Epsilon Toxin of *Clostridium perfringens*

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Etiology

The epsilon toxin is one of 12 protein toxins produced by *Clostridium perfringens*, a Gram positive, anaerobic spore-forming rod. There are five strains of *C. perfringens*, designated A through E. Each strain produces a unique spectrum of toxins. The epsilon toxin is made by types B and D. This toxin is a pore-forming protein; it causes potassium and fluid leakage from cells. In addition to the epsilon toxin, *Clostridium perfringens* type D strains produce alpha toxin and type B strains produce alpha and beta toxins. *C. perfringens* type B causes severe enteritis in young calves, foals, lambs and piglets. Type D causes enterotoxemia in sheep and goats and, on rare occasions, in cattle. All five strains can infect wounds in any species.

Table 1: Toxins produced by strains of Clostridium perfringens

Strain of *Clostridium perfringens*	Toxins
Type A	alpha
Type B	alpha, beta, epsilon
Type C	alpha, beta
Type D	alpha, epsilon
Type E	alpha, iota

Other *C. perfringens* toxins

Other *C. perfringens* toxins are also potential biological weapons. Alpha toxin is a necrotizing toxin produced by all five strains. The purified toxin can cause serious acute pulmonary disease, as well as vascular leak, hemolysis, thrombocytopenia and liver damage. It is expected to be lethal by aerosol. Beta toxin is a lethal necrotizing toxin found in types B and C. Theta toxin is an oxygen–labile cytolysin. This toxin can damage blood vessels, resulting in leukostasis, thrombosis, decreased perfusion and tissue hypoxia. Theta toxin also stimulates cytokine release and can cause shock. Two *C. perfringens* toxins, enterotoxin and beta2 toxin, are not associated with specific strains. Enterotoxin is the major toxin responsible from human food poisoning. Beta2 toxin has been associated with enteritis in pigs.

Transmission

The epsilon toxin could probably be transmitted in contaminated food, water or by aerosol.

Inactivation/ Decontamination

Methods of decontamination for the epsilon toxin do not appear to have been published. Proteins can generally be denatured by heating.

Infections in Humans

Epsilon toxin

There seems to be little or no information about the effects of epsilon toxin on humans. Extrapolating from studies with experimentally infected animals, neurologic signs or pulmonary edema may be possible.

Clostridium perfringens infections in humans

C. perfringens types B and D, which produce the epsilon toxin, are rarely isolated from humans. In humans, disease is usually caused by type A strains, typically associated with uncomplicated food poisoning, and type C strains, associated with

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necrotizing enteritis. All five types can be isolated from wound infections. *Clostridium perfringens* can produce the following syndromes in humans.

Food Poisoning and Necrotizing Enteritis

The symptoms of *C. perfringens* food poisoning usually appear 8 to 22 hours after ingestion of contaminated food. The clinical signs can include diarrhea, nausea, severe abdominal cramps and bloating. Vomiting and fever are not usually seen. Patients generally recover in a day or two, but the symptoms may persist for 1 to 2 weeks in the elderly and those with other illnesses. Complications and death are rare. Vaccines are not available.

Type C strains cause necrotizing enteritis, also known as enteritis necroticans or pig–bel disease. This disease is very rare in the United States. It is characterized by infection and necrosis of the intestines, with septicemia. Sloughing of the mucosa and intestinal perforation are common and infections are often fatal. A toxoid vaccine is available.

Wound Infections

Wound contamination can result in gas gangrene, clostridial cellulitis or superficial contamination. Gas gangrene (clostridial myonecrosis) results from the growth of *C. perfringens* with toxin production in a wound; the toxins cause local tissue necrosis, with further spread of the bacteria and systemic toxemia. The initial symptoms are usually fever and pain in the affected area. As the necrosis becomes more extensive, the muscles become mottled purple and edematous, with a foul–smelling exudate and gas bubbles. Toxemia results in massive hemolysis, severe shock and renal failure. Gas gangrene can be fatal within a short period of time without prompt and sometimes radical treatment.

In cases of clostridial cellulitis or superficial contamination, the organisms do not spread to healthy tissues. The onset is more gradual, pain is minimal and systemic toxemia is not usually seen. With treatment, the prognosis for anaerobic cellulitis is good. In cases of superficial contamination, the wound usually heals normally, although in some cases there may be an exudate and the infection may interfere with wound healing. Vaccines are not available for clostridial wound infections, including gas gangrene.

Infections in Animals

Species Affected

Type B and D strains, which contain the epsilon toxin, normally cause disease in cattle, sheep, goats, pigs and horses. The purified toxin could presumably affect most species.

Incubation Period

The incubation period for epsilon toxin administered by the oral or respiratory routes has not been published. Intravenous injection produces neurological signs in half an hour to 3 hours in young goats and lambs; after injection, neurologic signs appear in 2 minutes to an hour in calves.

Clinical Signs

Epsilon toxin

Intravenous injection of epsilon toxin causes neurologic signs in young lambs, goat kids and calves. In calves, the symptoms have included recumbency, hyperesthesia, convulsions, paddling, opisthotonus, loss of consciousness and dyspnea. In rats, intraperitoneal injection results in cerebral edema.

