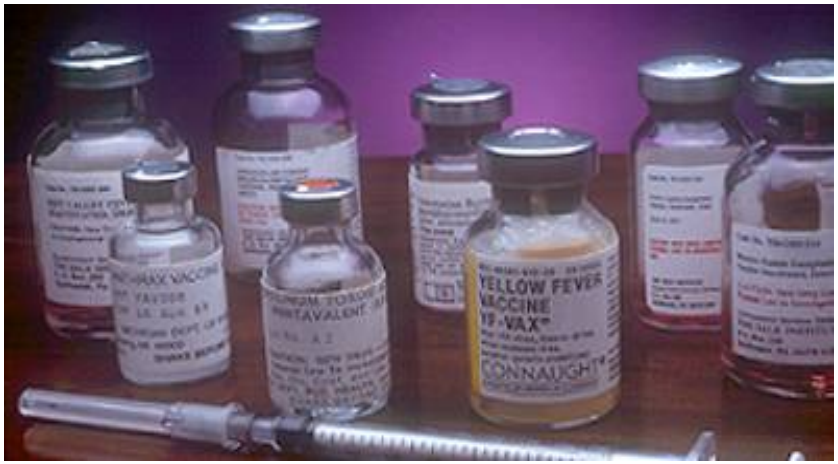
TABLE OF CONTENTS
Medical Management of Biological Casualties Course

LECTURES



USAMRIID OVERVIEW

Presented by

Colonel John P. Skvorak
Deputy Commander
U.S. Army Medical Research Institute of Infectious Diseases

Chain of Command

- U.S. Army Medical Command

  LTG Eric Schoomaker, Surgeon General

- U.S. Army Medical Research and Materiel Command

  MG George Weightman, Commander

- USAMRIID

  COL George W. Korch, Commander

Core Mission

Conduct basic and applied research on biological threats resulting in medical solutions (prophylactic vaccines, therapies and medical diagnostics) to protect the War Fighter.

USAMRIID is a subordinate laboratory of the U.S. Army Medical Research & Materiel Command

System of Systems Approach to Counter the Threat

CB Threats & Hazards

Agent Delivery → Doses on Target → Downwind Dispersal → Doses Absorbed → Symptoms

Medical Pretreatment

Individual & Collective Protection

Medical Diagnosis

Medical Treatment

Contamination Avoidance & NBC Battle Management(Detection, Identification, Reconnaissance & Warning)

Installation Force Protection

Decontamination, Restoration

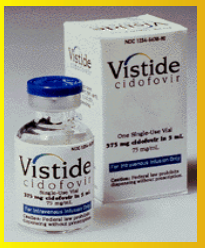
Sustained Combat Power

Full Spectrum of Medical CB Defense

PREVENTION

DETECTION

DIAGNOSIS
TREATMENT



Attack

YEARS | MONTHS | DAYS | HOURS | MINUTES | HOURS | DAYS

PLANNING OPPORTUNITY

REACTIVE

BIOLOGICAL DEFENSE

VACCINES

IMMUNE GLOBULINS

DRUGS

Diagnostics

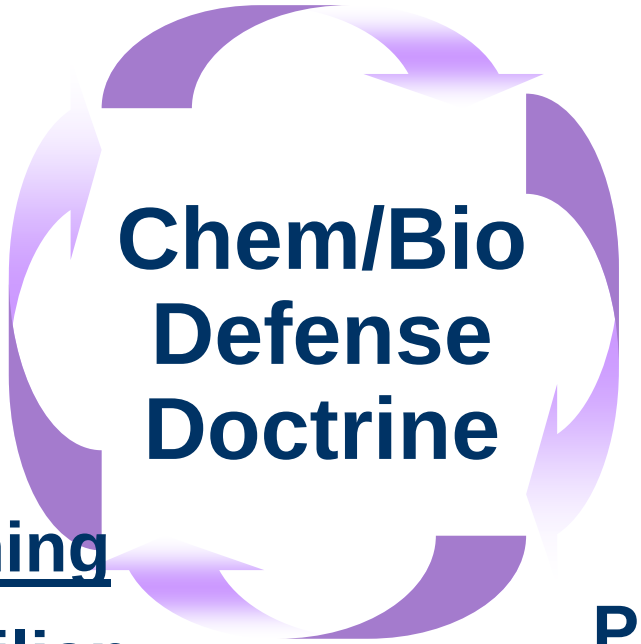
Protecting Warfighters

Intelligence

- → **Agent**
- → Delivery System
- → Organization
- → Time

Education & Training

- → **Military and Civilian Health Care Providers**
- → **Electronic Communication**
- → **Distance Learning**



Medical Countermeasures

- → **Vaccines**
- → **Diagnostics**
- → **Therapeutics**
- → **Medical solutions**

Physical Countermeasures

- → **Detection**
- → Physical Protection
- → Decontamination

CDC Biothreat Agents

Category A

Bacillus anthracis (anthrax)
Clostridium botulinum
Yersinia pestis
Variola major (smallpox) & other pox viruses
Francisella tularensis
LCM, Junin virus, Machupo virus, Guanarito virus
Lassa Fever
Hantaviruses
Rift Valley Fever
Dengue
Ebola
Marburg

Category B

Burkholderia pseudomallei
Brucella species (brucellosis)
Ricin toxin
Staphylococcus enterotoxin B
Diarrheagenic E.coli
Shigella species
Listeria monocytogenes
Yersinia enterocolitica)
Cryptosporidium parvum
Giardia lamblia
Toxoplasma
West Nile Virus
California encephalitis
EEE
Japanese Encephalitis Virus
Coxiella burnetti (Q fever)
Burkholderia mallei (glanders)
Epsilon toxin of C. perfringens
Typhus fever (Rickettsia rowazekii)
Pathogenic Vibrios
Salmonella
Campylobacter jejuni
Viruses (Caliciviruses, Hepatitis A)
Cyclospora cayatanensis
Entamoeba histolytica
Microsporidia
LaCrosse
VEE
WEE
Kyasanur Forest Virus

Most biothreats are zoonotic or emerging diseases

Category C

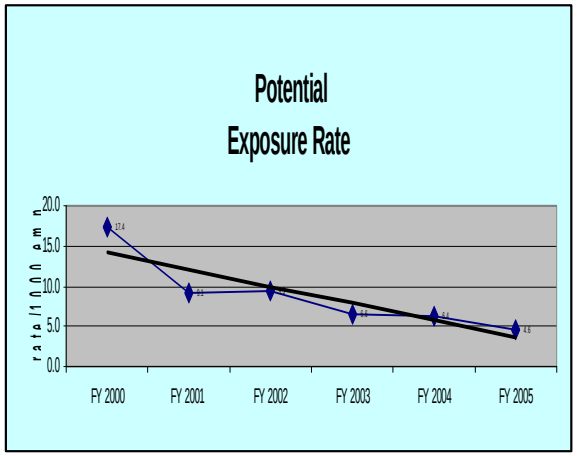
Emerging infectious disease threats (Nipah virus and additional hantaviruses).
NIAID priority areas:
Crimean-Congo Hemorrhagic fever virus
Tickborne encephalitis viruses
Yellow fever
Multi-drug resistant TB
Influenza
Other Rickettsias
Rabies

Red = DOD Threat Agents of concern

Unique Capabilities

Containment Laboratory Operations

Expert Bio Threat Knowledge



Biosafety/Biosurety

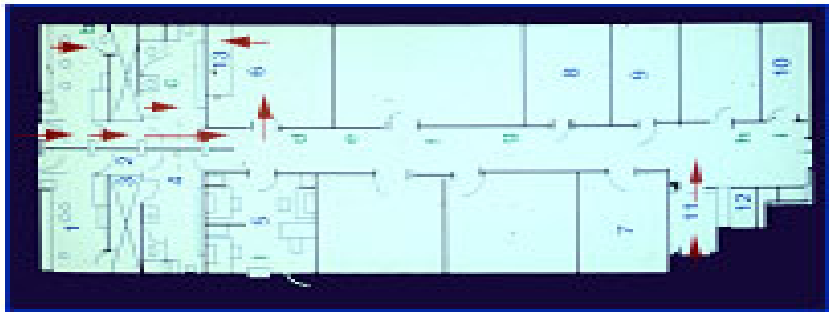
Clinical Studies

Training and Education

Rapid Response & SMART Teams

Medical Product R&D & GLP Studies

Unique Facilities



- **Largest collection of Biosafety Level 4**
- **Largest animal containment care facility**
- **Large Animal Care Facility (Farm)**
- **Unique *Center for Aerobiology***
- ***Field Laboratory Training Center***
- **Nation's Only BSL 4 Patient Care Suite**

USAMRIID's Current Operational Model

Leverage From External Sources

(selected examples)

Product Candidates

- Novel antimicrobials (ex. GSK, Siga, Chimerix)
- Vaccine candidates (ex.AlphaVax, NIAID VRC)
- Antisense Oligos (ex. AVI Biopharma)

Technologies

- Diagnostic systems (ex. BioVeris, Cephied)
- Antibodies (ex. BioFactura, Abgenix)
- Vaccine delivery devices (ex. PowderMed, B-D)
- Vaccine vectors (ex. Health Canada, Vical, Crucell)
- Adjuvants (ex. Coley Pharm., Chiron)

Concepts & Capabilities

- DNA vaccination
- siRNA
- Reverse genetics (ex. Univ. Wisconsin)
- Host targets for viral assembly (ex. Prosetta)
- Bioinformatics (ex. Diversa, TIGR, VBI, Los Alamos)
- Transgenic Mice (ex. Lexicon Genetics)

CRADAs/MTAs

~730 active agreements today!

USAMRIID

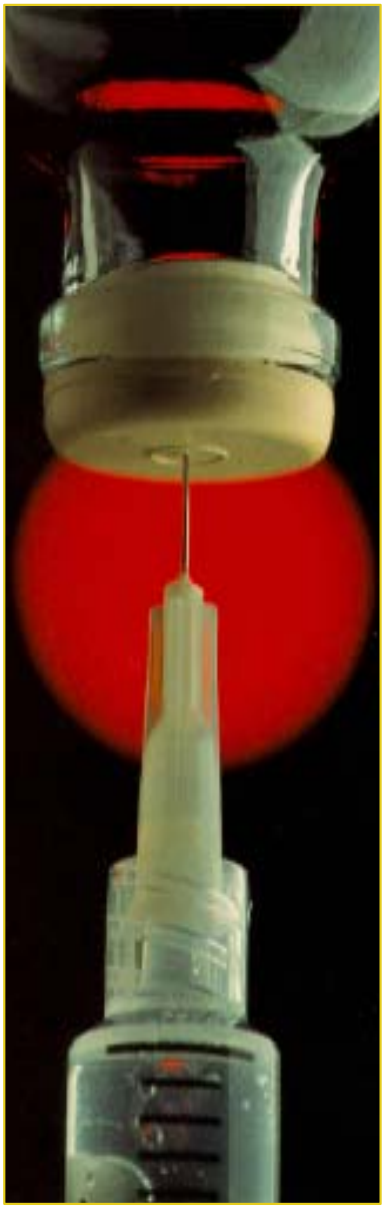
- Pathogenesis studies
- Adaptation of technology to biodefense countermeasures
- Animal model development
- Evaluation of countermeasure

Provide to Customers

- Product candidates
- Technical information

We apply cutting-edge approaches to our problem sets!

USAMRIID Biodefense Products to Protect the Nation



Available For Use Today	• Tularemia Vaccine (IND) • Venezuelan Equine Encephalitis (VEE) Vaccines (IND) • Eastern Equine Encephalitis (EEE) Vaccine (IND) • Western Equine Encephalitis (WEE) Vaccine (IND) • Botulinum Pentavalent Toxoid Vaccine (IND) • Smallpox Vaccine (cell culture derived vaccinia virus) • Botulinum Antitoxin (human & horse) • Vaccinia Immune Globulin • Ribavirin • Joint Biological Agent Identification System (JBAIDS) • Anthrax Gammaphage Diagnostic • Antibiotic Treatment of Pneumonic Plague and Inhalational Anthrax
In Advanced Development	• rPA-Based Anthrax Vaccine • Botulinum Neurotoxin Bivalent Vaccine • Venezuelan Equine Encephalitis Virus (V3526) Vaccine • Plague Vaccine (F1-V)
Emerging Products	• Ricin Vaccine • Ebola/Marburg Vaccine and Therapeutics • Cidofovir/ST-246 for Treatment of Smallpox • Staphylococcal Enterotoxin A/B Vaccine • Hantavirus Vaccines • Botulinum Neurotoxin Heptavalent Vaccine • Next-Generation Immunodiagnostics • Next-Generation EEE/WEE Vaccines • Burkholderia Vaccine and Therapeutics

USAMRIID scientists develop at least one new medical countermeasure per year.

Recent S&T Product Development Efforts

- Vaccines, diagnostics, therapeutics from USAMRIID S&T
  - Dynport
    - Plague F1-V vaccine in Stage II Clinical Trials
    - Botulinum Neurotoxin for serotypes A & B
    - First Licensed Real Time PCR Assay for Anthrax – JBAIDS Platform
  - NIAID/FDA
    - Animal Models for Plague, Anthrax
    - Postexposure rPA vaccine
  - CDC
    - Gammaphage assay - *B. anthracis*
- Transitioned products FY01-06
  - DTRA Program
    - VEE V3526 Vaccine
    - IV Cidofovir – postexposure therapeutic
  - MIDRP
    - Hantaan Virus Vaccine
- Near-term "ready to go" products (FY07-09)
  - Ricin mutagenized A-chain vaccine
  - Staphylococcal Enterovirus recombinant vaccine
- Mid to far term products (FY10 and out)
  - Filovirus therapeutic and vaccines
  - Amend drug Indicators for pneumonic plague
  - Burkholderia vaccine candidates
- IND outside of DoD development path
  - Orthopox Therapeutic: ST-246 (SIGA)
  - Anthrax Post-Exposure therapeutic (NIH)

Outbreak Investigations

- 1969 Venezuelan Equine Encephalitis (VEE), Honduras
- 1971 VEE in horses, Central America, southern Texas
- 1984 Eastern Equine Encephalitis in whooping cranes, Patuxent Wildlife Preserve, MD
- 1977 Rift Valley fever (RVF), animals and humans, Egypt
- 1983 Chikungunya fever, Indonesia
- 1988 Infant botulism, San Francisco
- 1988 RVF in animals and humans, Senegal and Mauritania
- 1989 Simian hemorrhagic fever, New Mexico
- 1990 Reston Ebola virus outbreak in primate colony, Reston, Virginia
- 1993 Hantavirus outbreaks in United States
- 1993 RVF outbreak in Egypt
- 1995 Ebola outbreak in Zaire
- 1995 VEE in Colombia
- 1996 Ebola Reston virus NHP outbreak, Alice, Texas
- 1997 Ebola Ivory Coast
- 1999 West Nile Virus
- 2000 West Nile Virus
- 2000 Anthrax outbreak, Minnesota
- 2000 Potential Ebola virus case, Uganda
- 2001 Support for Florida, New York City, and Washington, DC Anthrax cases
- 2003 SARS, 8 confirmed U.S. cases, CA, NJ, NM, NC, PA, UT, VA
- 2003 Monkeypox Indiana, Illinois, Wisconsin
- 2003 Malaria Outbreak among Marines in Liberia
- 2006 Plague Colorado
- 2006 Chikungunya fever, La Réunion, Mayotte, Maurice, Seychelles and India
- 2007 Burkholderia Australia
- 2007 RVF outbreak in Kenya
- 2007 Tuleremia in Thailand
- Ongoing Monkeypox in Democratic Republic of the Congo
- Ongoing Lassa fever in Sierra Leone

