# *Bacillus anthracis*

**Centers for Disease Control and Prevention**

October 31, 2001

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# Anthrax: Background

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# 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

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# 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

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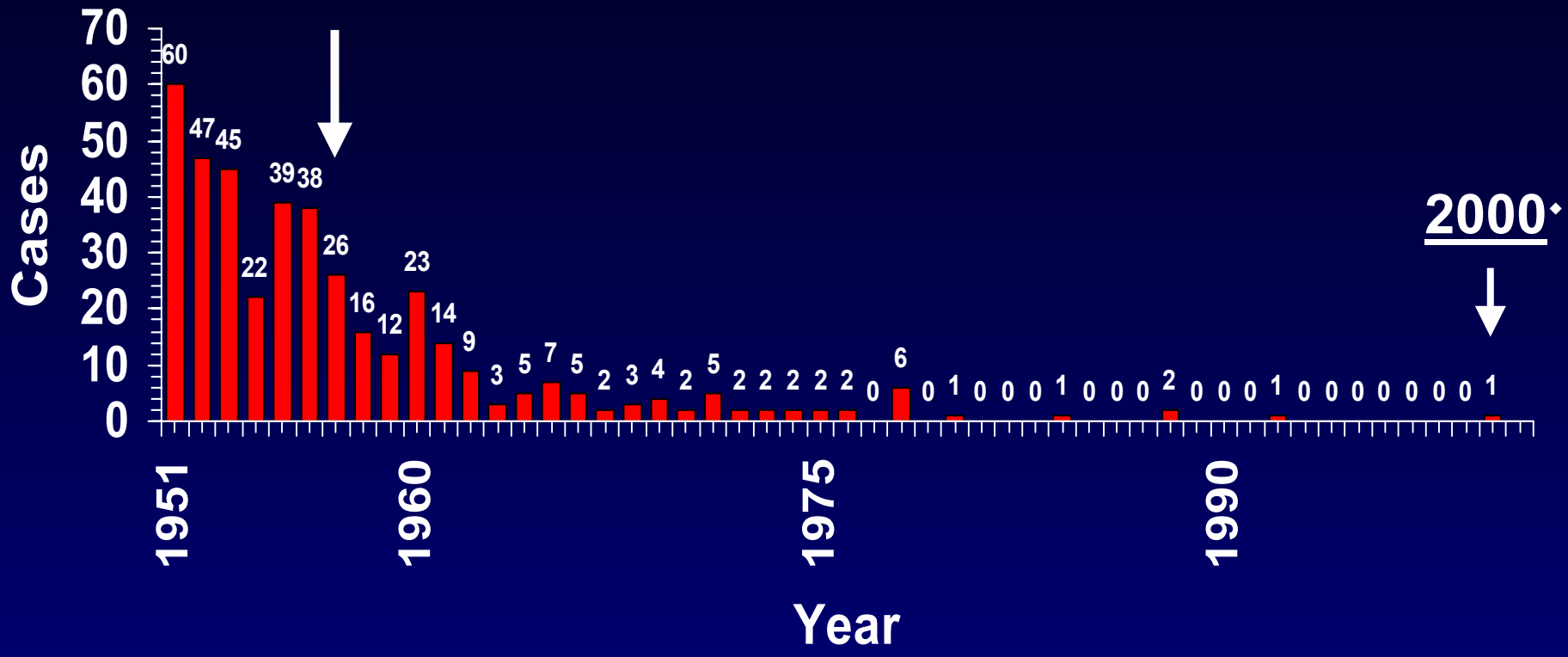
# Cases of Anthrax in the U.S., 1951–2000*
## (N = 409)

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.

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# 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

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# 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

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# Anthrax Bioterrorism Issues (2)

- Environmental assessment to determine exposures
- Decontamination
- Defining population at risk for pre-exposure immunization

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# 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

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# 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).

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# 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

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# 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

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# 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

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# 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)

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# 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%

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# 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)

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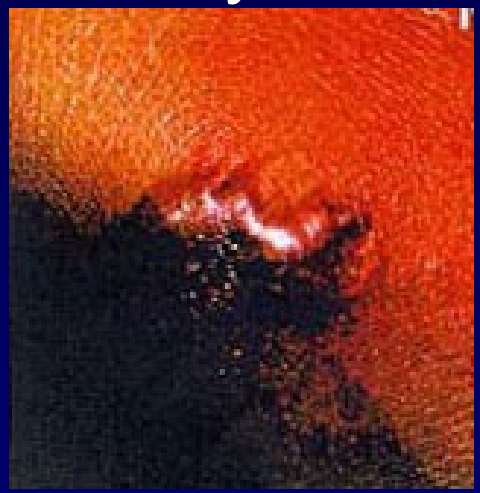
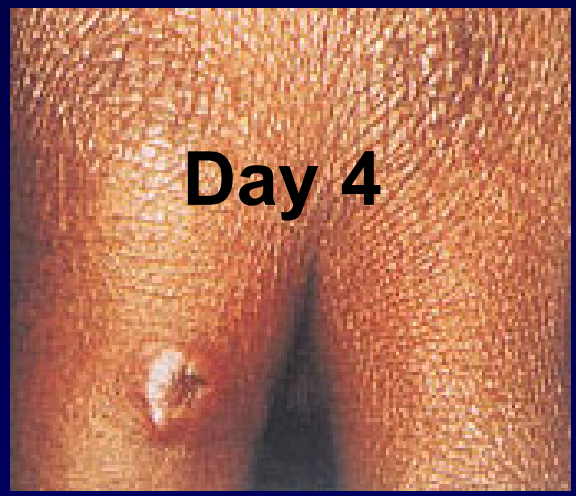
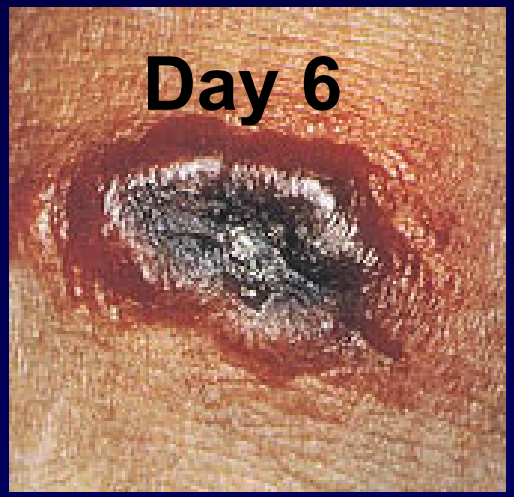
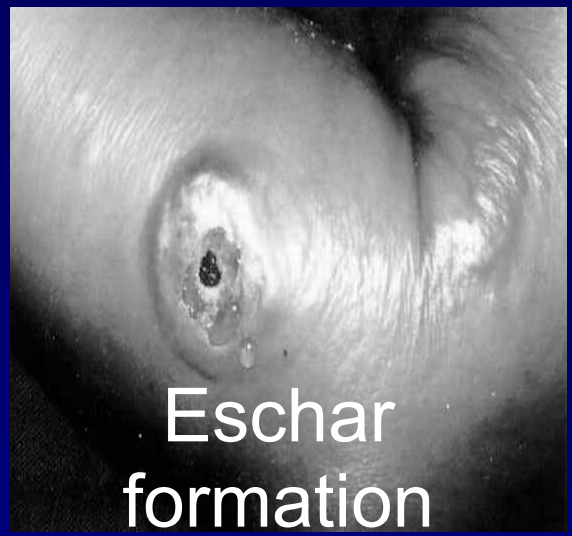
# Anthrax: Cutaneous

