclude sporulation in the intestines.

The enterotoxin is a simple polypeptide of a molecular weight of 36000± 4000; it contains 309 amino acid residues but only one cysteine residue; it is inactivated by pronase and *B. subtilis* protease but not by trypsin, chymotrypsin, papain or bromelin.

Enterotoxin is heat labile with 90% destruction in 4 minutes at 60˚C.

It has similarities with *V. cholerae* enterotoxin; it causes a transient increase in capillary permeability with fluid accumulation and diarrhea; there is increased secretion of water, sodium and chloride and decreased absorption of glucose. Enterotoxin causes desquamation of villi epithelium.

In patients suffering from *C. perfringens* food poisoning 0.2 – 36 μg of enterotoxin is found per gram feces.

Only toxigenic types A and C, seldom type D, of *C. perfringens* produce enterotoxin.

C. perfringens spores can be isolated from healthy persons; these spores are probably formed in the colon rather than in the small intestines.

Immunity
After an incident of disease, circulating antibodies that neutralize enterotoxin are found in the blood but not in the intestines.

C. perfringens food poisoning does not confer immunity.

Reservoirs
C. perfringens is found in human and animal intestinal tracts and soil.

Normal healthy humans are 50 to 100% carriers of *C perfringens*, and excrete around 10^3 spores per gram of feces; people recovering excrete more than 10^5 per gram.

Frequency of animal shedders of *C. perfringens:*
Swine — 18%
Rats — 41%
Chicken — 88% (60% produced enterotoxin)
Cattle — 80% (68 % produced enterotoxin)

Cattle and pigs that are exhausted at the time of slaughter may have *C. perfringens* in the mesenteric lymph nodes and muscle tissue.

Growth and survival
- Growth temperature ranges from 6.5 – 47 ˚C.
- Cells frozen at –18˚C — 4% survived for 180 days.
- Spores frozen at –18˚C — 11% survived for 180 days.
- Storage at 5˚C was found more lethal than freezing at –18˚C.
- A 1.2 – 3.4 kGy of gamma rays kills 90% of spores.
- The limiting water activity for growth is 0.95 – 0.96.
- The limiting NaCl concentration is 5 – 8%.
- Upper redox potential permitting growth +31 mV at pH 7.7, +230 mV at pH 6

Detection of *C. perfringens*
Many different media have been proposed (see Compendium of Methods for the Microbiological Examination of Foods)
- Sulfite cycloserine agar is convenient for pour plates
- Neomycin blood agar for surface plating
- Most probable numbers (MPN) can be determined in cooked meat or liver medium followed by streaking on neomycin blood agar.
-Enterotoxin can be detected by ELISA test.

References
Downes, F.P. and Ito, K. (eds). Compendium of Methods for the Microbiological Examination of Foods, 4th ed, American Public Health Association, Washington, DC.

Hauschil, A., and Hilsheimer, R. 1971. Purification and characteristics of the enterotoxin of *Clostridium perfringens* type A. Can. J. Microbiol. 17: 1425-1433.

Hobbs, B. 1979. *Clostridium perfringens* gastroenteritits. pp. 131-171. *In* H. Riemann and F.L. Bryan (eds) 1979. Food-borne Infections and Intoxications, 2d ed, Academic Press, New York.

Labbe, R.G., and Juneja, V.k. 2006. *Clostridium perfringens* gestroenteritis. pp. 137-184. *In* H. Riemann and D.O. Cliver (eds). Foodborne Infections and Intoxications, 3rd ed, Academic Press, New York.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P., and Tauxe, R. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5(5): 607-625.

Stark, R., and Duncan, C. 1972. Purification and bichemical properties of *Clostridium perfringens* type A enterotoxin. Infect. Immun. 6:662-673.