A Changed World
The Next Generation Biothreat

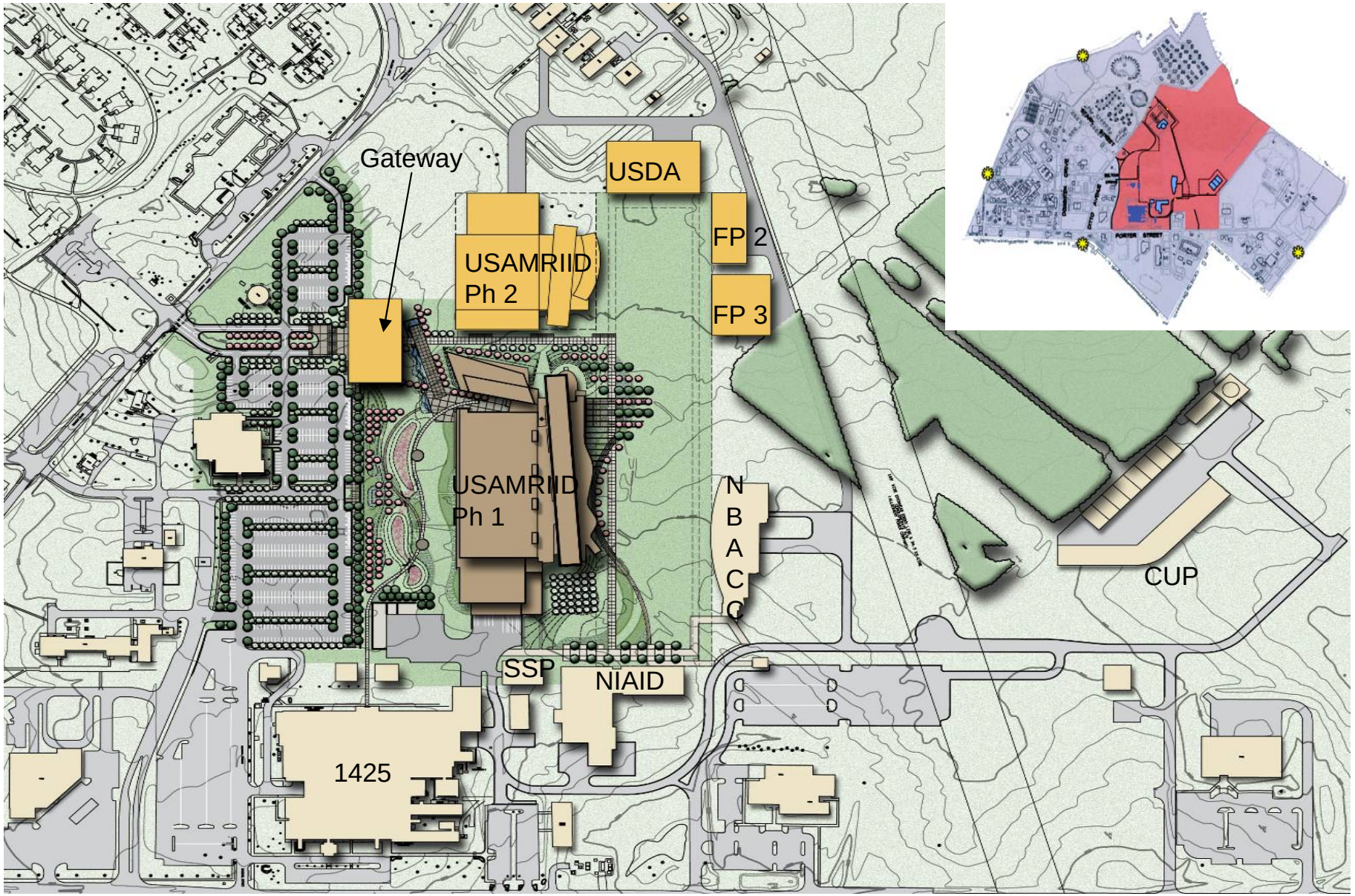
- **The new biological threat respects no borders; knows no boundaries**
- **Over 80 biological threats of concern to both military and civilian populations**
- **Unknown number of emerging & genetically engineered threats**

Next Generation Threat Demands Next Generation Capabilities

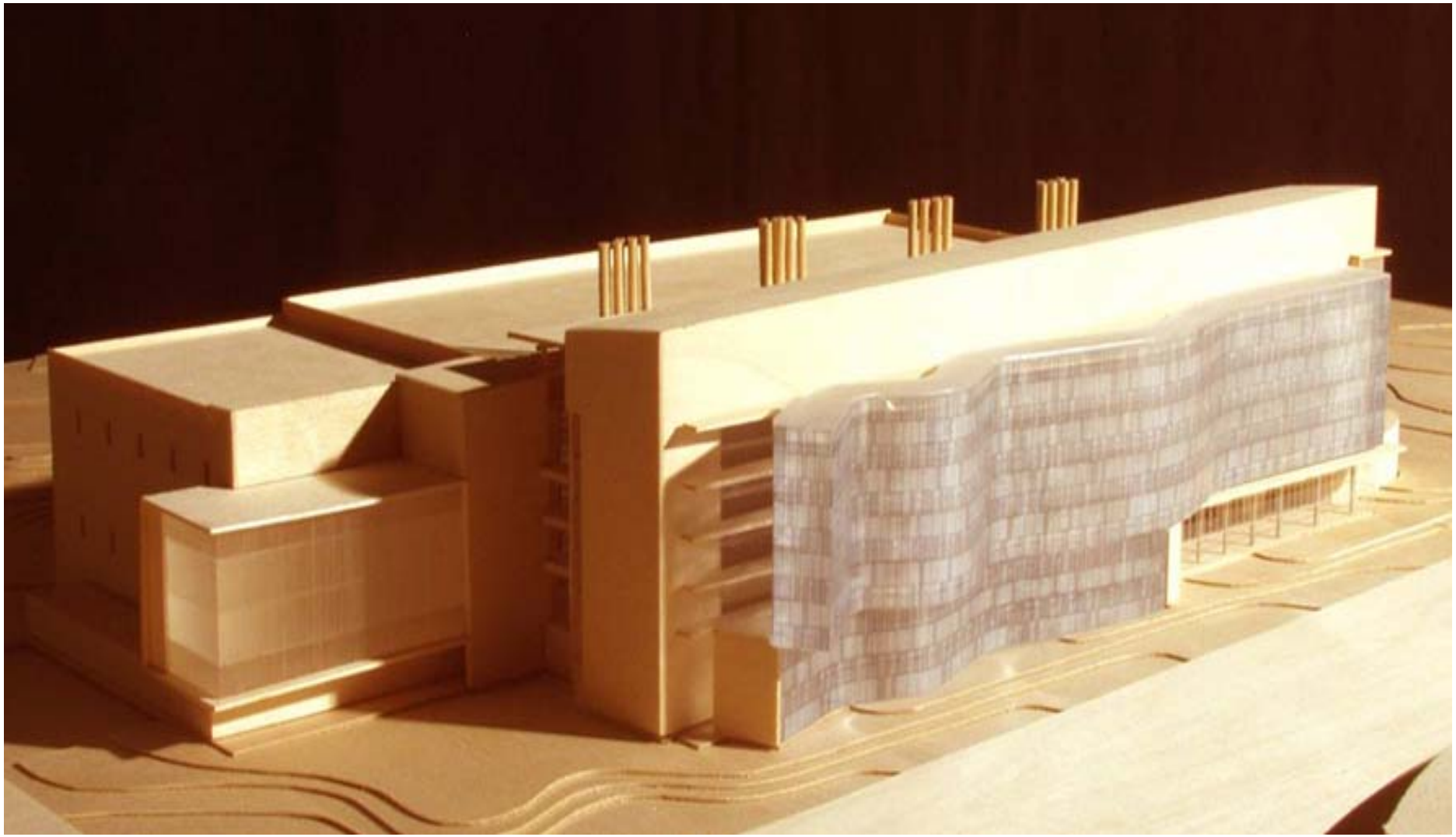
National Interagency Biodefense Campus (NIBC)

- **Congressional mandate for interagency coordination and collaboration**
- **NIBC Partners**
  - **USAMRIID**
    - Lead laboratory for test and evaluation (T&E) of medical defense products
  - **National Institute of Allergy and Infectious Diseases Integrated Research Facility (NIAID IRF)**
    - Focus on disease process and clinical outcomes using hospital tools such as imaging and physiologic monitoring
  - **U.S. Department of Agriculture (USDA)**
    - Pathogenesis and genomics of plant diseases
  - **Department of Homeland Security (DHS) National Biodefense Analysis and Countermeasures Center (NBACC)**
    - Threat characterization and forensic expertise
  - **Centers for Disease Control and Prevention (CDC)**
    - Environmental Biology

Proposed Site of the New UAMRIID Facilities



Providing Quality Medical Products to Conserve the Fighting Strength



USAMRIID

Introduction to Biological Warfare, Biological Terrorism & the Threat

COL Zygmunt F. Dembek, MS

PhD, MS, MPH

USAMRIID, Fort Detrick, MD

May 2008

DEFINITIONS

BIOLOGICAL WARFARE

The intentional use of microorganisms or toxins derived from living organisms to produce death or disease in humans, animals, or plants

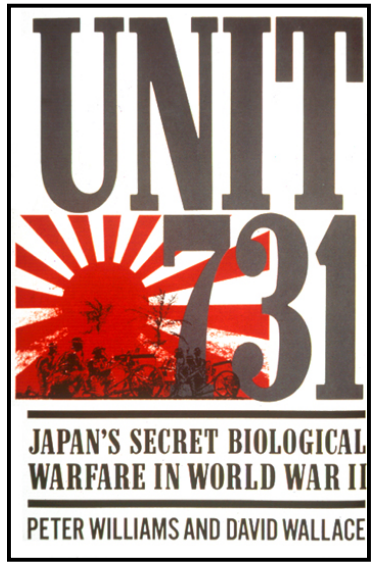
BIOLOGICAL TERRORISM

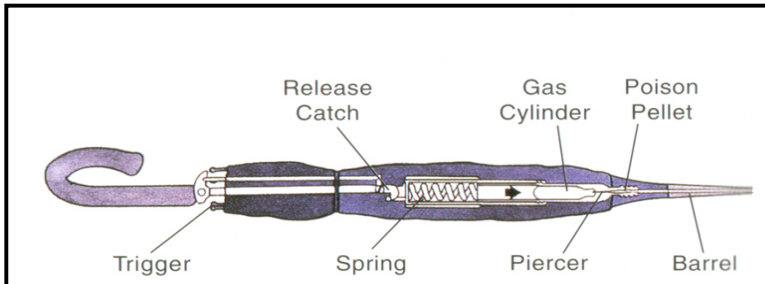
The threat or use of biological agents by individuals or groups motivated by political, religious, ecological, or other ideological objective.*

* W. Seth Carus, 1998. Bioterrorism and Biocrimes, Center for Counterproliferation Research, National Defense University

BW Historical Events

- 1346 Kaffa – Plague
- 1763 French and Indian War
- 1914 German WWI
- 1925 Geneva Convention
- 1937 Japan WW II – Unit 731
- 1972 Biological Weapons Convention
- 1978 Ricin (Assassination)
- 1979 Sverdlovsk – Anthrax
- 1984 Rajneeshees Cult
- 1995 Aum Shinrikyo (Tokyo Sarin Gas Incident)
- 2001 Anthrax Mail Attacks





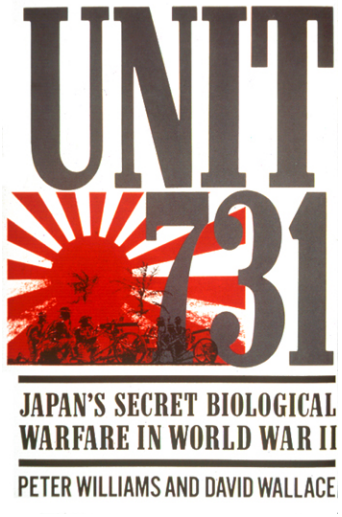
Geneva Protocol of 1925

"Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare"

- Use was 'justly condemned by general opinion of the civilized world'
- Prohibits first use only
- Not ratified by U.S.

Japan's Unit 731

- 1939-45: human research in Ping Fang, Manchuria
- October 1940: Chekiang province epidemic of bubonic plague
- 3000 POWs died in experiments using anthrax, botulism, brucellosis, cholera, dysentery, gas gangrene, tetrodotoxin, meningococcal infection, plague, etc.



Japan’s Unit 731

US Offensive Program

US Offensive Program

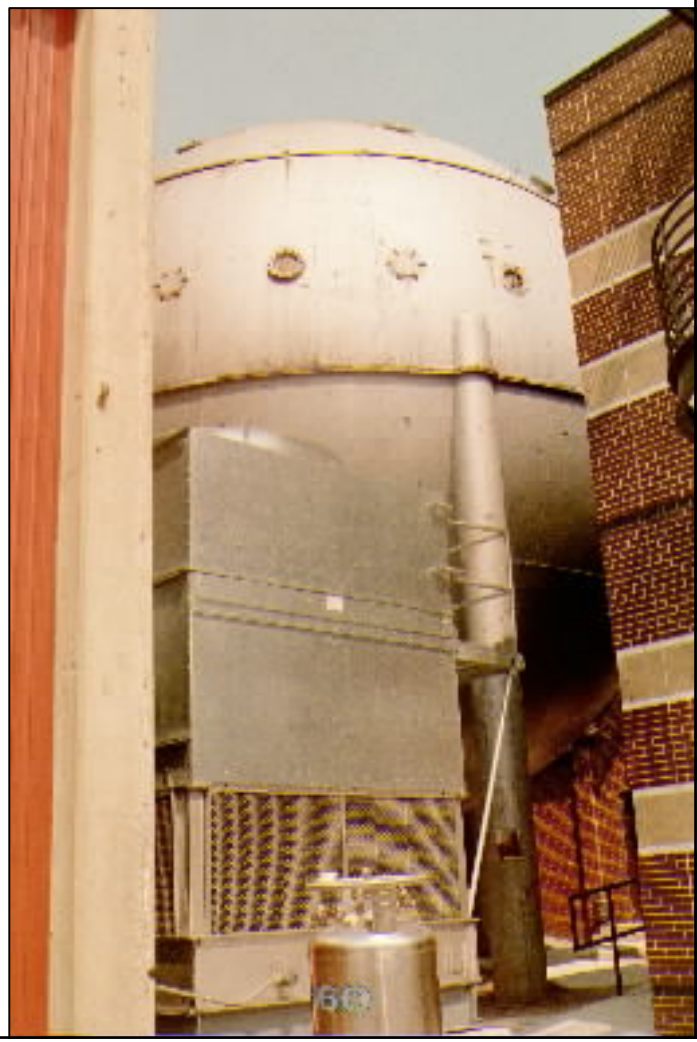
Major Facilities

- Camp Detrick - Frederick, MD, 1943 -1969
- Vigo Production Facility - Terre Haute, IN (1944-1946)
- Production Facility - Pine Bluff, AR
- Camp Terry (Plum Island, NY)
- Testing Sites
  - Horn Island, MS
  - Dugway Proving Ground, UT

Doctrine: Primary deterrence and retaliate, if necessary

US Offensive Program

- Project Whitecoat – 1954
  - Medical Research Volunteers
  - Seventh Day Adventists
  - *C. burnetii, F. tularensis*, SEB
  - Outdoor test sites as well as Ft Detrick's "8-Ball" aerosol facility



US Offensive Program

US Offensive Program

- 1969 President Nixon Renounces U.S Program!