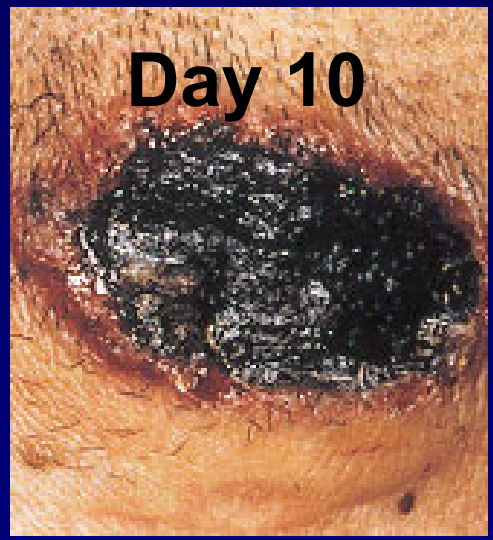
Vesicle development Day 2



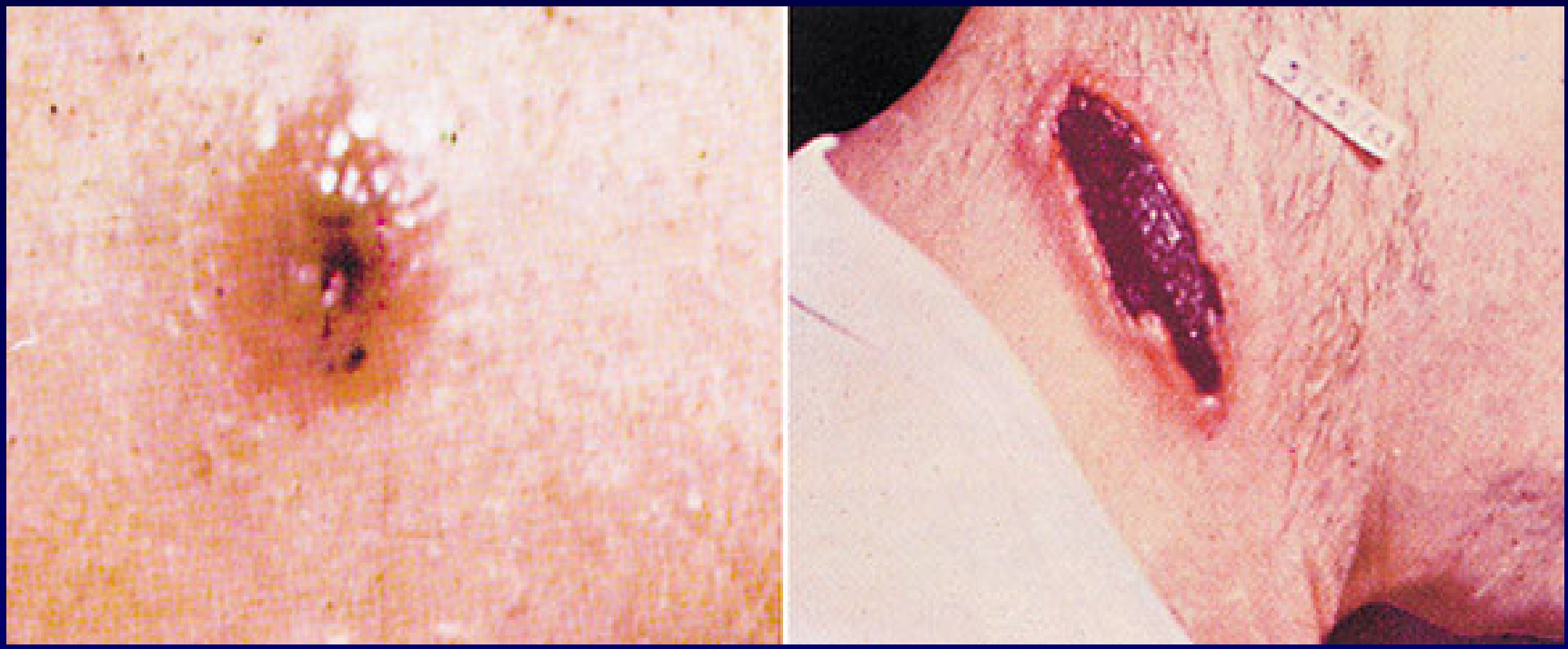


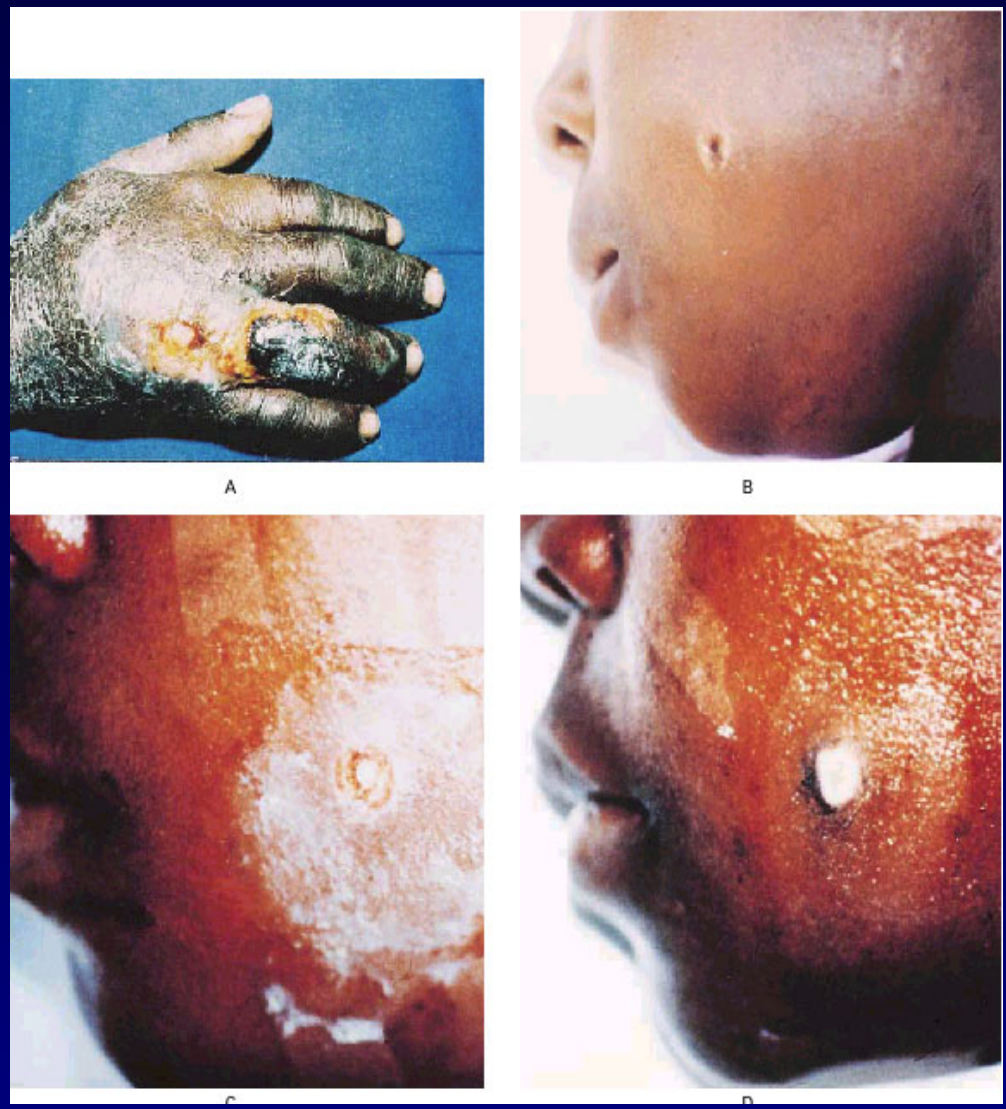



