Bacillus anthracis

Centers for Disease Control and Prevention

October 31, 2001
Anthrax: Background
Anthrax: Basics

- Caused by the spore-forming bacterium, *Bacillus anthracis*
- Zoonotic disease in herbivores (e.g., sheep, goats, cattle) follows ingestion of spores in soil
- Human infection typically acquired through contact with anthrax-infected animals or animal products or atypically through intentional exposure
- Three clinical forms
  - Cutaneous
  - Inhalational
  - Gastrointestinal
Epidemiology of Anthrax in the 21st Century

• Agricultural, farm workers exposed to infected animals (rare)
• Non-industrial: laboratorians through close contact with *B. anthracis* spores or civilians exposed to contaminated imported animal products (rare)
• Industrial: processors of wool, hair, hides, bones, or other animal products (now rare)
• Intentional/bioterrorist: inhalational and cutaneous exposure to *B. anthracis* spores through U.S. mail
Cases of Anthrax in the U.S., 1951–2000*
(N = 409)

Animal (Stern's) vaccination started in 1957, after OK enzootic. Recommended for use in animals in endemic areas thereafter.

*Only 18 of these cases were inhalational; the remainder were cutaneous.
*One cultured case (cutaneous) reported in 2000 from North Dakota.
Anthrax: Current Issues in the U.S.

- Anthrax remains an endemic public health threat through annual epizootics.

- *B. anthracis* is one of the most important pathogens on the list of bioterrorism threats
  - Aerosolized stable spore form
  - Human LD50 8,000 to 40,000 spores, or one deep breath at site of release
Anthrax Bioterrorism Issues (1)

- Surveillance for cutaneous and inhalational disease to identify attack
- Targeting prevention strategies
  - Rapidly identify exposed populations
  - Conduct epidemiologic investigation with environmental testing
  - Supply postexposure prophylaxis
  - Trace route of vehicle of exposure
Anthrax Bioterrorism Issues (2)

- Environmental assessment to determine exposures
- Decontamination
- Defining population at risk for pre-exposure immunization
Threat Assessment of Anthrax

• FBI and other law enforcement authorities are investigating intentional exposures as criminal acts.
• Until source of exposures is eliminated, exposure to *B. anthracis* and subsequent clinical illness may continue.
• Clinicians and laboratorians should be vigilant for *B. anthracis* infection, particularly among mail handlers.
• CDC will provide updated information at [www.bt.cdc.gov](http://www.bt.cdc.gov)
Threat Assessment

• Clinical laboratorians should be alert to *Bacillus* species, particularly in specimens from previously healthy patients with rapidly progressive respiratory illness or cutaneous ulcer.

• If *B. anthracis* is suspected, laboratories should immediately notify the healthcare provider and local and state public health staff.

• For rapid identification of *B. anthracis*, state and local health departments should access the Laboratory Response Network for Bioterrorism (LRN).
Exposure Situation Management: *B. anthracis* in Envelope

- Antimicrobial prophylaxis for those potentially exposed
- Environmental samples
  - Surface swabs
  - Nasal swabs of potentially exposed persons (if ≤7 days)
- Refine list of potentially exposed persons
  - Not exposed: stop treatment
  - Likely exposed: continue treatment for 60 days total
Anthrax: Case Definition

Confirmed Case:
  • Clinically compatible illness confirmed by isolation of *B. anthracis* or other laboratory evidence based on at least two supportive laboratory tests

Suspected Case:
  • Clinically compatible illness with one supportive lab test or linked to a confirmed environmental exposure
Anthrax: Exposure Classification

Exposure, laboratory-confirmed:
• Epidemiologically linked to a plausible environmental exposure, with laboratory evidence of *B. anthracis* from a nasal swab or other clinical sample

Exposure, not laboratory-confirmed:
• Epidemiologically linked to a plausible environmental exposure, without laboratory evidence of *B. anthracis*
Anthrax: Clinical Information

Cutaneous
Inhalational
Gastrointestinal
Anthrax: Cutaneous

- Begins as a papule, progresses through a vesicular stage to a depressed black necrotic ulcer (eschar)
- Edema, redness, and/or necrosis without ulceration may occur
- Form most commonly encountered in naturally occurring cases
- Incubation period: 1–12 days
- Case-fatality:
  - Without antibiotic treatment—20%
  - With antibiotic treatment—1%
Anthrax: Inhalational (1)