DESTROYED U.S. BIOLOGICAL WARFARE AGENTS

Lethal

- *B. anthracis*
- Botulinum toxins
- *F. tularensis*

Anticrop

- Wheat stem rust
- Rye stem rust
- Rice blast

Incapacitating

- *Brucella suis*
- VEE virus
- SEB
- Q fever agent

1972 Biological Weapons Convention

- EIF March 26, 1975
  - Signed and ratified by 140 countries
  - Signed and not ratified by 18 countries
- Never to develop, produce, stockpile, acquire or retain any biological agent for other than peaceful purposes
- Facilitate exchange of equipment, materials, and information on use of biological agents for peaceful purposes
- Prohibits for non-peaceful purposes:
  - Acquisition, production, stockpiling
  - Weapons, delivery means
  - Transfer of supplies, equipment, etc.
- Lack of verification provision

BRIEF THREAT OVERVIEW:
STATE SPONSORED PROGRAMS

RUSSIA: BIOPREPARAT WORLD'S LARGEST, MOST ADVANCED

SOVIET BW PROGRAM PRIORITIES

Soviet ”Criterion Rating” as a Measure of Probable Use of Bioagents as Biological Weapons

Smallpox	26
Plague	23
Anthrax	21
Botulism	21
VEE	20
Tularemia	20
Q Fever	20
Marburg	18
Influenza	17
Melioidosis	17
Typhus	15

SVERDLOVSK INCIDENT

- Accidental release of **~1 gram** of anthrax spores from a Soviet military compound
- Resulted in ≥ 66 human deaths
- As a result, biological weapons production moved to Stepnogorsk, Kazakhstan

GEOGRAPHIC DISTRIBUTION OF THE CASES:

- As this satellite image clearly suggests virtually all of the cases occurred in a narrow band directly Southeast from compound 19.

- The two cases which did not usually live or work Southeast turned out to be reservists who had spent Saturday April the 2nd on an adjacent military compound.

“THERE WERE MORE INSTITUTES WORKING ON PLAGUE IN THE USSR THAN PERSONNEL WORKING ON PLAGUE IN THE USA”

- Dr. Ken Alibek, Deputy Chief of Biopreparat

SOVIET BW PROGRAM

BW MANUFACTURING FACILITIES MINISTRY OF DEFENSE

- Sverdlovsk: **Anthrax**
  - Stockpiled: > 100 tons
  - Annual Production Capacity: > 1000 tons
- Kirov: **Plague**
  - Stockpiled: 20 tons
  - Annual Production Capacity: ~ 200 tons
- Zagorsk: **Smallpox**
  - Stockpiled: 20 tons
  - Annual Production Capacity: ~ 100 tons

FSU

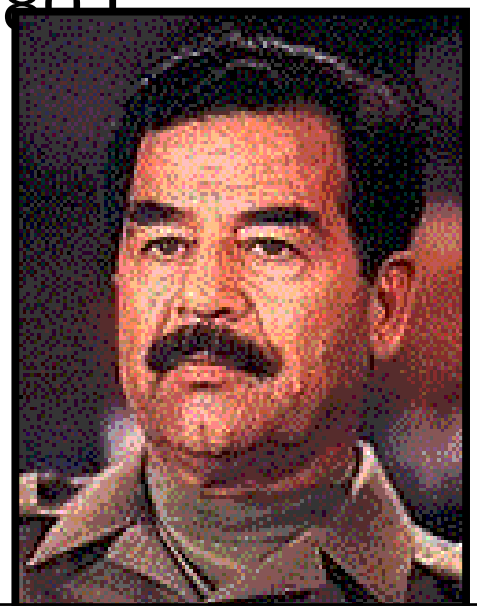
50,000 Employees Downsized to under 25,000
Where are the rest?

OFFENSIVE BW PROGRAM: IRAQ

1995 disclosures to UNSCOM:

	Produced	Weaponized
Botulinum toxin	19,000 Liters	10,000 L
Anthrax spores	8,500 L	6,500 L
Aflatoxin	2,200 L	1,58[illegible] L

UN Doc S/1995/864, 11 OCT 1995



DECEMBER 1990, IRAQIS FILLED:

- R400 bombs
  - 100 with Botulinum toxin,
  - 50 with anthrax
  - 16 with aflatoxin
- SCUD warheads (al Hussein)
  - 13 with Botulinum toxin
  - 10 with anthrax
  - 2 with aflatoxin

1,500 L fermenters

Al Hakeem Biological Production Facility

BIOLOGICAL WEAPONS PROGRAMS

CONFIRMED

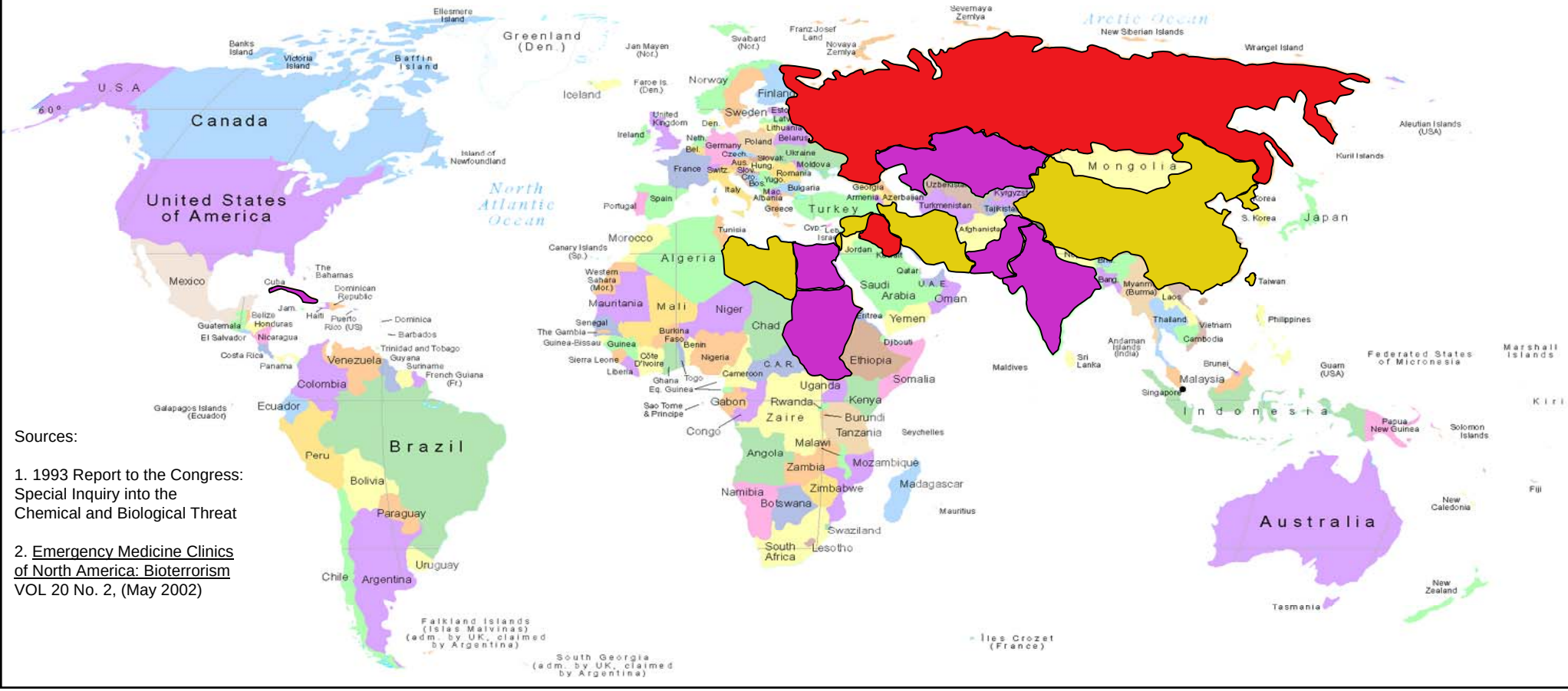
RUSSIA
IRAQ

SUSPECTED

IRAN, SYRIA, LIBYA, CHINA, NORTH KOREA, ISRAEL, TAIWAN

POSSIBLE

SUDAN, INDIA, PAKISTAN, KAZAKSTAN, CUBA, EGYPT



Sources:

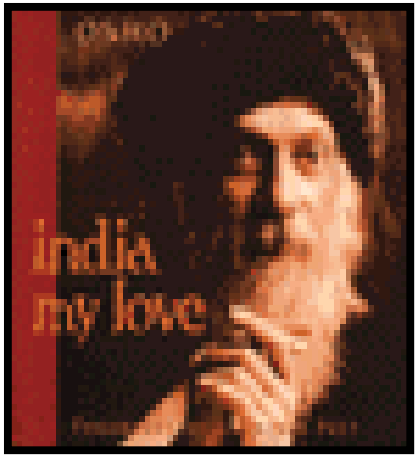
1. 1993 Report to the Congress: Special Inquiry into the Chemical and Biological Threat

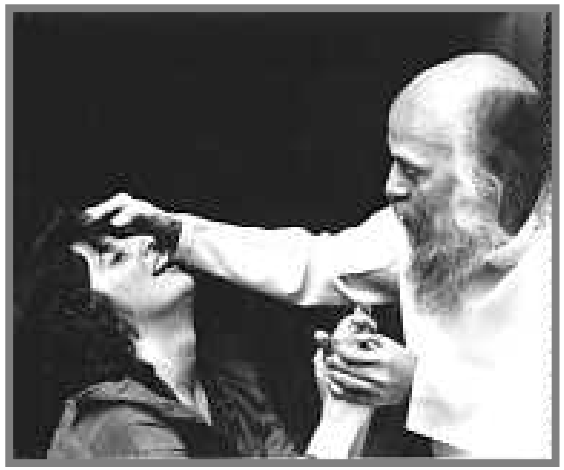
2. Emergency Medicine Clinics of North America: Bioterrorism VOL 20 No. 2, (May 2002)

BRIEF THREAT OVERVIEW: TERRORIST ORGANIZATIONS AND LONE INDIVIDUALS

RAIJNEESHEE CULT

- The Dalles, Oregon, 1984
- Contaminated salad bars
  - *S. typhimurium*
- 751 cases of enteritis





Lone Individuals

Dr. Mitsuru Suzki:

- A physician trained in Bacteriology
- Used sponge cakes to infect colleagues with dysentery
- Used bananas to infect members of his family with typhoid
- Estimated 120 people were infected and 4 died 1964-1966

Diane Thompson

- Large medical center in Texas - October - November 1996
- 12 of 45 lab staff ill
- Muffins and doughnuts
  - *Shigella dysenteriae* type 2
- Laboratory stack culture source
- Unknown motive

WHY WOULD A TERRORIST CONSIDER BIOLOGICAL WEAPONS?

ADVANTAGES OF BW:

- Easy to obtain
- Relatively easy and inexpensive to produce
- Readily available delivery modes
- Dissemination over large areas
- Difficult to detect (odorless, colorless)
- Large numbers of casualties possible
- Even threat of use would create fear, panic
- Perpetrators escape days before effects are seen

Cost Comparison

Cost (km2) to produce mass casualties

Agent	$$
BW Agents	1
Nerve Agents	600
Nuclear Weapons	800
Conventional Weapons	2000

ADVANTAGES OF BW

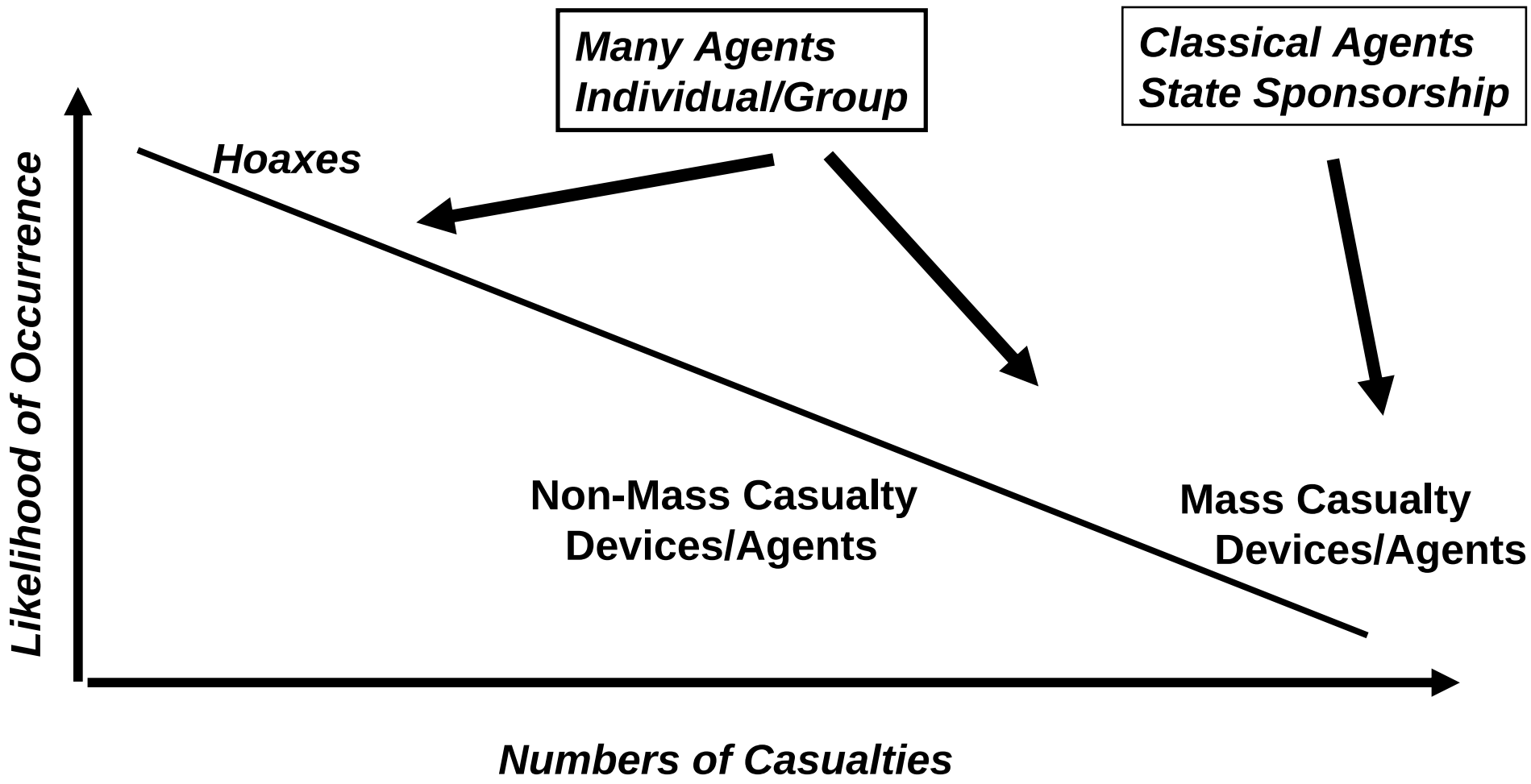
HYPOTHETICAL AIRCRAFT DISSEMINATION OF 50 KG OF AGENT ALONG A 2 KM LINE UPWIND OF A POPULATION CENTER OF 500,000*