# Anthrax: Cutaneous



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*

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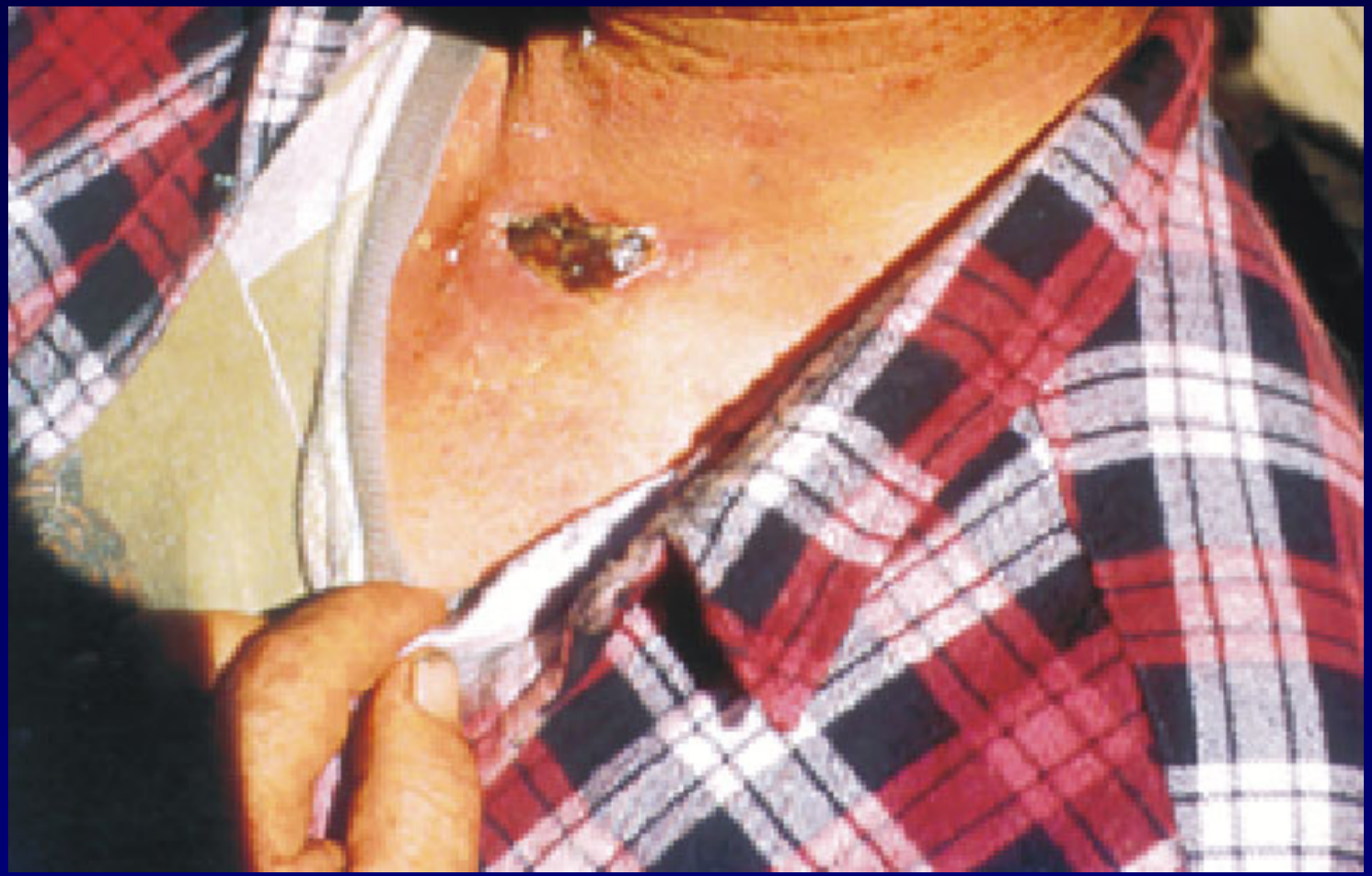
# Anthrax: Cutaneous