- A brief prodrome resembling a “viral-like” illness, characterized by myalgia, fatigue, fever, with or without respiratory symptoms, followed by hypoxia and dyspnea, often with radiographic evidence of mediastinal widening.
- Meningitis in 50% of patients
- Rhinorrhea (rare)
Anthrax: Inhalational (2)

- Extremely rare in United States (20 reported cases in last century)
- Incubation period: 1–7 days (possibly ranging up to 42 days)
- Case fatality:
  - Without antibiotic treatment—97%
  - With antibiotic treatment—75%
Anthrax: Gastrointestinal

- Abdominal distress, usually accompanied by bloody vomiting or diarrhea, followed by fever and signs of septicemia
- Gastrointestinal illness sometimes seen as oropharyngeal ulcerations with cervical adenopathy and fever
- Develops after ingestion of contaminated, poorly cooked meat.
- Incubation period: 1–7 days
- Case-fatality: 25–60% (role of early antibiotic treatment is undefined)
Anthrax: Cutaneous

- **Vesicle development**
  - Day 2

- **Eschar formation**
  - Day 4

- **Day 6**

- **Day 10**
Anthrax: Cutaneous

Left, **Forearm lesion on day 7**—vesiculation and ulceration of initial macular or papular anthrax skin lesion. Right, **Eschar of the neck on day 15** of illness, typical of the last stage of the lesion. From Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases*. Vol 1. Washington, DC: AFIP; 1976:119. AFIP negative 71-1290–2.
Anthrax: Cutaneous

NEJM 1999; 341: 815–826
Anthrax: Cutaneous

Healing after treatment
Anthrax: Cutaneous

Ulcer and vesicle ring

Black eschar. Redness remains
Anthrax: Cutaneous

Notice the edema and typical lesions
Anthrax: Inhalational

- Mediastinal widening

JAMA 1999;281:1735–1745
Mediastinal Widening and Pleural Effusion on Chest X-Ray in Inhalational Anthrax
Differential Diagnosis of Cutaneous Anthrax

- Spider bite
- Ecthyma gangrenosum
- Ulceroglandular tularemia
- Plague
- Staphylococcal or streptococcal cellulitis
- Herpes simplex virus
Differential Diagnosis of Inhalational Anthrax

- Mycoplasmal pneumonia
- Legionnaires’ disease
- Psittacosis
- Tularemia
- Q fever
- Viral pneumonia
- Histoplasmosis (fibrous mediastinitis)
- Coccidioidomycosis
- Malignancy
Differential Diagnosis of Gastrointestinal Anthrax

• Acute appendicitis
• Ruptured viscus
• Diverticulitis
• Diseases that cause acute cervical lymphadenitis or acute gastritis
• Dysentery
Anthrax: Diagnosis

Cutaneous

- Gram stain, polymerase chain reaction (PCR), or culture of vesicular fluid, exudate, or eschar
- Blood culture if systemic symptoms present
- Biopsy for immunohistochemistry, especially if person taking antimicrobials
Anthrax: Diagnosis

Inhalational

- Chest X-ray—widened mediastinum, pleural effusions, infiltrates, pulmonary congestion
- Affected tissue biopsy for immunohistochemistry
- Any available sterile site fluid for Gram stain, PCR, or culture
- Pleural fluid cell block for immunohistochemistry
Anthrax: Diagnosis

Gastrointestinal

- Blood cultures
- Oropharyngeal (OP) swab collection
Laboratory Criteria for Identification of *B. anthracis* (1)

- From clinical samples, such as blood, cerebrospinal fluid (CSF), skin lesion (eschar), or oropharyngeal ulcer
  - Encapsulated gram-positive rods on Gram stain
- From growth on sheep blood agar:
  - Large gram-positive rods
  - Nonmotile
  - Nonhemolytic
Laboratory Criteria for Identification of *B. anthracis* (2)