Agent	Distance Km	Casualties	Fatalities
Rift valley fever	1	35,000	400
Tick borne encephalitis	1	35,000	9,500
Typhus	5	85,000	19,000
Brucellosis	10	100,000	500
Q-fever	>20	125,000	150
Tularemia	>20	125,000	30,000
Anthrax	>20	125,000	95,000

*Health Aspects of Chemical and Biological Weapons, WHO, 1970


LARGE
AREA
COVERAGE

THE BIOLOGICAL TERRORIST SPECTRUM



HIGHEST THREAT AGENT CHARACTERISTICS

- Dispersed in aerosol
- Highly lethal Agent
- Production capability / knowledge available
- Lack of treatment or vaccine
- Communicable
- Mere threat of use creates panic

The New Threat

Osama Bin Laden

Al-Zawaheri

- Development of new highly trained terrorist organizations
- Increasingly these organization's use new technologies such as the internet and wireless communications
- Tend to operate independently and very difficult to locate and interdict

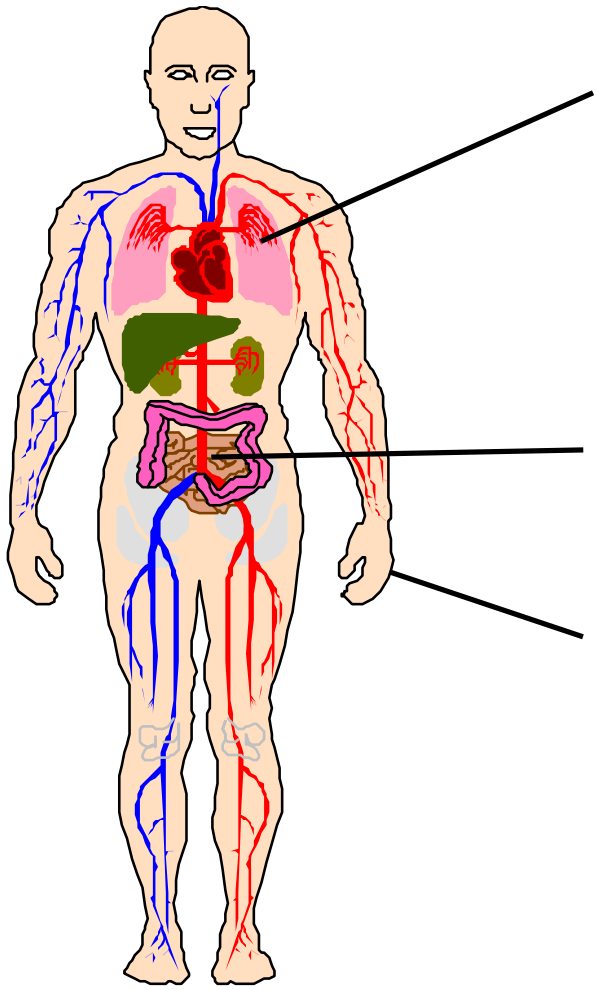
HOW ARE BW AGENTS DELIVERED?

BW WEAPONS DELIVERY

- To target individuals or small groups:
  - Letters or packages containing cells or spores
  - Injections of toxins
  - Local poisoning of food, water or agricultural products

- To target large gatherings:
  - Aerosol sprays of spores or cells from aircraft, trucks or other vehicles, or bombs and other munitions

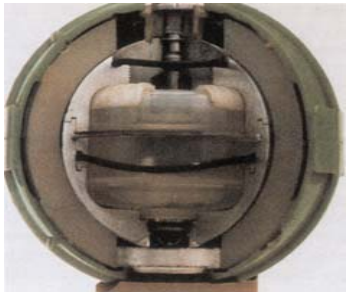
PORTALS OF ENTRY



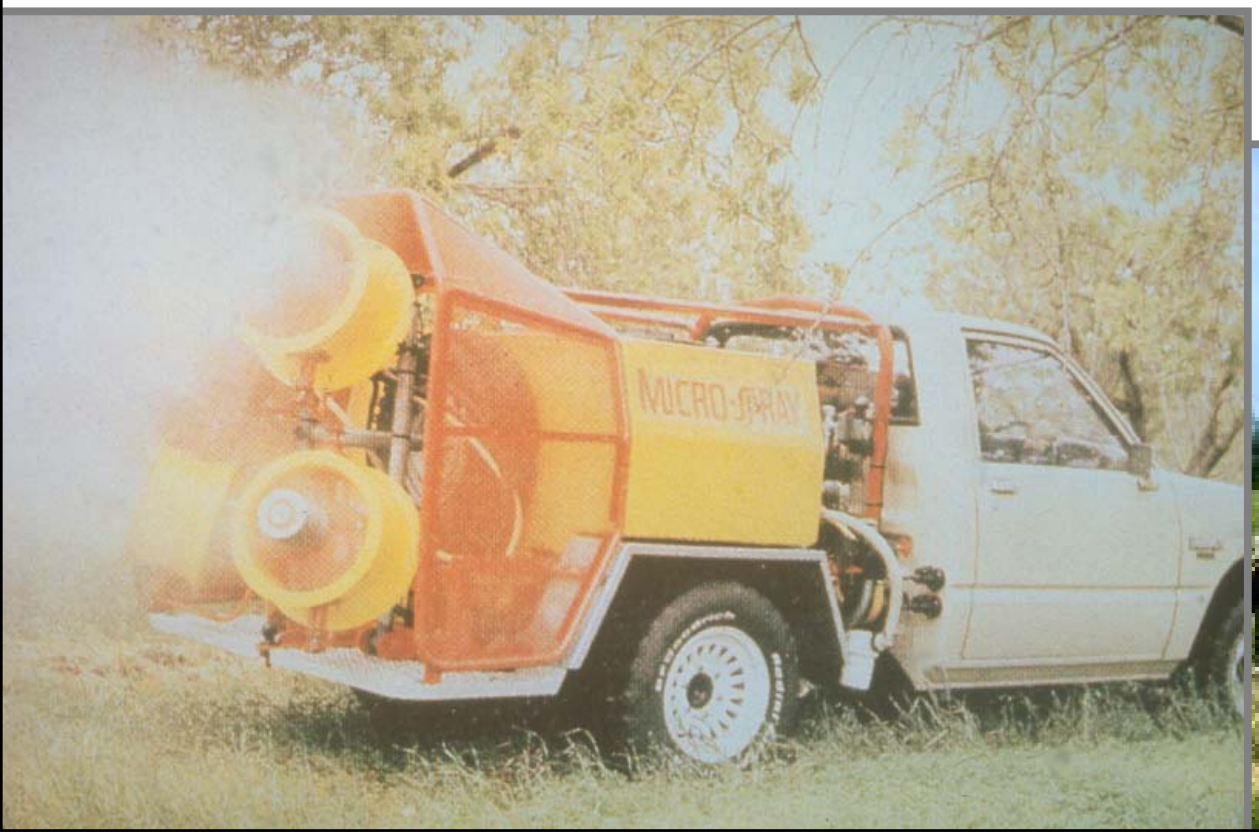
- Respiratory (Lungs)
  - Focal infection (pneumonia)
  - Susceptible to aerosol delivery
  - Can spread to other parts of the body
- GI tract
  - Food/water delivery
- Skin / mucous membranes
  - Skin is effective barrier vs BW agents (except T-2 mycotoxins)
  - Abrasions, wounds, exposed mucosal surfaces-potential portals of entry

DELIVERY SYSTEMS

- Aerosol delivery: Optimal
  - Generation of particles 1-5 microns
    - Settle in lower respiratory tract
    - Not detectable by our senses
  - Larger - mucocilliary clearance
  - Smaller - exhaled
- Explosive munitions: Poor
  - Heat, light from explosion inactivate agent
  - Inefficient production of particles of 1-5 u size



OTHER DISSEMINATION OPTIONS




Inversion Layer
(late PM – early AM)
Light Wind

BIOLOGICAL WARFARE AGENT CLASSIFICATION

POTENTIAL BW AGENTS*

Bacteria	Viruses	Toxins
Anthrax	Smallpox	Botulinum
Plague	Rift Valley Fever	Ricin
Q-Fever	Crimean-Congo HF	SEB
Brucellosis	VEE	T2 Mycotoxins
Tularemia		Saxitoxin
Cholera		*C. perfringens* toxin
Glanders		
Melioidosis		

*NATO AMedP-6(B)1996; Annex B unclassified
*Not to be interpreted as sanctioned “threat list”

CLASSIFICATION
BIOLOGICAL AGENTS

Type	Use	Operational
Pathogens	Antipersonnel	Transmissible
Toxins	Anti-animal	Lethal
Bio-modulators	Anti-plant	Incapacitating
	Anti-material	

CDC Classification

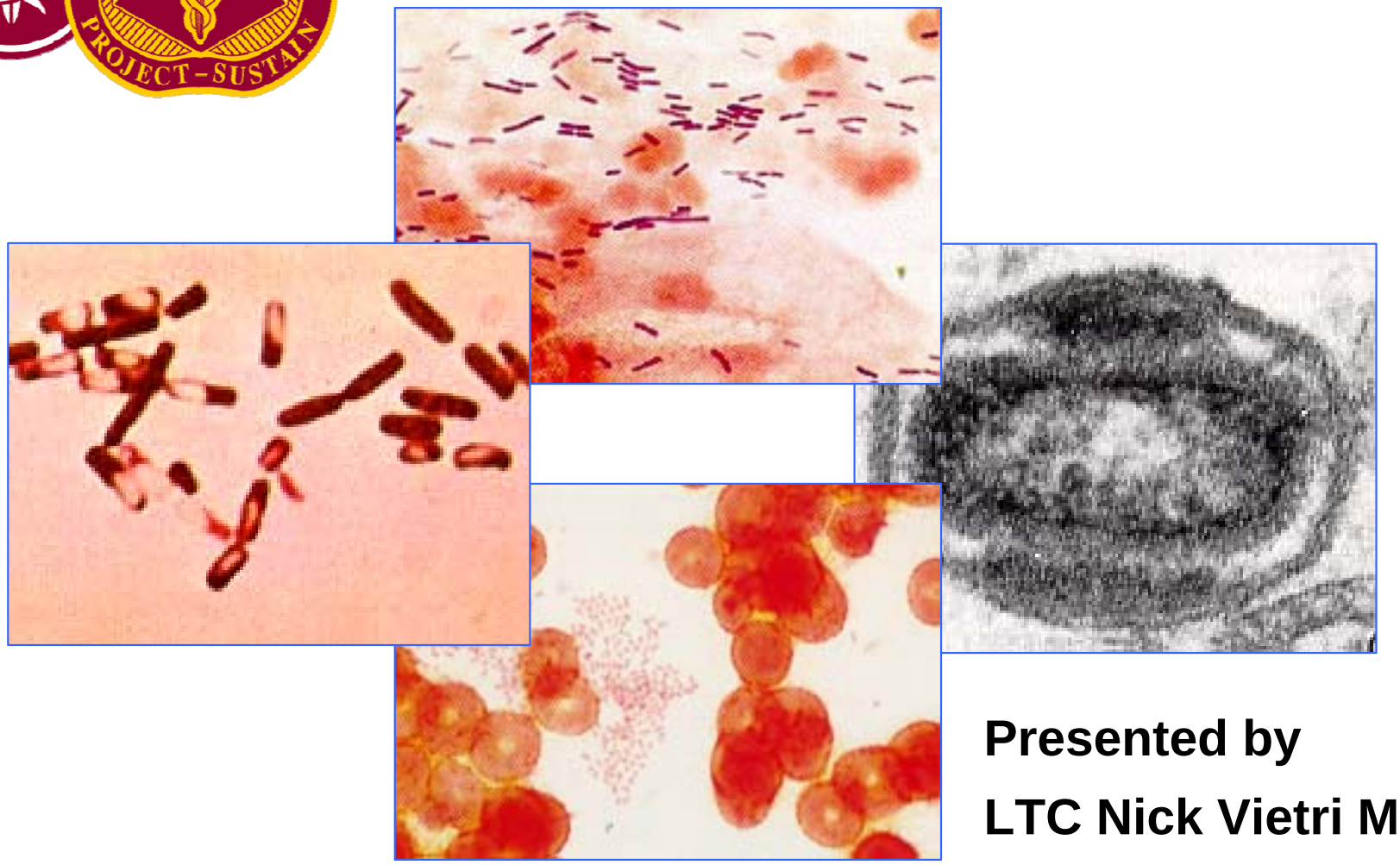
- Category “A” Agents
  - Anthrax
  - Botulism
  - Plague
  - Smallpox
  - Tularemia
  - Viral hemorrhagic fevers
- Category “B/C” Agents
  - Brucellosis
  - Glanders
  - Melioidosis
  - Psittacosis
  - Q Fever
  - Typhus Fever
  - Viral encephalitis
  - Toxins
  - Food Safety Threats
  - Water Safety Threats
  - Nipah virus
  - Hantavirus

USAMRIID

QUESTIONS?

USAMRIID

Anthrax



Presented by

LTC Nick Vietri MC

Bacteriology Division

USAMRIID

Lesson Objectives

- Discuss the epidemiology of naturally occurring anthrax disease
- Discuss the microbiology of anthrax
- Discuss the pathogenesis of anthrax disease
- Identify the clinical manifestations of anthrax in humans, to include cutaneous, oropharyngeal, gastrointestinal, and inhalational anthrax
- Outline the diagnosis of anthrax disease, to include cutaneous, oropharyngeal, gastrointestinal, and inhalational anthrax

Lesson Objectives

- Discuss the appropriate medical management of anthrax disease, to include cutaneous, oropharyngeal, gastrointestinal, and inhalational anthrax
- Discuss the appropriate post exposure prophylaxis of inhalational anthrax
- Identify anthrax vaccine characteristics, to include components, administration, common side effects, and efficacy
- Identify the DOD policy on AVA use

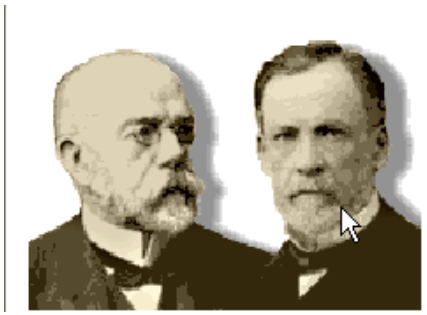
“So they took soot from a furnace and stood in the presence of Pharaoh. Moses scattered it toward the sky, and it caused festering boils on man and beast.”