Healing after treatment

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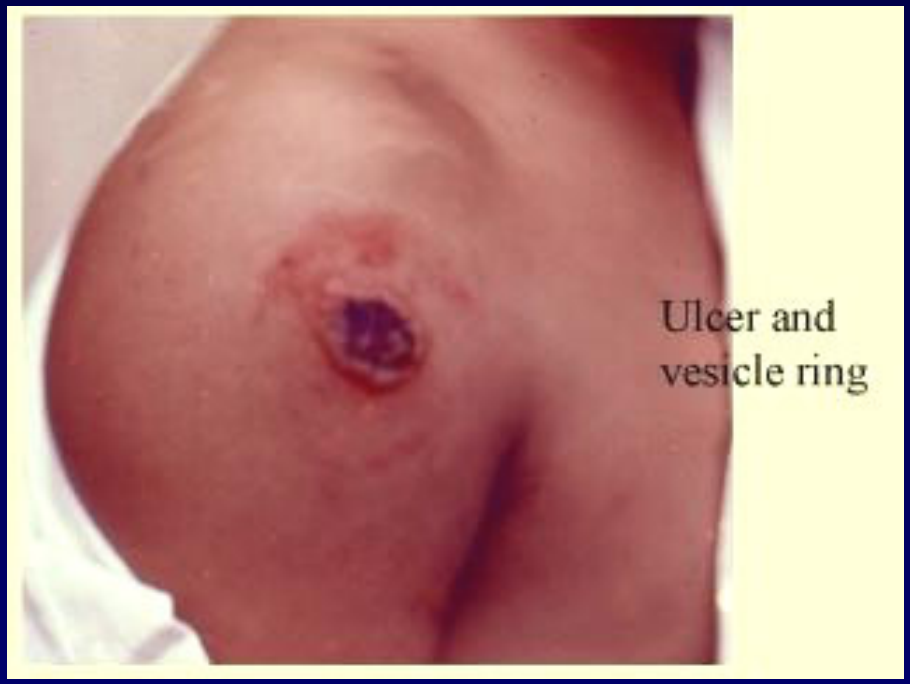
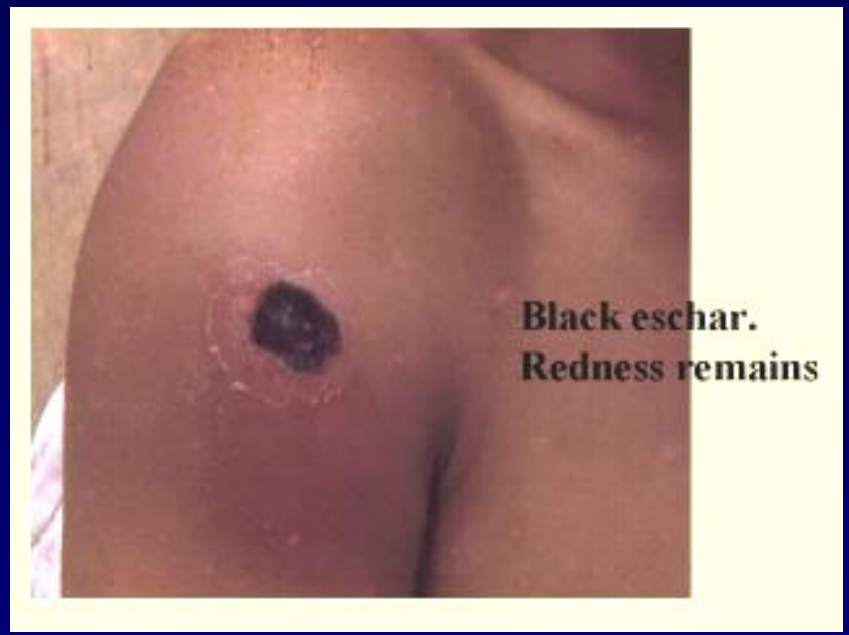
# Anthrax: Cutaneous