- Rapid screening assay (PCR- and antigen-detection based) for use on cultures and directly on clinical specimens
- Confirmatory criteria for identification of *B. anthracis*
  - Capsule production
  - Lysis by gamma-phage
  - Direct fluorescent antibody assay (DFA)
### Recommended Postexposure Prophylaxis to Prevent Inhalational Anthrax

<table>
<thead>
<tr>
<th></th>
<th>Initial Therapy</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(/including pregnant women and immunocompromised)</td>
<td>Ciprofloxacin 500 mg PO BID</td>
<td>60 days</td>
</tr>
<tr>
<td></td>
<td>OR Doxycycline 100 mg PO BID</td>
<td></td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin* 10–15 mg/kg PO Q 12 hrs</td>
<td>60 days</td>
</tr>
<tr>
<td></td>
<td>OR Doxycycline: if susceptible &gt;8 yrs and &gt;45 kg: 100 mg PO BID</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;8 yrs and ≤45 kg: 2.2 mg/kg PO BID</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤8 yrs: 2.2 mg/kg PO BID</td>
<td></td>
</tr>
</tbody>
</table>

*Ciprofloxacin not to exceed 1 gram daily in children

Patient information sheets at [www.bt.cdc.gov](http://www.bt.cdc.gov)
**Cutaneous Anthrax Treatment Protocol** for Cases Associated with Bioterrorist Events

<table>
<thead>
<tr>
<th>Category</th>
<th>Initial Therapy (Oral)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Ciprofloxacin</td>
<td>60 days*</td>
</tr>
<tr>
<td>(Including pregnant women and immunocompromised)</td>
<td>500 mg BID OR Doxycycline 100 mg BID</td>
<td></td>
</tr>
<tr>
<td>Children (including immunocompromised)</td>
<td>Ciprofloxacin** 10–15 mg/kg Q 12 hrs OR Doxycycline: &gt;8 yrs and &gt;45 kg: 100 mg BID &gt;8 yrs and ≤45 kg: 2.2 mg/kg BID ≤8 yrs: 2.2 mg/kg BID</td>
<td>60 days*</td>
</tr>
</tbody>
</table>

**Ciprofloxacin not to exceed 1 gram daily in children.**  
**60-day duration is to prevent inhalational anthrax.**  
Patient information sheets at www.bt.cdc.gov

*Source MMWR 2001;50:909–19
### Inhalational Anthrax Treatment Protocol* for Cases Associated with Bioterrorist Events (1)

<table>
<thead>
<tr>
<th>Category</th>
<th>Initial therapy (intravenous)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Ciprofloxacin</td>
<td>Switch to oral therapy when clinically appropriate:</td>
</tr>
<tr>
<td>(Including pregnant women** and</td>
<td>400 mg Q 12 hrs OR</td>
<td>Ciprofloxacin 500 mg BID OR</td>
</tr>
<tr>
<td>immunocompromised)</td>
<td>Doxycycline 100 mg Q 12 hrs AND One or two additional</td>
<td>Doxycycline 100 mg BID</td>
</tr>
<tr>
<td></td>
<td>antimicrobials</td>
<td>Continue for 60 days (IV and PO combined)</td>
</tr>
</tbody>
</table>

**High death rate from infection outweighs risk of antimicrobials

Patient information sheets at www.bt.cdc.gov

*Source  MMWR 2001;50:909–19
**Inhalational Anthrax Treatment Protocol** for Cases Associated with Bioterrorist Events (2)

<table>
<thead>
<tr>
<th>Category</th>
<th>Initial therapy (intravenous)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (including immuno-</td>
<td>Ciprofloxacin 10–15 mg/kg Q 12 hrs</td>
<td>Switch to oral therapy when clinically appropriate:</td>
</tr>
<tr>
<td>compromised)</td>
<td>OR</td>
<td>Ciprofloxacin 10–15 mg/kg Q 12 hrs</td>
</tr>
<tr>
<td></td>
<td>Doxycycline &gt;8 yrs and &gt;45 kg: 100 mg Q 12 hrs</td>
<td>OR</td>
</tr>
<tr>
<td></td>
<td>Doxycycline &gt;8 yrs and &lt;45 kg: 2.2 mg/kg Q 12 hrs</td>
<td>Doxycycline &gt;8 yrs and &gt;45 kg: 100 mg BID</td>
</tr>
<tr>
<td></td>
<td>&lt;8 yrs: 2.2 mg/kg Q 12 hrs AND One or two additional antimicrobials</td>
<td>&gt;8 yrs and &lt;45 kg: 2.2 mg/kg BID &lt;=8 yrs: 2.2 mg/kg BID</td>
</tr>
</tbody>
</table>