Natural infections with type B strains

C. perfringens type B is associated with enterotoxemia in young calves, lambs and foals. The symptoms may include listlessness, recumbency and a fetid, blood–tinged diarrhea. Many lambs die without clinical signs. In calves, the clinical signs include diarrhea, dysentery, abdominal pain, opisthonos and convulsions. Affected calves can die very quickly, but some survive for a few days or recover.

Natural infections with type D strains

C. perfringens type D is associated with enterotoxemia in sheep, goats and, rarely, cattle or young horses. Typically, sudden death is seen in lambs in good condition. In some animals, excitement, incoordination, convulsions or opisthonos can be seen. Affected lambs may circle or push their heads against fixed objects. Diarrhea sometimes occurs. In adult sheep, the symptoms can include weakness, incoordination and convulsions, with death within 24 hours.

In goats, the course of disease can be peracute, acute or chronic. The clinical signs vary from watery diarrhea, sometimes containing blood, to sudden death. Neurologic signs are common. Death may occur after several weeks.

In mildly affected calves, stupor may be seen for a few days, sometimes followed by recovery. Severely affected calves develop convulsions, mania and blindness, with death typically occurring within a few hours.

Communicability

Specific information is not available for the epsilon toxin. Toxins are not generally transmitted from animal to animal or to people.

Diagnostic Tests

The epsilon toxin can be detected in the small intestinal fluid of animals with type B or D enterotoxemia. It can be identified in a mouse neutralization test (MNT) or with enzyme–linked immunosorbent assays (ELISAs). A latex agglutination test has been developed and a cytotoxicity

assay using MDCK cells has been published. Polymerase chain reaction (PCR) assays can identify the epsilon toxin gene, if it is present.

Treatment and Vaccination

Epsilon toxin

Treatment would most likely be supportive. Hyperimmune serum might be helpful if given soon after exposure. In mice, a variety of drugs, including reserpine, diazepam, apomorphine, gamma–butyrolactone, and phenothiazine and butyrophenone derivatives can prevent death or delay the effects of epsilon toxin, but only if they are given before exposure. A genetically modified toxin can protect mice against lethal challenge.

Natural infections with type B and D strains

In natural infections with type B strains, hyperimmune serum and antibiotics may be helpful. Toxoid vaccines can prevent type B and D enterotoxemia.

Morbidity and Mortality

Epsilon toxin

In mice, the LD_{50} is 0.78 nanograms, given intravenously. In one experiment, neurologic signs were seen in all six goat kids given intravenous injections of 250, 185 or 120 mouse lethal doses 50% (MLD50)/kg body weight, and in two of three kids given 60 MLD50/kg. In this experiment, all five lambs injected with 250 or 120 MLD50/kg also developed neurologic signs. No symptoms were seen in six kids and three lambs that received 15, 30 or 45 MLD50/kg. One lamb given 60 MLD50/kg also remained normal.

Natural infections with type B and D strains

C. perfringens type B infections are usually seen in young calves, lambs and foals. Lamb dysentery typically occurs in animals less than 3 weeks old. Type D enterotoxemia is most common in young lambs, particularly single nursing lambs, animals in feedlots and animals on lush, green pastures. It tends to occur after excessive ingestion of feed, milk or grain; any factor that slows the transit of food through the intestines and encourages the production of toxin can predispose an animal to enterotoxemia. The case fatality rate for both type B and type D infections is high.

Post–Mortem Lesions

Epsilon toxin

Intravenous injection of epsilon toxin results in severe pulmonary edema in young goats and calves and sometimes in lambs. In calves, perivascular proteinaceous edema has sometimes been found in the internal capsule, thalamus and cerebellar white matter. In one study, the brains of lambs but not goats contained perivascular proteinaceous edema and hemorrhages. In rats, intraperitoneal injection of epsilon toxin results in cerebral edema.

Natural infections with type B strains

The major lesion is hemorrhagic enteritis with mucosal ulceration. The affected region of the intestine is a deep bluish purple and resembles an infarction.

Natural infections with type D strains

The rumen and abomasum typically contain a large amount of food. Undigested feed may also be seen in the ileum. Young lambs may have only a few hyperemic areas on the intestine and fluid in the pericardial sac. In older animals, there may be hemorrhages on the myocardium, or petechiae and ecchymoses on the intestinal serosa and abdominal muscles. Pulmonary edema and congestion are common in older animals. Hemorrhagic or necrotic enteritis is sometimes seen in goats. In some animals, the kidneys are rapidly autolyzed after death; this sign is not consistently present.

Internet Resources

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov

Clostridium perfringens Genotyping. Clostridial Enteric Disease Unit, Department of Veterinary Science and Microbiology, University of Arizona
http://microvet.arizona.edu/research/ClostridiumWeb/genotyping.htm

Clostridium perfringens Toxins. Northstar Preparedness Network
http://www.preparednessnetwork.org/northstar/warfare/cpt.html

Material Safety Data Sheets–Canadian Laboratory Center for Disease Control
http://www.hc–sc.gc.ca/pphb–dgspsp/msds–ftss/index.html#menu

Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627

NATO Handbook on the Medical Aspects of NBC Defensive Operations. Part II – Biological Annex B. Clinical Data Sheets for Selected Biological Agents
http://www.fas.org/nuke/guide/usa/doctrine/dod/fm8–9/2appb.htm#b06

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

USAMRIID's Medical Management of Biological Casualties Handbook
http://www.vnh.org/BIOCASU/toc.html

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