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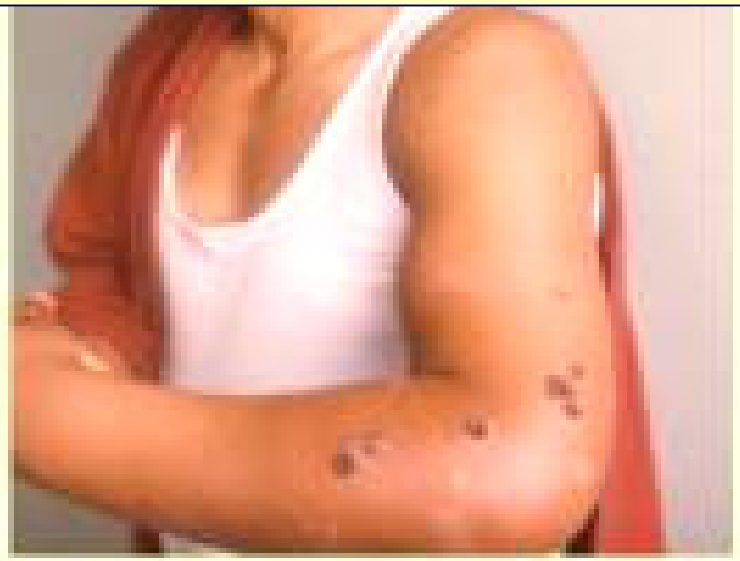
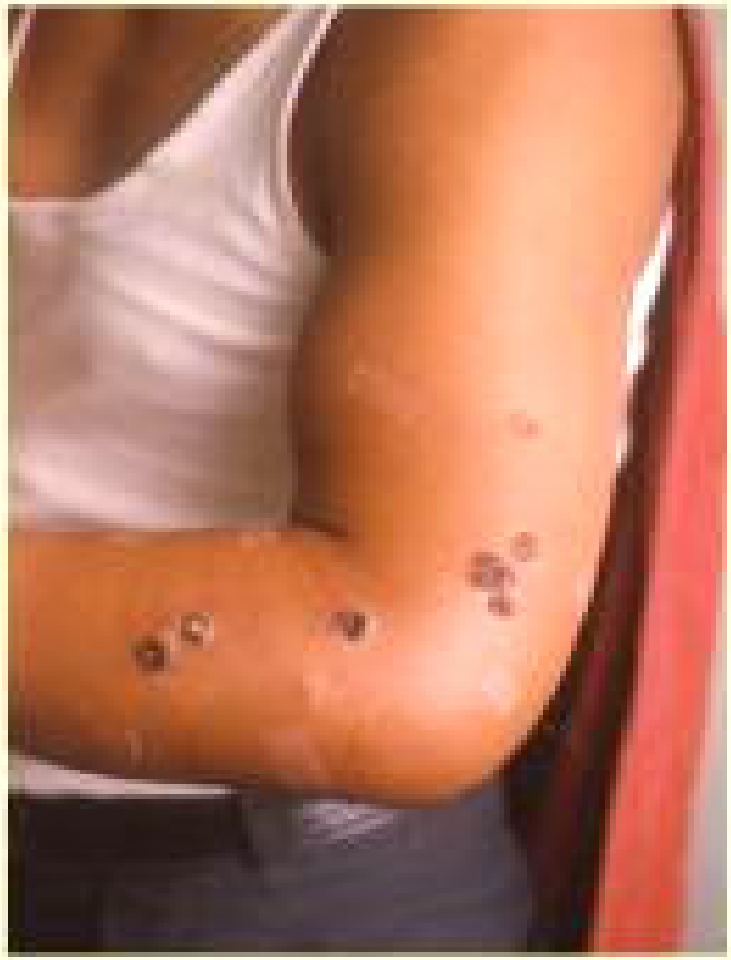
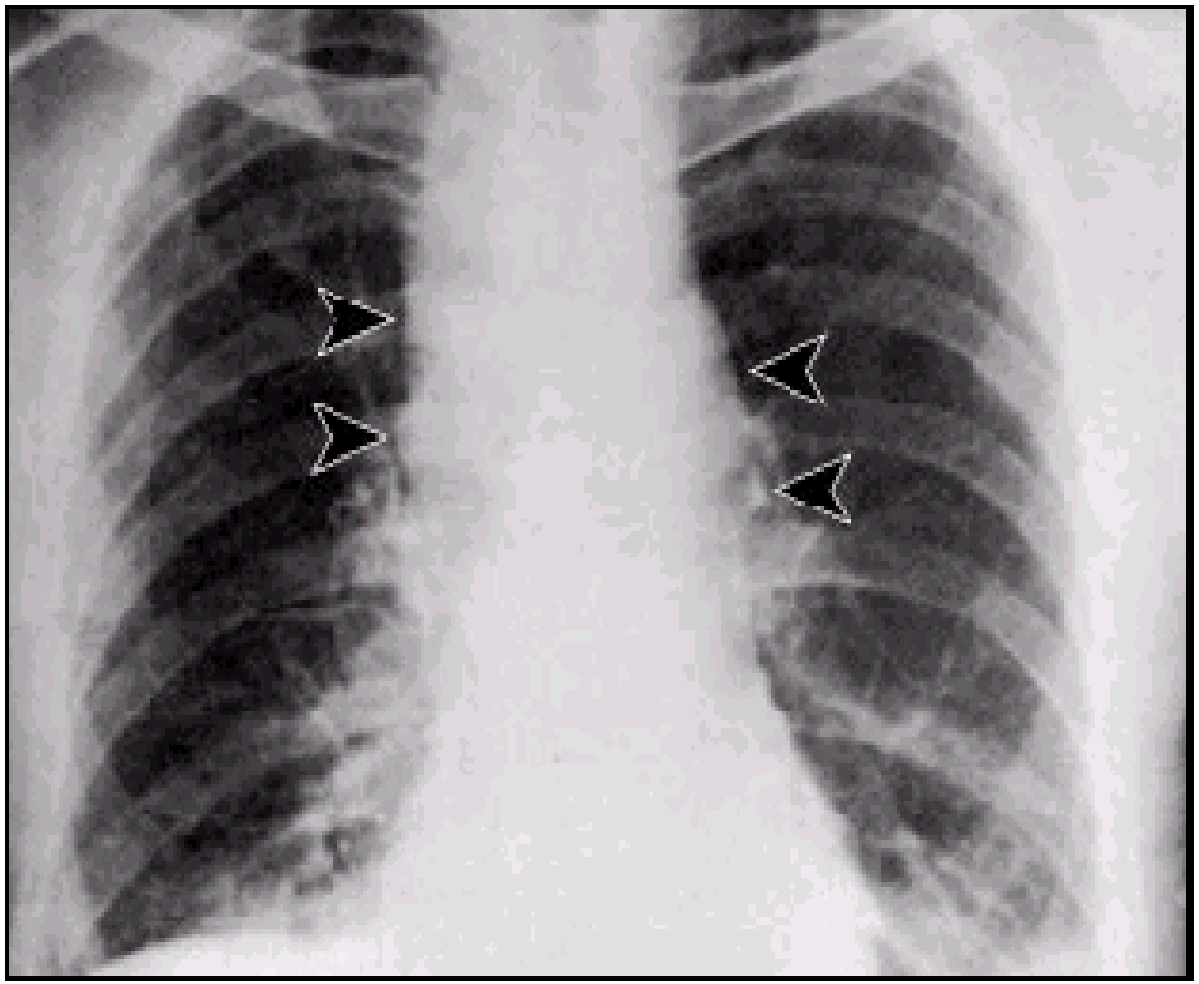
# Anthrax: Cutaneous