**Ciprofloxacin not to exceed 1 gram daily**  
**Continue for 60 days (IV and po combined)**

Patient information sheets at [www.bt.cdc.gov](http://www.bt.cdc.gov)

*Source MMWR 2001;50:909–19*
Immune Protection Against Anthrax

- Live cellular vaccines
  - "Sterne" type live spore (toxigenic, noncapsulating)
  - Former USSR STI live spore (toxigenic, non-capsulating)
  - "Pasteur" type (mixed culture, reduced virulence)
- Sterile, acellular vaccines
  - US "anthrax vaccine adsorbed" (AVA)—not licensed for use in civilian populations
  - UK "anthrax vaccine precipitated" (AVP)
- Recombinant PA research vaccines
  - Al³⁺; Freund’s; Saponin, Monophosphoryl lipid A; Ribi
Anthrax: Laboratory Information
Specimen Collection and Handling

- Primary isolation and Gram stain can be conducted at the hospital or clinical level.
- Most clinical samples and suspect isolates will be handled via the Laboratory Response Network for Bioterrorism (LRN) and state public health laboratories (www.bt.cdc.gov).
- Triage of specimens at CDC by the Rapid Response and Advanced Technology (RRAT) Laboratory.
Laboratory Response Network (LRN)

• LRN links state and local public health laboratories with advanced capacity laboratories — including clinical, military, veterinary, agricultural, water, and food-testing laboratories.

• Laboratorians should contact their state public health laboratory to identify their local LRN representative.
LRN Criteria for Identification of *B. anthracis* (1)

**LRN level A:** Rule-out and presumptive identification criteria

a. From clinical samples, such as blood, CSF, skin lesion (eschar), or oropharyngeal ulcer: encapsulated gram-positive rods

b. From growth on sheep blood agar: Large gram-positive rods

c. Nonmotile

d. Nonhemolytic on sheep blood agar
LRN Criteria for Identification of *B. anthracis* (2)

Many LRN laboratories use rapid screening assays (PCR for nucleic acid amplification and TRF immunoassay for antigen detection) on cultures and directly on clinical specimens.

**LRN confirmatory criteria for identification of *B. anthracis*** is

- Capsule production and visualization and lysis by gamma-phage or
- Direct fluorescent antibody assays (DFA) for capsule antigen and cell wall-associated polysaccharide
Presumptive Identification of *B. anthracis* (1)

Direct smears from clinical specimens

- Encapsulated broad rods in short chains, 2–4 cells. India ink will demonstrate capsule (Gram stain will not)
- *B. anthracis* not usually present in clinical specimens until late in course of disease
Presumptive Identification of *B. anthracis* (2)

Smears from sheep blood agar or other routine nutrient medium

- Non-encapsulated broad rods in long chains
- Encapsulated bacilli grow only in nutrient agar supplemented with 0.8% sodium bicarbonate in presence of 5% CO$_2$ (Note: this procedure is performed in Level B/LRN laboratories)
B. anthracis: Presumptive Identification

Clinical specimen (blood, CSF, etc.)
- Gram stain
- Capsule production

Isolate on SBA
- Colony morphology
- Hemolysis
- Motility
- Spores
- Gram stain
- Malachite green
B. anthracis:
Confirmatory Identification

Isolate

Phage lysis

Capsule

Horse blood (M’Fadyean Stain)

Bicarbonate media (M’Fadyean stain India ink stain)

DFA

Capsule antigen Cell wall

DFA
Gram Stain Morphology of *B. anthracis*

- Broad, gram-positive rod: 1–1.5 x 3–5 μ
- Oval, central to subterminal spores: 1 x 1.5 μ with no significant swelling of cell
- Spores usually NOT present in clinical specimens unless exposed to atmospheric O₂
B. anthracis