Exodus 9:10

Anthrax

- Anthrax- disease of antiquity
- 5th and 6th plagues described in Exodus may have been anthrax in domesticated animals, followed by cutaneous anthrax in humans (circa 1491 BC)
- Described by Hippocrates (300 BC)
- Virgil described an epidemic suggestive of anthrax in Rome in the first century B.C.

Anthrax



- 18th century- the first careful clinical descriptions of anthrax in animals and humans
- *B. anthracis*- is closely associated the origins of medical microbiology and immunology
- Robert Koch-Anthrax first disease for which a microbial cause was definitively established
- Pasteur and Greenfield- first disease for which a live bacterial vaccine was shown to be effective (1881)

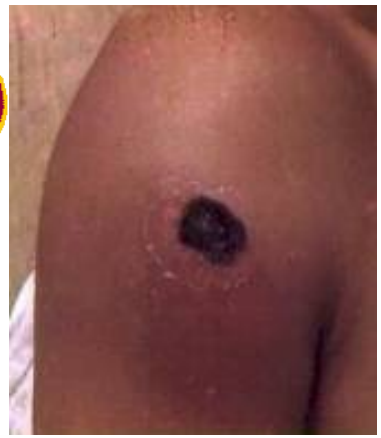
- Mid 1800's- inhalational anthrax recognized as a significant problem among British wool sorters
- Associated with mohair (goat) from Asia Minor and alpaca from Peru

Inhalational Anthrax

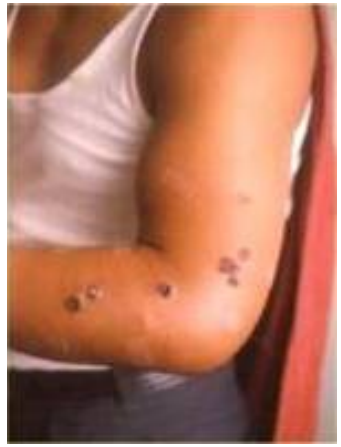
- 1905- Studies by F.W. Eurich demonstrated that the presence of *B. anthracis* was associated with "general dirtiness" and blood contamination of the animal fibers.
- Wool disinfection station established in Liverpool
- Formaldehyde appeared to be the best disinfectant
- No new U.K. inhalational cases since 1939*

Inhalational Anthrax

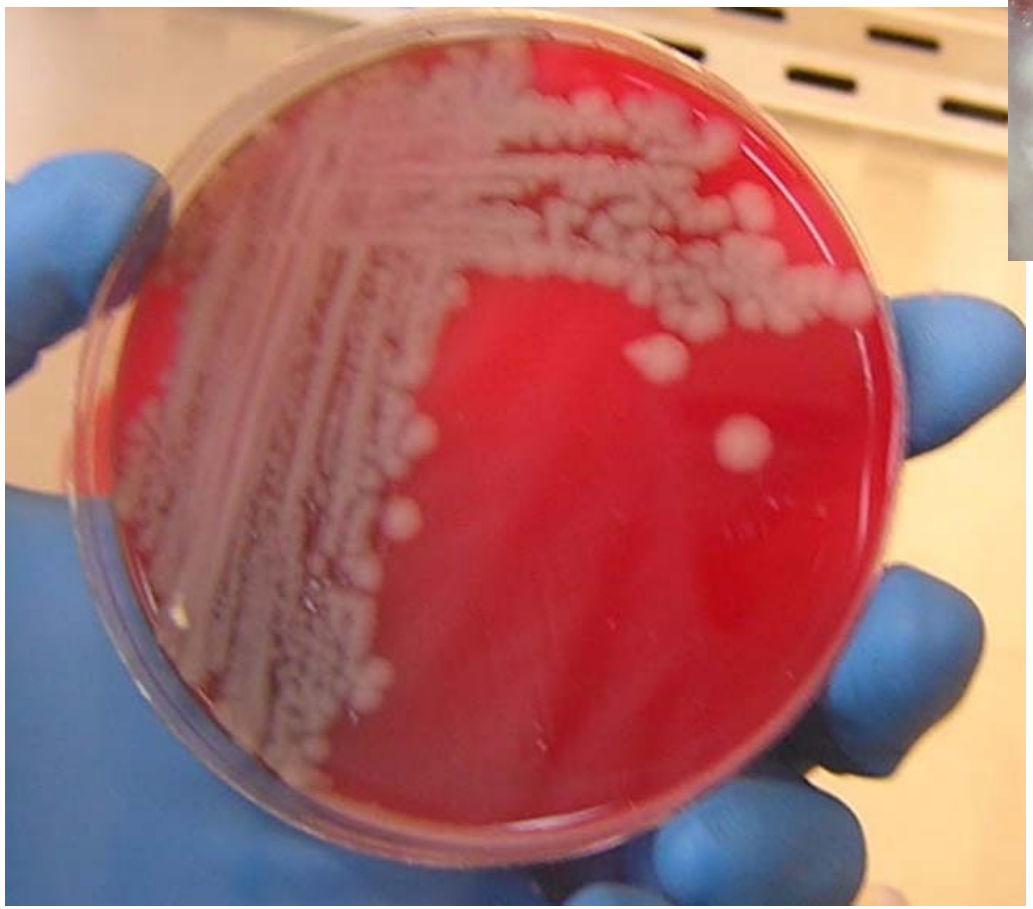
- In the U.S. there were only 21 reported inhalational cases from 1900 to 1978
- Sverdlovsk-1979 there were 79 cases with 68 deaths?
- In 2001 there were 11 inhalational cases associated with bio-terrorism in the U.S.
- February 2006- New York city, 44 year old male drum maker with goat hides from Africa
- July 2006- Scotland, 50 year old male drum maker



Microbiology

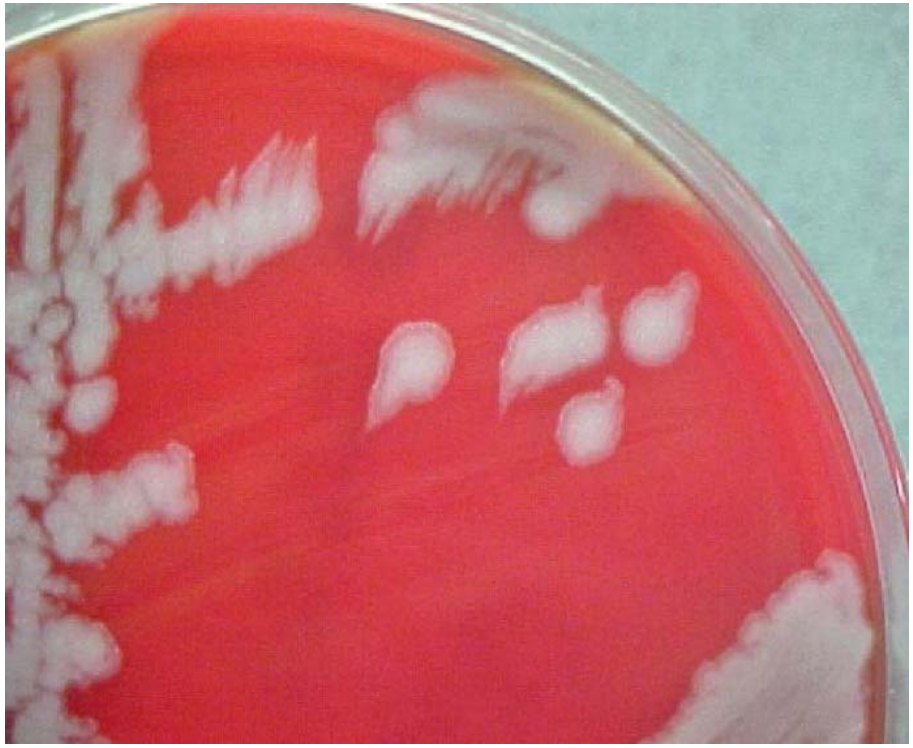


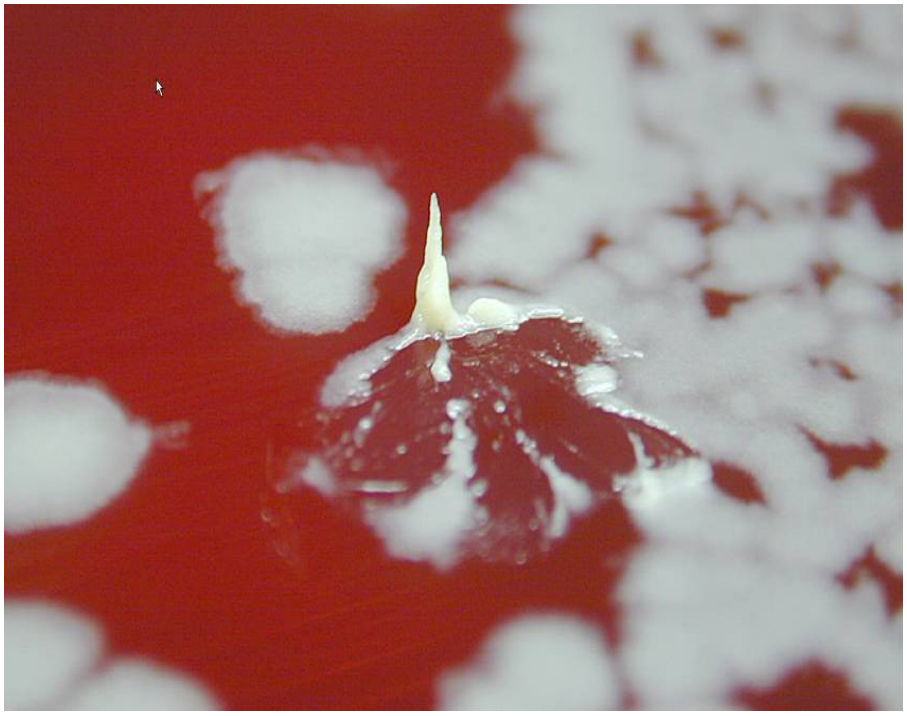
- *Bacillus anthracis*- derives from Greek word for coal, anthrakis-disease causes black coal like skin lesions
- *B. anthracis*- large Gram-positive spore-forming bacillus (1-1.5 µm x 3-10 µm)
- Grows readily on sheep blood agar
- Non-hemolytic or rarely weakly hemolytic



Microbiology

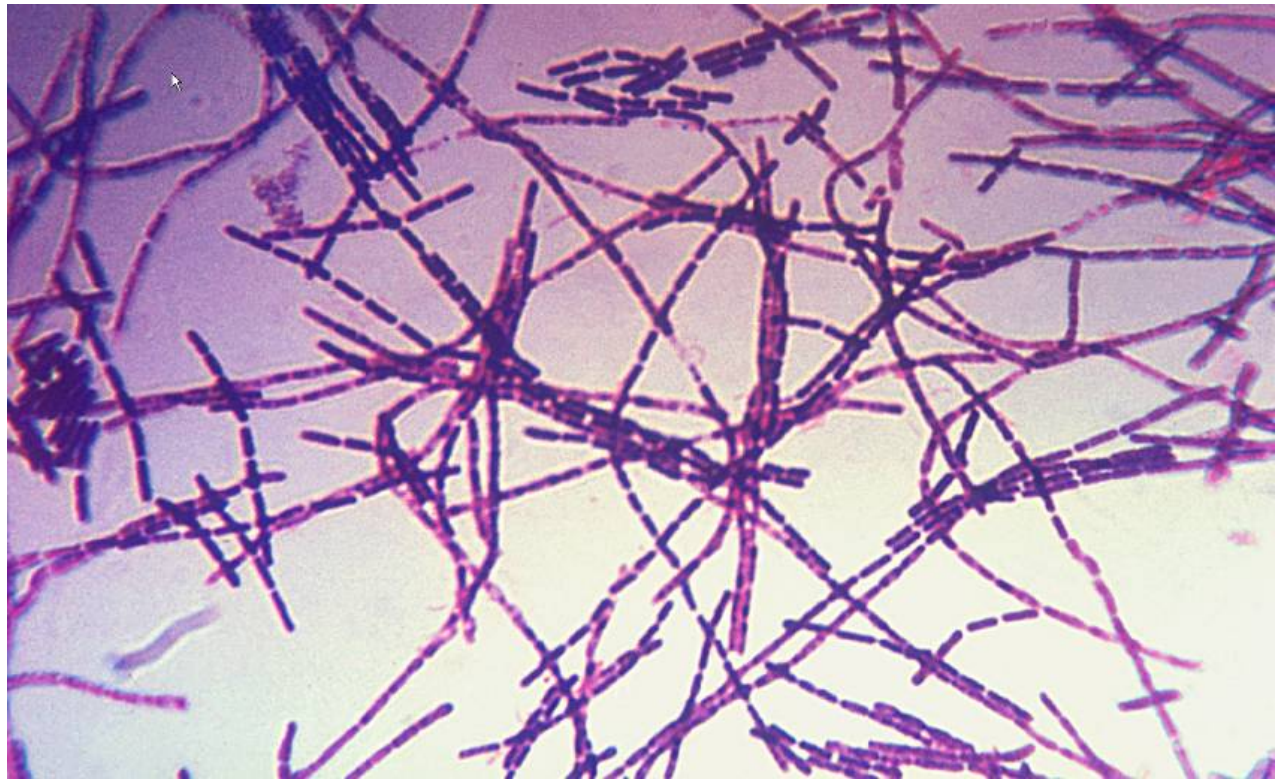
- Colonies are large, rough and grayish-white
- One day old colonies show irregular tapered, curved outgrowths “Medusa head”
- Colonies are tenacious
- Prominent capsule with grown on sodium bicarbonate agar with CO_2