Notice the edema and typical lesions





# Anthrax: Inhalational



- Mediastinal widening

*JAMA 1999;281:1735–1745*

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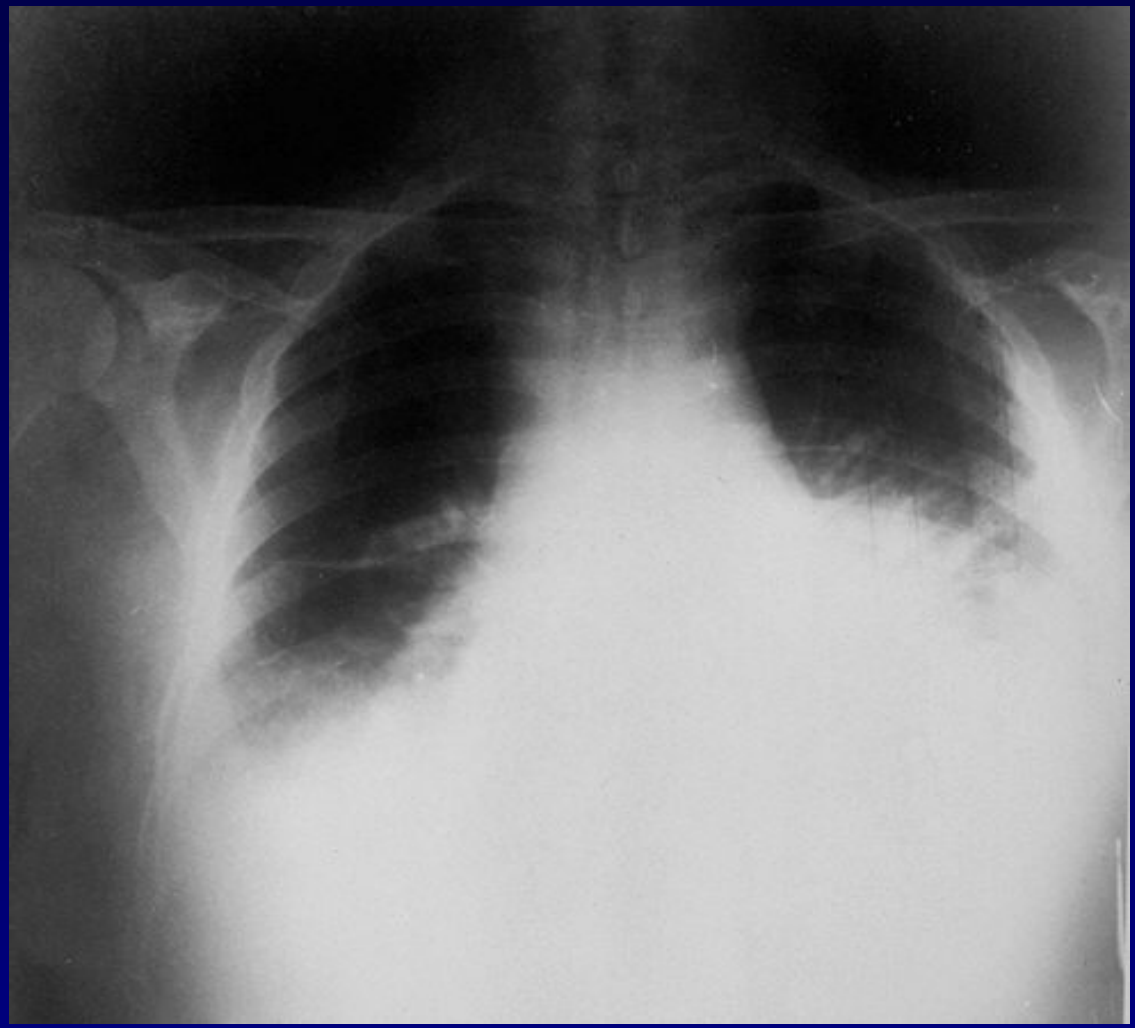
# Mediastinal Widening and Pleural Effusion on Chest X-Ray in Inhalational Anthrax



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# 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

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# 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

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# 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

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# 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)

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# Recommended Postexposure Prophylaxis to Prevent Inhalational Anthrax

| | Initial Therapy | Duration |
|---|---|---|
| Adults (including pregnant women and immunocompromised) | Ciprofloxacin 500 mg PO BID OR Doxycycline 100 mg PO BID | 60 days |
| Children | Ciprofloxacin* 10–15 mg/kg PO Q 12 hrs OR Doxycycline: >8 yrs and >45 kg: 100 mg PO BID >8 yrs and ≤45 kg: 2.2 mg/kg PO BID ≤8 yrs: 2.2 mg/kg PO BID | 60 days Change to amoxicillin if susceptible |