- Gram-positive, spore-forming, non-motile bacillus
Colony Characteristics of *B. anthracis* (1)

- After incubation on a blood agar plate for 12–24 hours at 35–37°C, well-isolated colonies are 2–5 mm in diameter; heavily inoculated areas may show growth in 6–8 hours.
- Gray-white, flat or slightly convex colonies are irregularly round, with edges that slightly undulate, and have “ground glass” appearance.
- Often have comma-shaped protrusions from colony edge (“Medusa head” colonies).
Colony Characteristics of *B. anthracis* (2)

- Tenacious consistency (when teased with a loop, the growth will stand up like beaten egg white)
- Nonhemolytic (weak hemolysis may be observed under areas of confluent growth in aging cultures and should NOT be confused with real $\beta$-hemolysis)
- Will not grow on MacConkey agar
- Nonmotile
Presumptive Identification Key for *B. anthracis*

- Nonhemolytic
- Nonmotile
- Encapsulated (requires India ink to visualize capsule)
- Gram-positive, spore-forming rod
Packaging and Transporting Protocol (1)

• Specimen packaging and labeling same as for any infectious substance
• If specimen is a dry powder or paper material, place in plastic self-sealing bag (e.g., Ziploc®) with biohazard label, and follow steps 1–4 (next slides)
• If specimen is a clinical specimen, place biohazard label on specimen receptacle, wrap receptacle with absorbent material, and follow steps 1–4 (next slides)
Packaging and Transporting Protocol (2)

1. Place the bag or specimen receptacle into a leak-proof container (with tight cover) labeled “biohazard.”

2. Place container into a second leak-proof container (with tight cover) also labeled “biohazard” and no larger than a one-gallon paint can.
   a) For a clinical specimen, place an ice pack (not ice) in the second container to keep specimen cold.
   b) For a nonclinical specimen (e.g., paper or powder) omit ice pack.
Packaging and Transporting Protocol (3)

3. Place the second container into a third leak-proof container (with tight cover) labeled “biohazard” and no larger than a five-gallon paint can.

4. Both the second and third containers should meet state and federal regulations for transport of hazardous material and be properly labeled.
Packing and Labeling Infectious Substances

Cross Section of Proper Packing

- Waterproof Tape
- Culture
- Specimen ID Label
- Biohazard Label
- Absorbent Packing Material

Primary Receptacle
Absorbent Packing Material
Culture
Biohazard Label

Secondary Packaging

Name, Address, & Telephone Number of Shipper

Outer Packaging

Infectious Substance Label
Transporting Specimens to the DOH Public Health Lab

- Coordinate with DOH Public Health Lab and LRN
- Local FBI personnel may transport specimens if bioterrorism is suspected
- When specimens are shipped by commercial carrier, ship according to state and federal shipping regulations
- Contact shipping company, public health laboratory and local FBI
Disinfection and Disposal (1)

Effective sporicidal solutions
• Commercially-available bleach, 0.5% hypochlorite (1 part household bleach to 9 parts water)
• Rinse off concentrated bleach to avoid caustic effects
• Approved sporicidal agents
Disinfection and Disposal (2)

Surfaces and non-sterilizable equipment
• Wipe work surfaces before and after use with a sporicidal solution
• Routinely clean non-sterilizable equipment with a sporicidal solution

Contaminated instruments (pipettes, needles, loops, micro slides)
• Soak in a sporicidal solution before autoclaving
Disinfection and Disposal (3)

Accidental spills of material known or suspected to be contaminated with *B. anthracis*

- Contamination involving fresh clinical samples:
  - Flood with sporicidal solution, soak for 5 minutes, then clean.

- Contamination involving lab samples (e.g., culture plates or blood cultures) or spills in areas below room temperature:
  - Gently cover spill, then liberally apply sporicidal solution.
  - Soak for 30 minutes, then clean.
  - Autoclave or incinerate any soiled cleaning materials.

Incinerate or steam sterilize cultures, infected material, and suspect material.
Summary

- Public health preparedness is needed.
- Early detection and response is critical.
- Communications networks (e.g., HAN, Epi-X, LRN) are key to success.