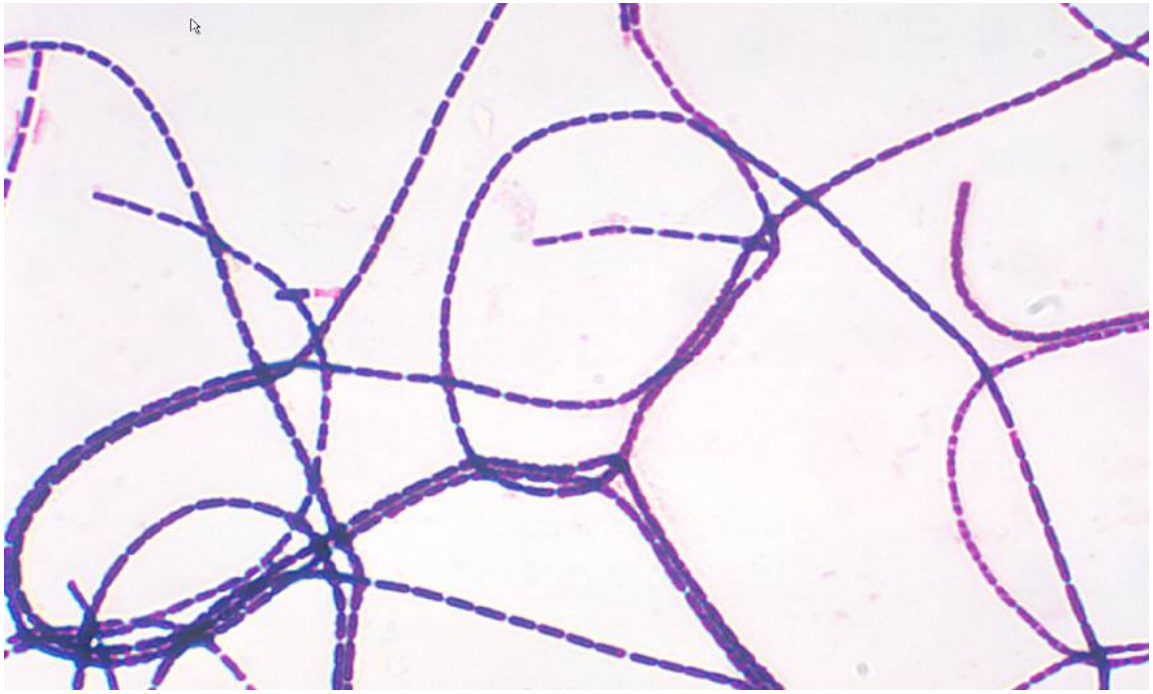


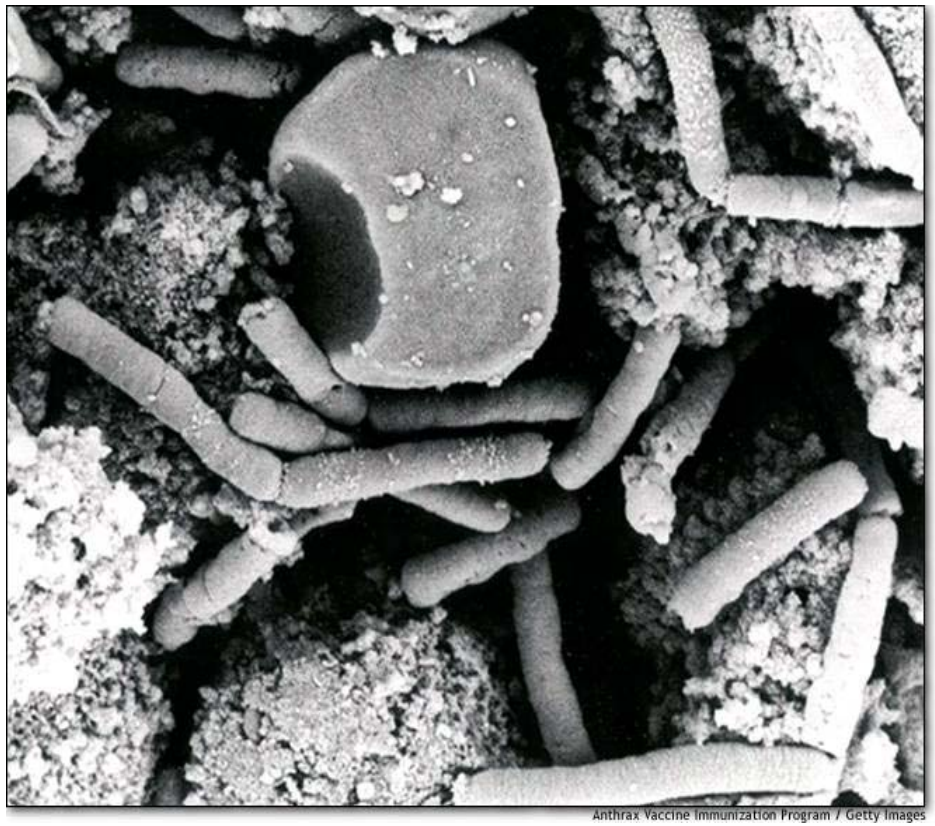


B. anthracis

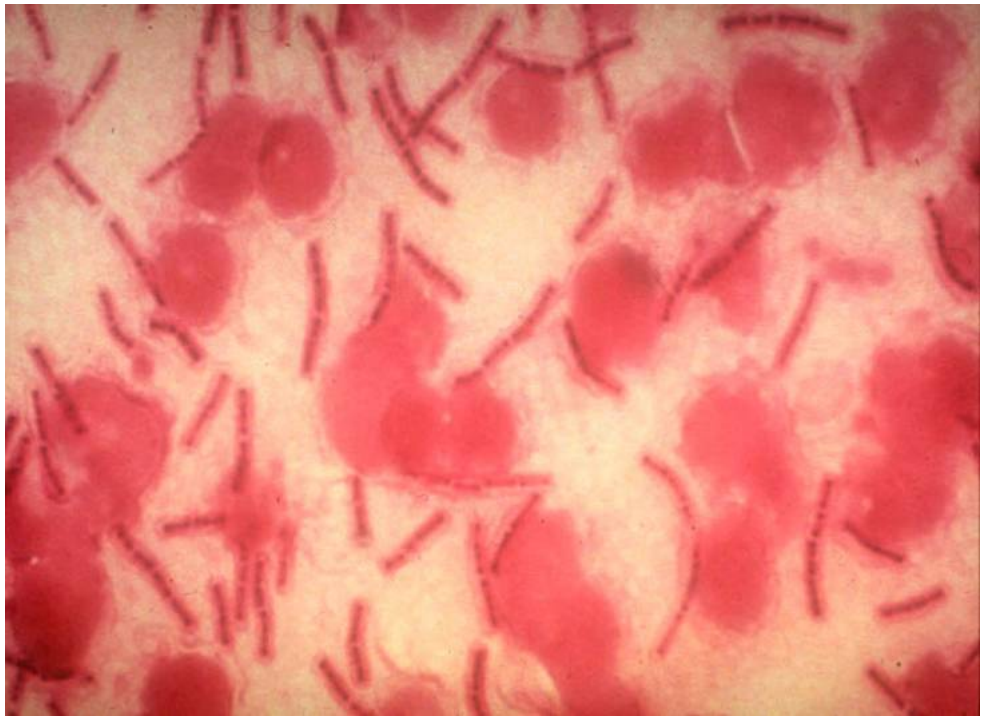
- *B. anthracis* is derived from ancestral member of the *Bacillus cereus* group
- *B. anthracis* grows in long chains *in-vitro*
- Specimens isolated from *in-vivo* growth demonstrate shorter chains
- Spores- form when nutrients are exhausted, and can last for decades





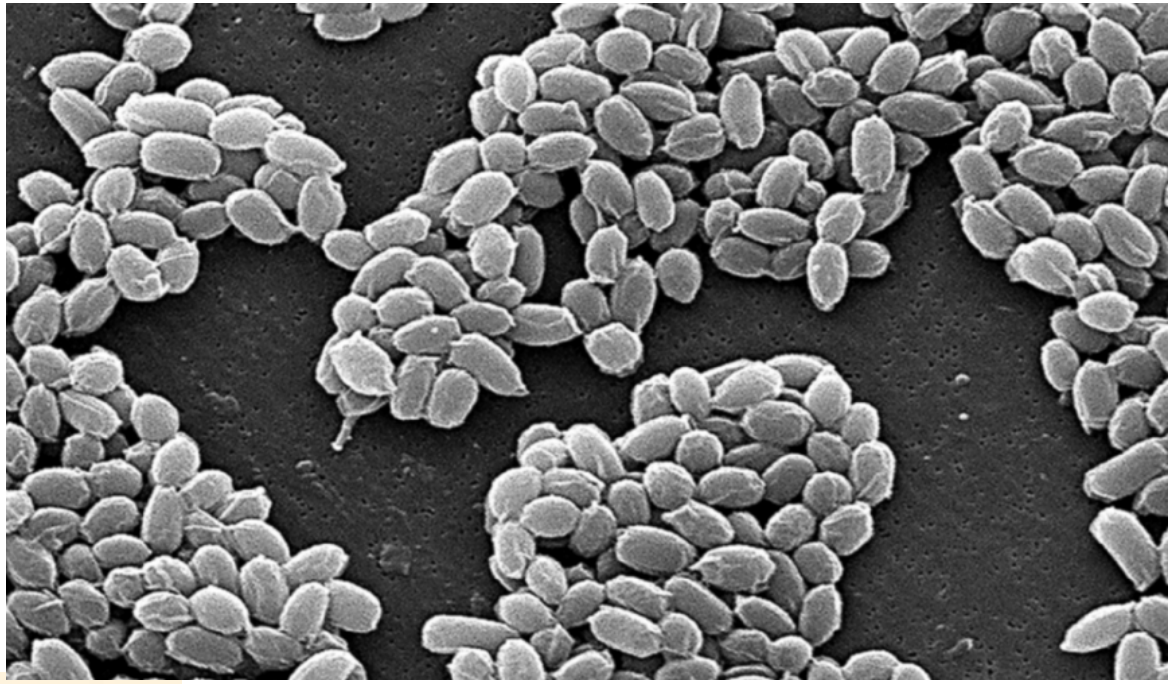


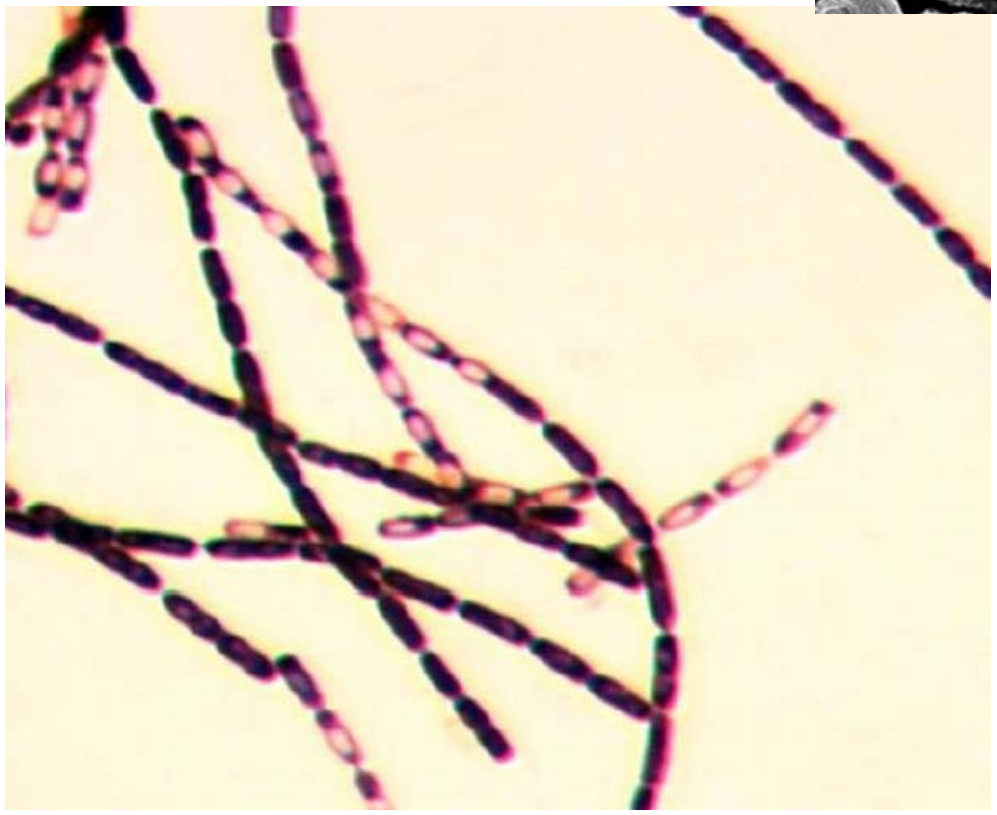
Anthrax Vaccine Immunization Program / Getty Images



B. anthracis spores

- Spore-dormant form of *B. anthracis*
- Highly resistant to adverse environmental conditions
  - Heat, ultraviolet and ionizing radiation, pressure and chemical agents
- Able to survive for long periods of in soils which accounts for the ecological cycle of the organism



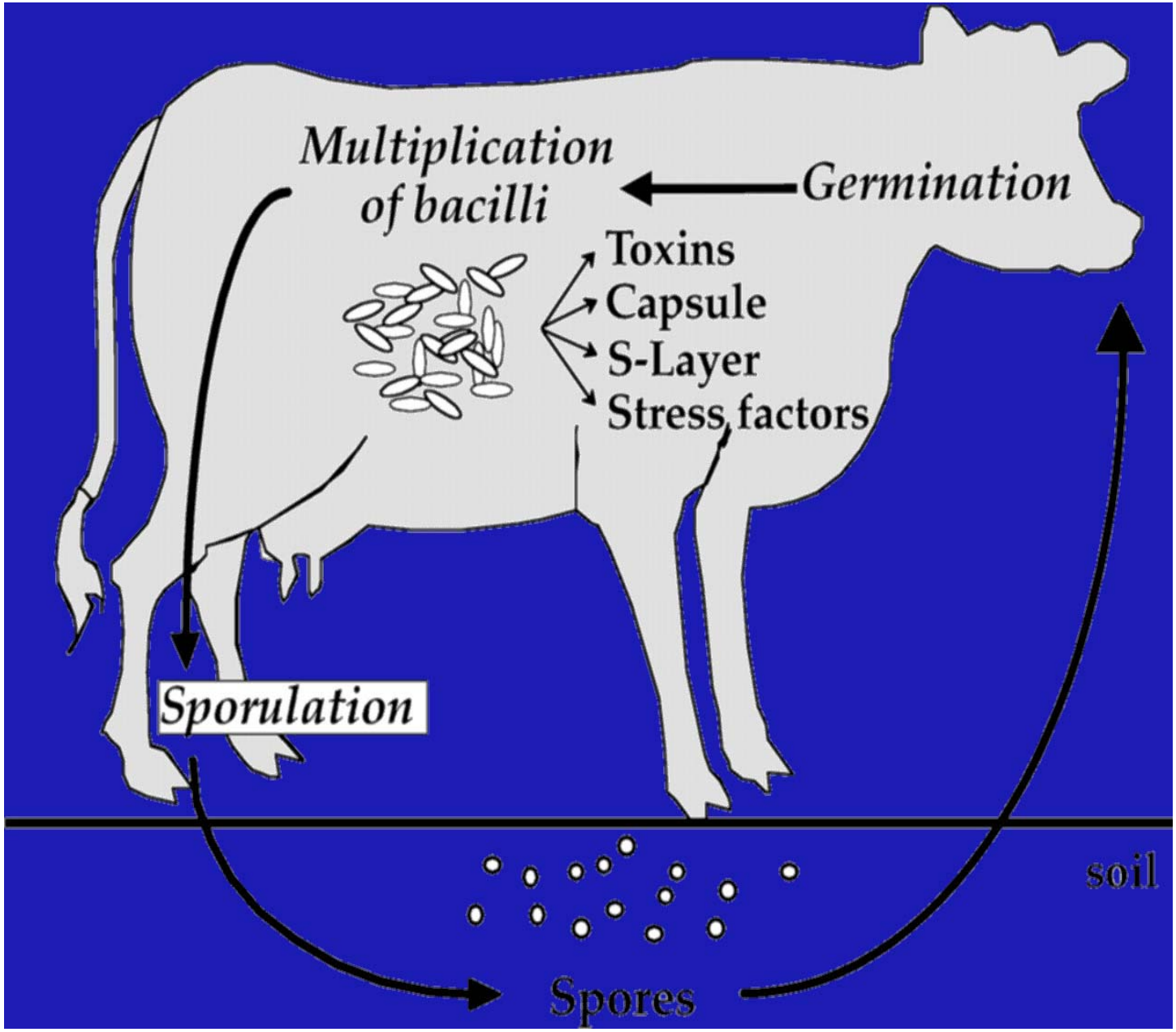


Microbiology

- *B. anthracis* spores reestablish vegetative growth when they are ingested by herbivores and germinate in an environment rich in amino acids, nucleosides and glucose
- Vegetative cells multiply rapidly and express virulence factors that kill the host