*Ciprofloxacin not to exceed 1 gram daily in children

Patient information sheets at www.bt.cdc.gov

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# Cutaneous Anthrax Treatment Protocol* for Cases Associated with Bioterrorist Events

| Category | Initial Therapy (Oral) | Duration |
|---|---|---|
| Adults (Including pregnant women and immunocompromised) | Ciprofloxacin 500 mg BID OR Doxycycline 100 mg BID | 60 days◆ |
| Children (including immuno-compromised) | Ciprofloxacin** 10–15 mg/kg Q 12 hrs OR Doxycycline: >8 yrs and >45 kg: 100 mg BID >8 yrs and ≤45 kg: 2.2 mg/kg BID ≤8 yrs: 2.2 mg/kg BID | 60 days◆ |

**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

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# Inhalational Anthrax Treatment Protocol* for Cases Associated with Bioterrorist Events (1)

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

**High death rate from infection outweighs risk of antimicrobials

Patient information sheets at www.bt.cdc.gov

*Source MMWR 2001;50:909–19

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# Inhalational Anthrax Treatment Protocol* for Cases Associated with Bioterrorist Events (2)

| Category | Initial therapy (intravenous) | Duration |
|---|---|---|
| Children (including immuno-compromised | Ciprofloxacin 10–15 mg/kg Q 12 hrs OR Doxycycline >8 yrs and >45 kg: 100 mg Q 12 hrs >8 yrs and ≤45 kg: 2.2 mg/kg Q 12 hrs ≤8 yrs: 2.2 mg/kg Q 12 hrs AND One or two additional antimicrobials | Switch to oral therapy when clinically appropriate: Ciprofloxacin 10–15 mg/kg Q 12 hrs OR Doxycycline >8 yrs and >45 kg: 100 mg BID >8 yrs and ≤45 kg: 2.2 mg/kg BID ≤8 yrs: 2.2 mg/kg BID |

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

Patient information sheets at www.bt.cdc.gov

*Source MMWR 2001;50:909–19

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# 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
  - $Al^{3+}$; Freund's; Saponin, Monophosphoryl lipid A; Ribi

# Anthrax:
# Laboratory Information

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# 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

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# 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.

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# 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

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# Presumptive Identification of *B. anthracis* (1)

**Direct smears from clinical specimens**

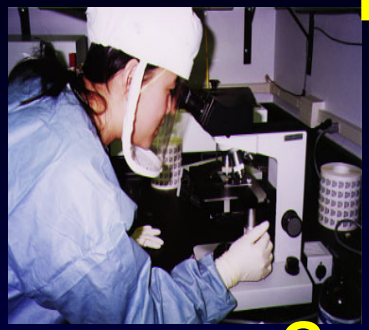
- Encapsulated broad rods in short chains, 2–4 cells. India ink will demonstrate capsule (Gram stain will not)
- *B. anthracis* not usually present in clinical specimens until late in course of disease

# 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)

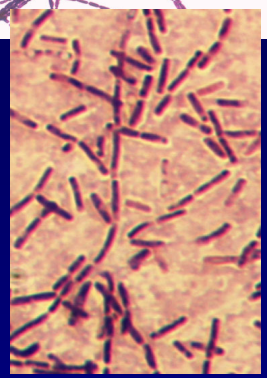
# *B. anthracis:* Presumptive Identification

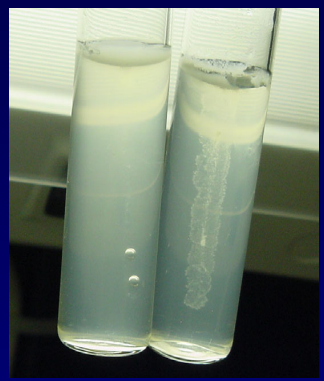


Clinical specimen (blood, CSF, etc.)

- Gram stain
  - Capsule production
- Isolate on SBA

  - Colony morphology
  - Hemolysis
  - Motility
  - Spores
    - Gram stain
    - Malachite green

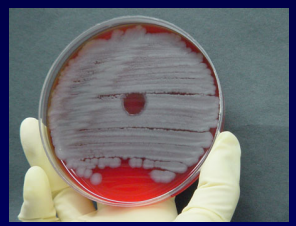




# *B. anthracis:* Confirmatory Identification

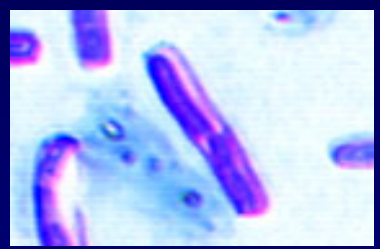
**Isolate**

**Phage lysis**



**Capsule**

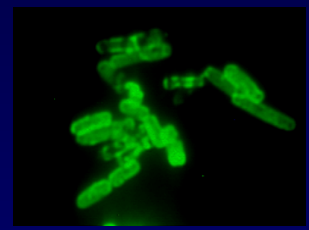
**Horse blood (M'Fadyean Stain)**



**Bicarbonate media (M'Fadyean stain India ink stain)**

**DFA**

**Capsule antigen Cell wall**



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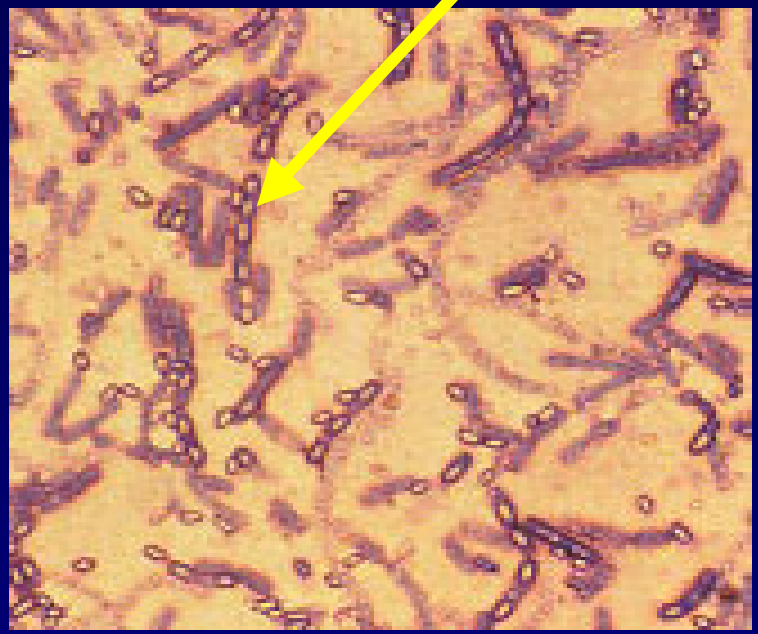
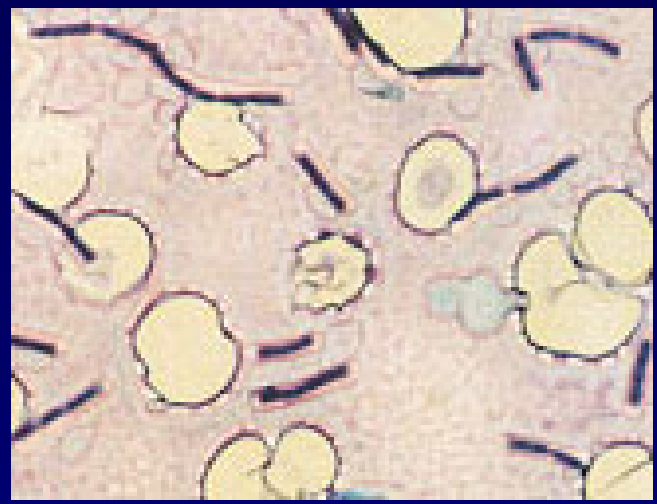
# Gram Stain Morphology of *B. anthracis*

- 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 $O_2$

# *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 β-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

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# 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.

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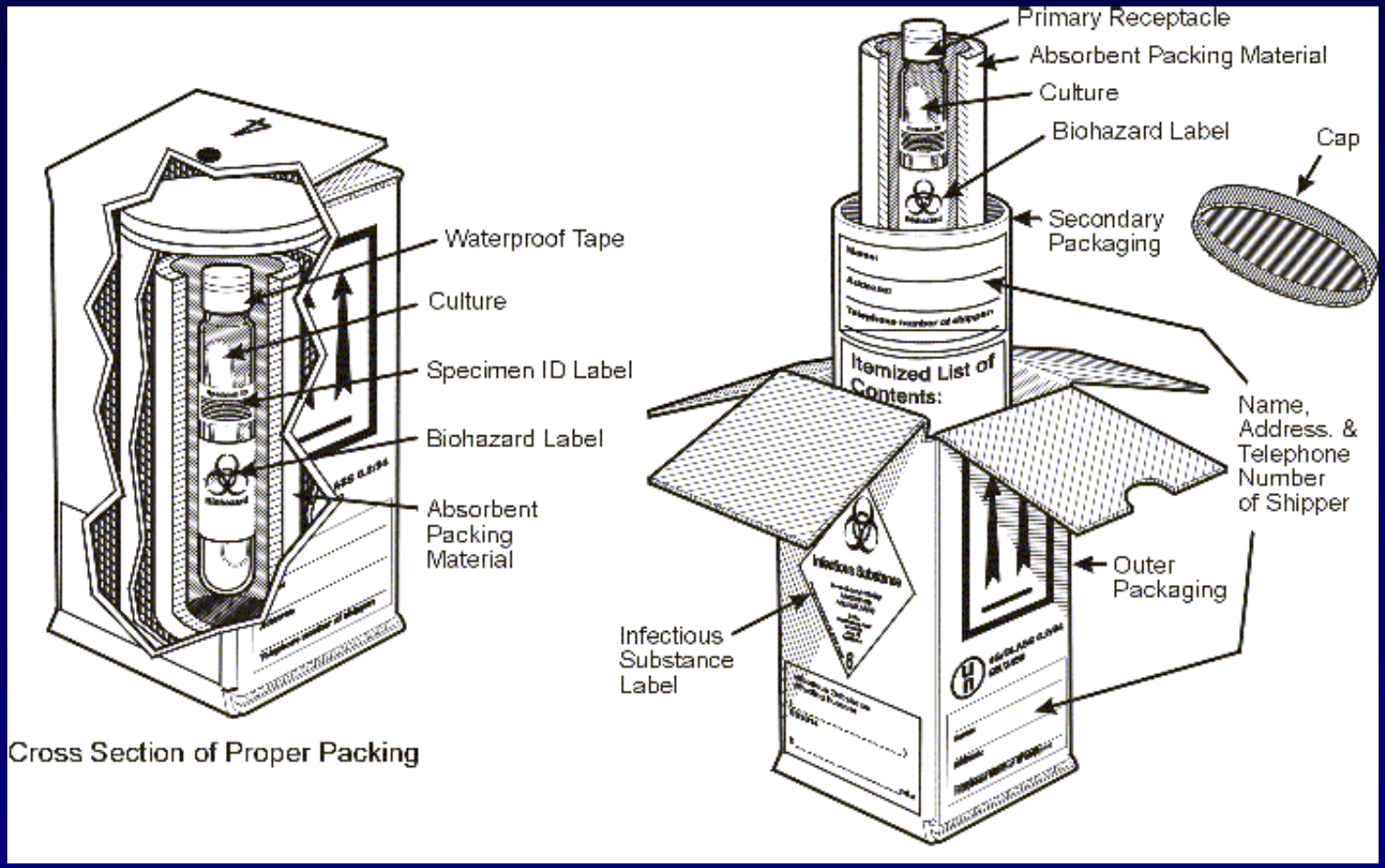
# Packaging and Transporting Protocol (3)

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



# 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

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# 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.

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