Microbiology

- *B. anthracis* bacilli shed by the dying animal will sporulate on contact with air and contaminate the soil
- The proportion of vegetative cells that become a dormant spore is variable
- Typically anthrax contamination lasts only months or a few years due to microbial competition


Multiplication
of bacilli
Germination
Toxins
Capsule
S-Layer
Stress factors
Sporulation
soil
Spores

Epidemiology

- However spores can survive for extended periods
  - 40 years in soil
  - 80 years in a vial
  - 200 years from bones from an archeological site

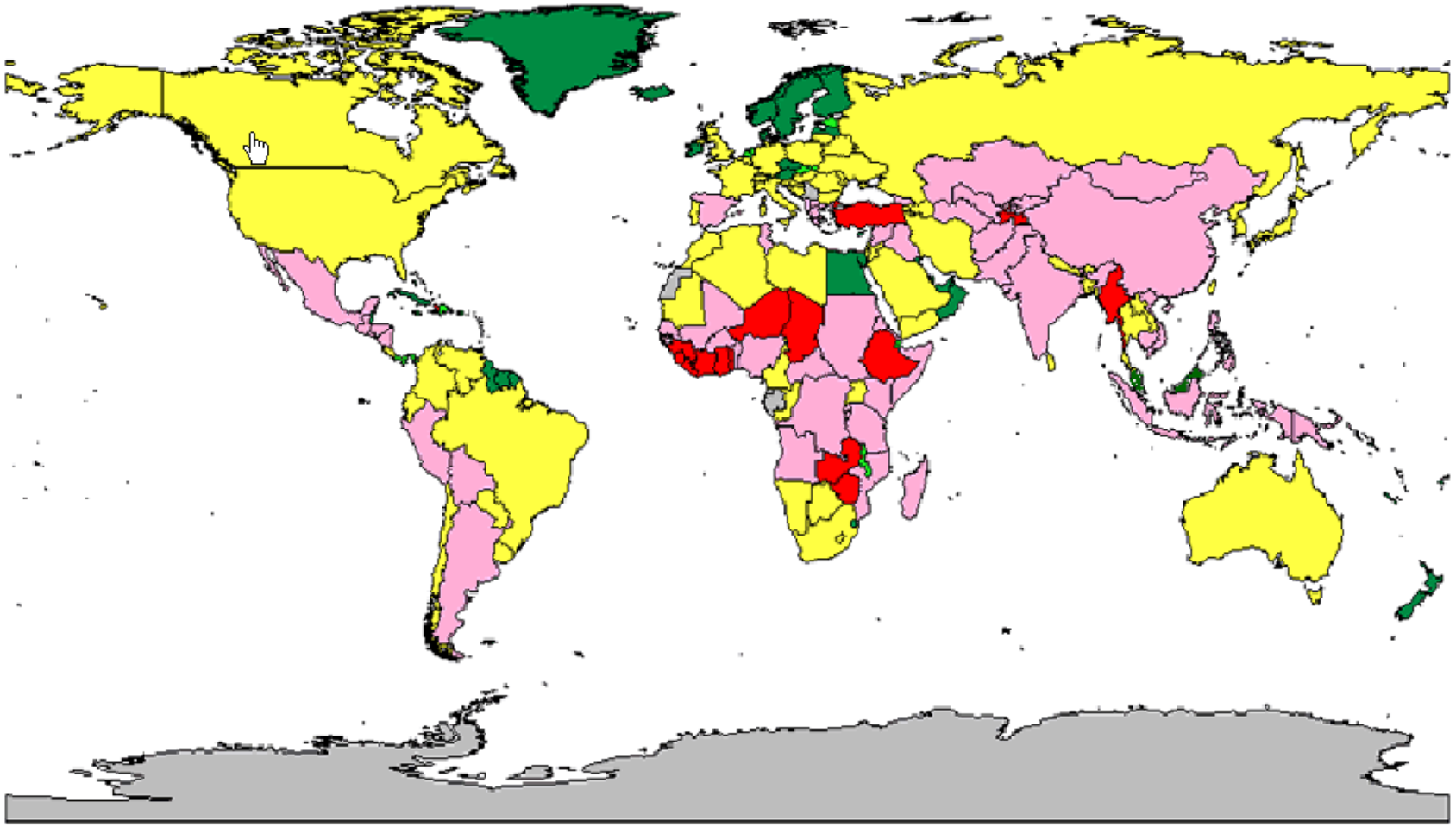
Gruinard Island

- *B. anthracis* weapons testing in 1943-1944 was conducted on a British island off the coast of Scotland
- Estimated 4 x 10^{14} anthrax spores were deposited on the island
- Spores were still detected >40 years later
- 1987 the 520 acre island was decontaminated with 280 tons of formaldehyde diluted in sea water

Gruinard Island is in the Scottish Highlands

Epidemiology

- Biting flies can transmit the disease from animal to animal or to humans
- Non-biting flies or vultures may transmit spores mechanically from one site to another


Hyperendemic / epidemic
Endemic
Sporadic
Probably free
Free
Unknown

Epidemiology

- *B. anthracis* is endemic or hyper-endemic
  - Most areas of Middle East, equatorial Africa
  - Mexico, Central America
  - Chile, Argentina, Peru and Bolivia
  - Myanmar, Vietnam Cambodia, Thailand
  - Papua New Guinea, China
  - Some Mediterranean countries

Epidemiology

- In the rest of the world, cases of anthrax occur only sporadically
- In U.S., since 1990 animal cases in Kansas, Nebraska, North Dakota, South Dakota, Missouri, California, Nevada, Texas and Oklahoma

Epidemiology

- Modes of Transmission (human cases)
  - Contact with infected tissues of dead animals (butchering) leading to cutaneous anthrax
  - Consumption of contaminated undercooked meat leading to gastrointestional or oropharyngeal anthrax
  - Contact with contaminated hair, wool or hides which can lead to either inhalational or cutaneous anthrax

Epidemiology

- Modes of Transmission (human cases)
  - Laboratory exposure
  - Person to person transmission-rarely reported with cutaneous anthrax but not recognized with inhalational or gastrointestinal anthrax

Epidemiology

- True incidence of anthrax is difficult to determine due to poor reporting
- 1958- between 20,000-100,000 cases annually world wide
- US early 1900's-127 cases per year to less than 10 cases since the 1960's
- More then 95% of all cases are cutaneous
- No gastrointestinal cases in U.S

Epidemiology

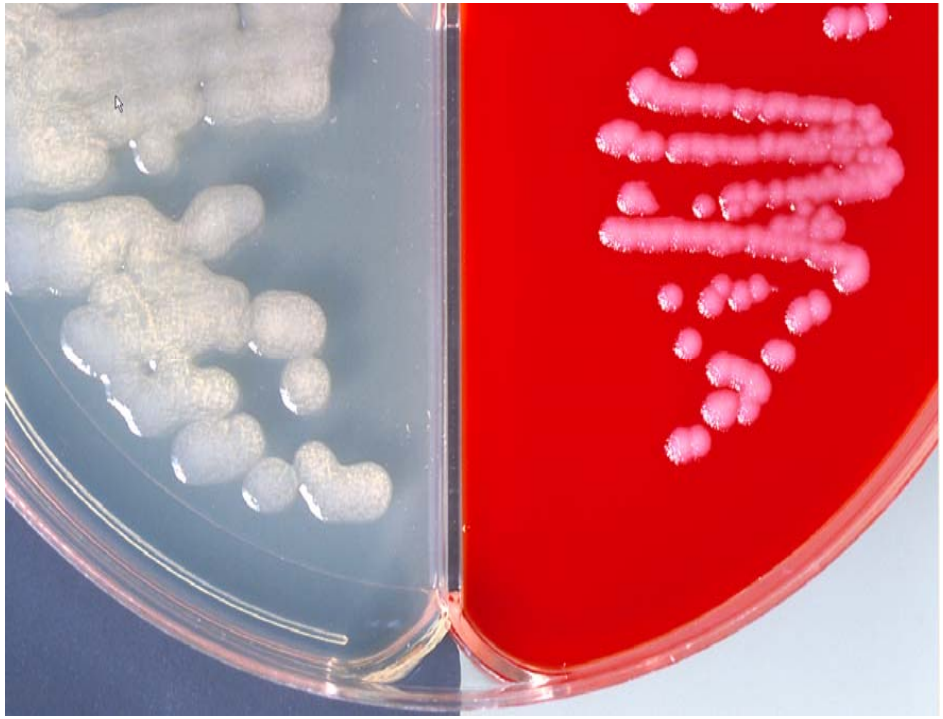
- In the 1980's and 1990's the global total of human anthrax cases decreased to around 2000 per year
- Human cases generally follow animal disease occurrence
- Most common in Africa, Middle East and parts of Southeast Asia

Pathogenesis

- *B. anthracis* produces three known virulence factors
  - Anti-phagocytic capsule (poly-D-glutamic acid) encoded on small plasmid-pX02
  - two binary exotoxins, (lethal toxin, edema toxin) encoded on large plasmid-pX01
- Virulence of all *B. anthracis* strains require both plasmids, pX01 and pX02

poly-D-glutamic acid capsule

- Provides resistance to phagocytosis
- Provides resistance to lysis by serum cationic proteins
- Needed for dissemination in a murine model of infection



Anthrax Toxins

- Plasmid pX01
  - Encodes three toxin genes, *pagA*, *lef* and *cya* which produce Protective antigen (PA), Lethal factor (LF) and Edema factor (EF) respectively
- The three exotoxins combine to produce two binary toxins:
  - PA + LF = Lethal Toxin (LT)
  - PA + EF = Edema Toxin (ET)

Anthrax Toxins

- LT- Zn^{++}metalloprotease which cleaves mitogen activated protein kinase kinases which are important in cell signal transduction and cell proliferation
- LT- causes apoptosis of activated macrophages and release of IL-1 and TNF-alpha
- ET- is a adenylate cyclase that converts adenosine triphosphate to cyclic adenosine monophosphate (cAMP)
- Increased cAMP leads to impaired water homeostasis and edema.
- ET-also inhibits neutrophil function

Toxin Pathogenesis

- Three identified host cell receptors for anthrax toxins
  - ART/tumor endothelial marker 8 (TEM8)
  - Capillary morphogenesis protein 2 (CMG2)
  - A recently discovered co-receptor called low-density lipoprotein receptor-related protein 6 (LRP6)
- PA binds to these receptors on the eukaryotic cell surface and is cleaved by the protease furin

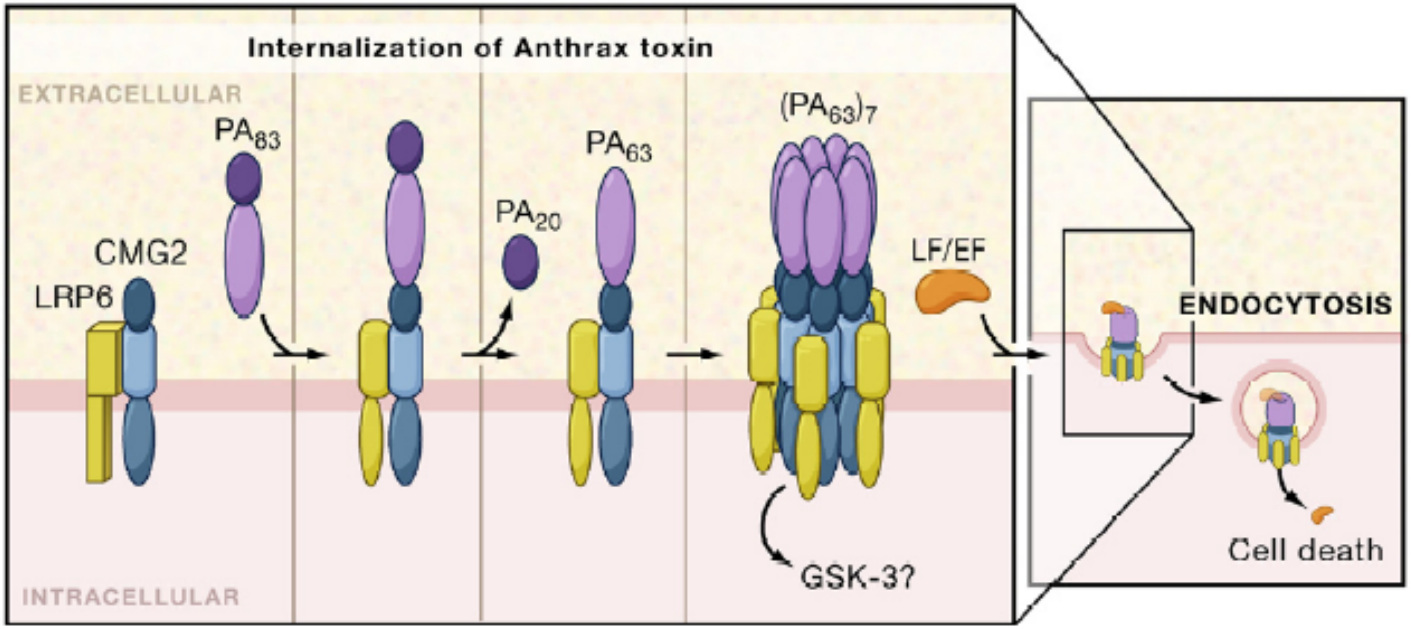
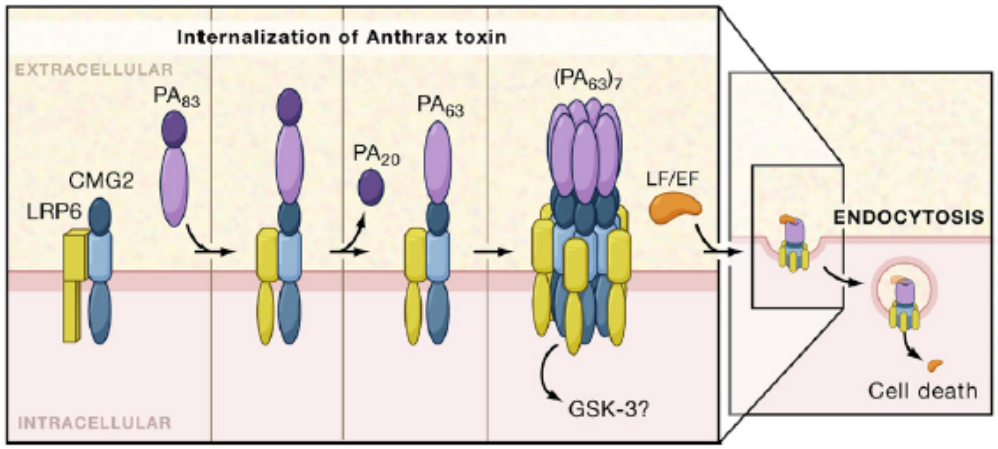


Figure 1. Model for Anthrax Toxin Endocytosis

The receptors for anthrax toxin CMG2/TEM8 (blue) form a complex with LRP6 (yellow) through the extracellular domains of both proteins. Upon binding of PA_{83}, a possible conformational change (depicted as the conversion of a rectangle to an oval) occurs in LRP6 that is propagated to the C-terminal cytoplasmic domain. Furin cleavage leads to the release of PA_{20} and the formation of a heptameric pore precursor. Binding of lethal factor (LF) or edema factor (EF) to the pore precursor is followed by endocytosis, which is known to be a clathrin-dependent process. It is currently unknown at which point after binding to the receptor the internalization signal from LRP6 is transferred to another protein (perhaps GSK-3), but the transfer likely follows heptamer formation or the binding of LF/EF.

Toxin Pathogenesis

- After PA 83 is cleaved, the receptor bound PA 63 forms a heptamer pore precursor
- Binding of LF or EF to the pore precursor is followed by endocytosis into the cell



Toxin Pathogenesis

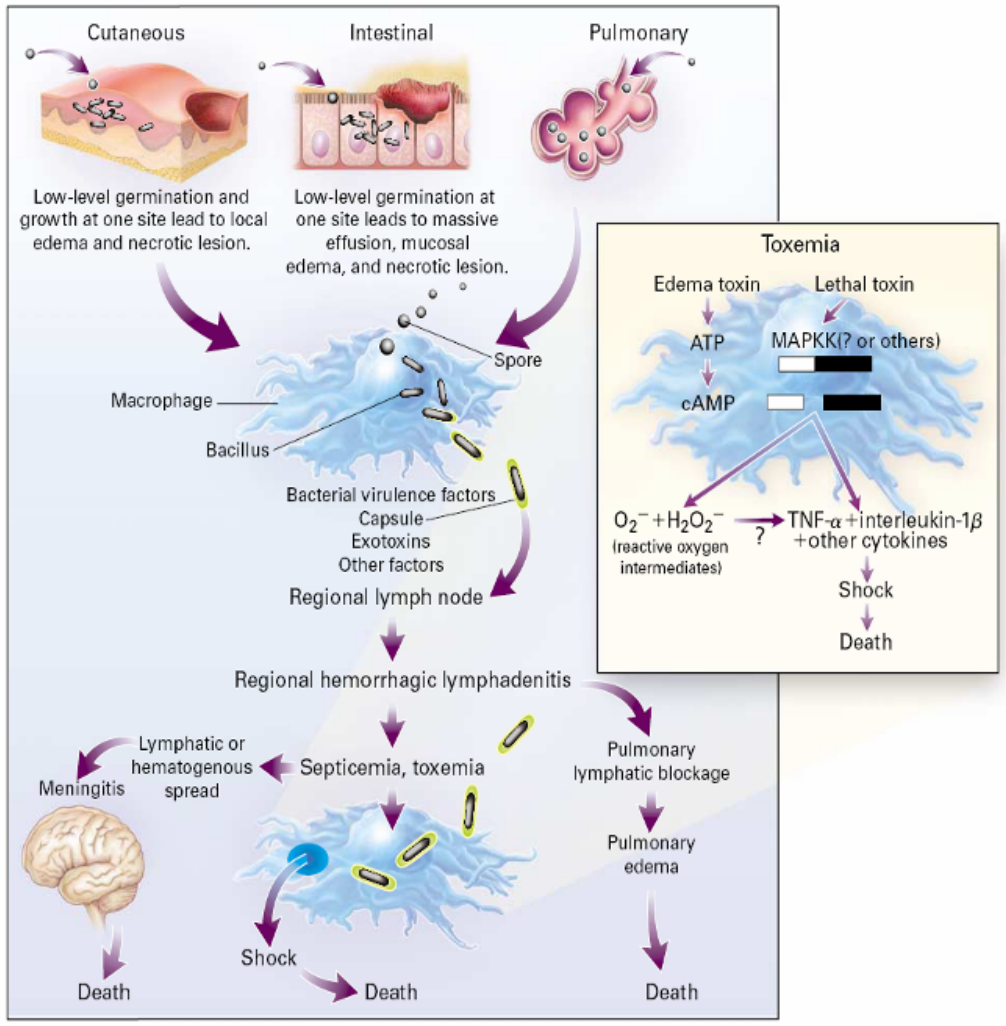
- In the endocyte LT and ET are then able to enter the cell cytoplasm and exert their effects.
- Anthrax toxin targets are neutrophils and macrophages although other cells are likely involved as well.

Anthrax Gene Regulation

- pX01 encodes *atxA* a transcriptional activator of toxin production
  - *atxA* transcription is up-regulated with high CO_2 tension and elevated temperature (37C)
- AtxA protein also up-regulates the capsule biosynthetic genes (*capBCA*) on pX02
- Evidence suggests that AtxA also regulates a large number genes, including chromosomal genes of *B. anthracis*

Anthrax Pathogenesis

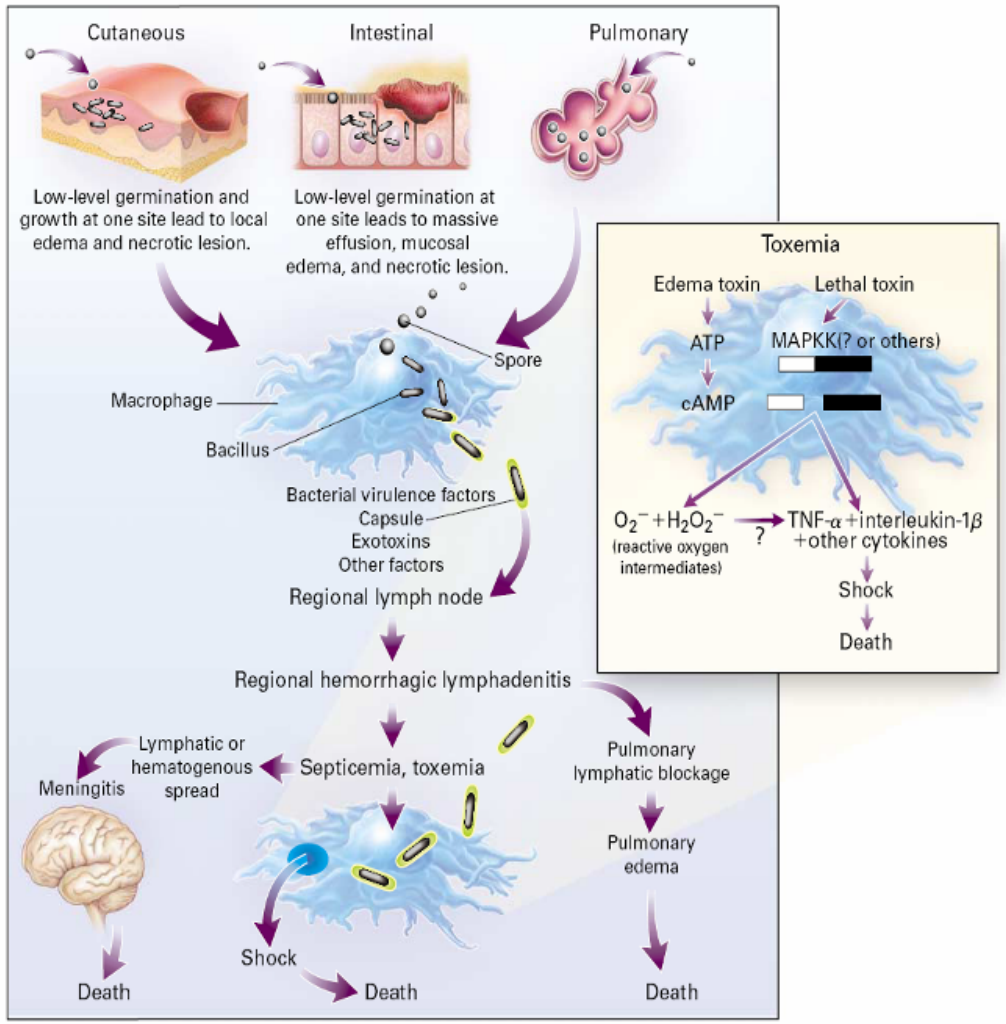
- Introduction of spore through the skin or mucosa
- Spore germinates into bacillus after ingestion by macrophages
- Capsule and Toxins are produced
- Bacilli leave the macrophages and multiply in the lymphatics



Dixon, T.C et al. New Eng. Jour. Med. 1999; 341:815-826.

Anthrax Pathogenesis

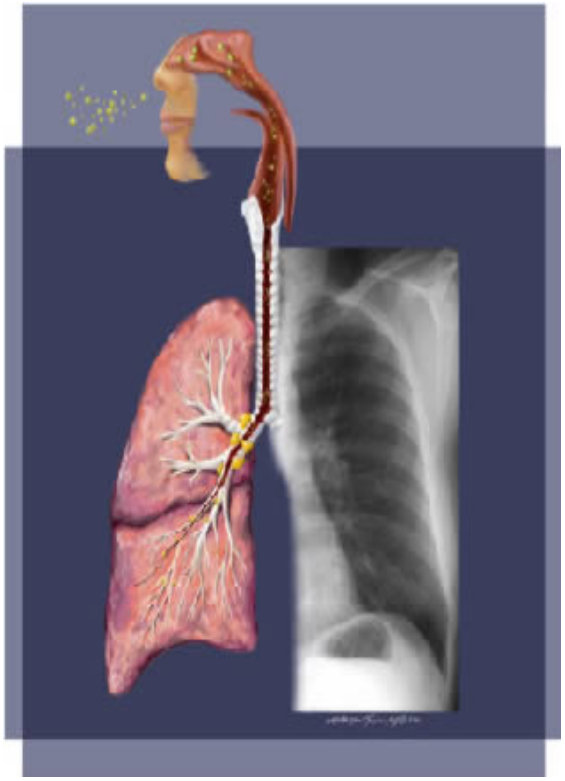
- Organisms spread to the draining lymph node
- Pathological findings include hemorrhagic, edematous and necrotic lymphadenitis
- With tracheobronchial lymph node involvement (inhalational anthrax), a hemorrhagic necrotizing mediastinitis results

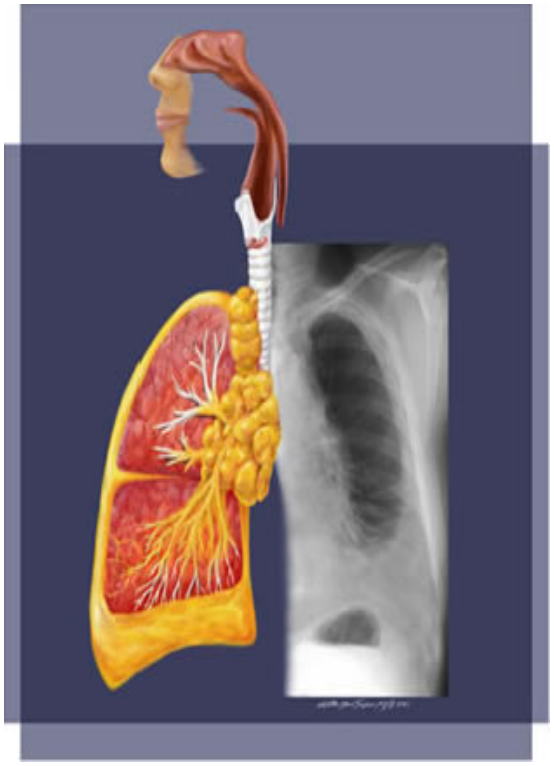


Dixon, T.C et al. New Eng. Jour. Med. 1999; 341:815-826.

Anthrax Pathogenesis

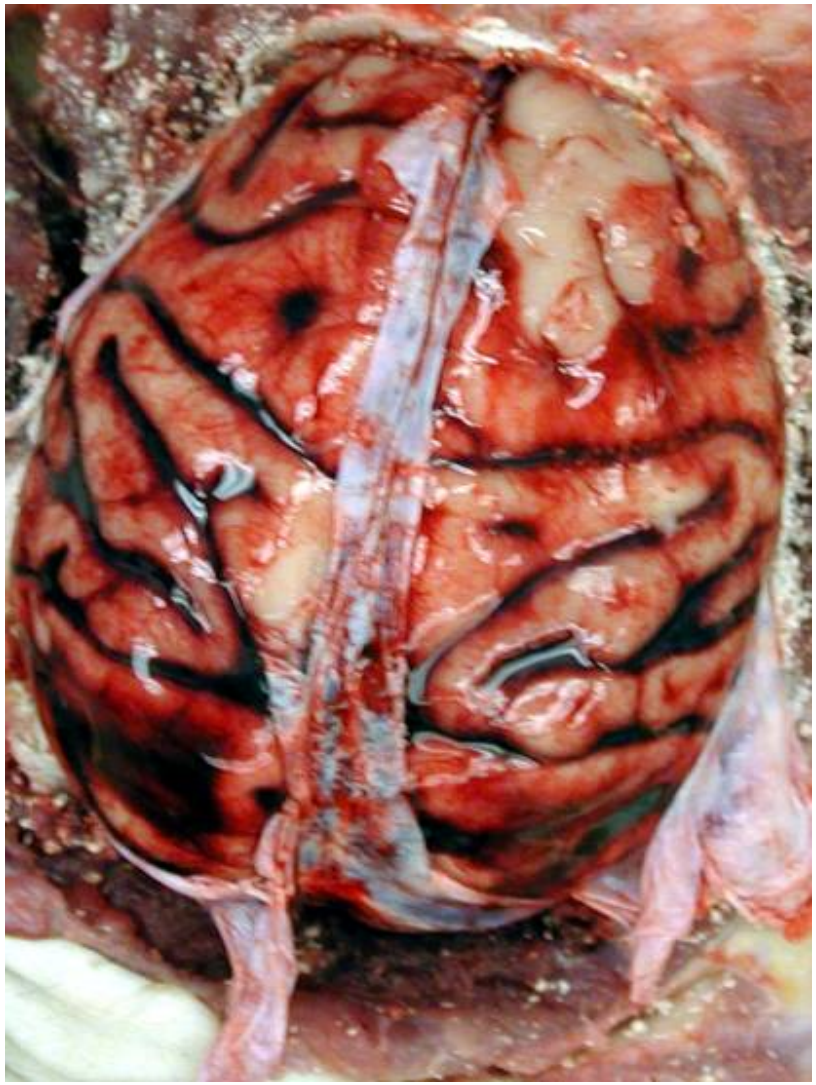
- Bacteria then spread through the lymph causing bacteremia and organ seeding
- Death is likely due to respiratory failure associated with lymphatic obstruction and pulmonary edema, overwhelming bacteremia and septic shock



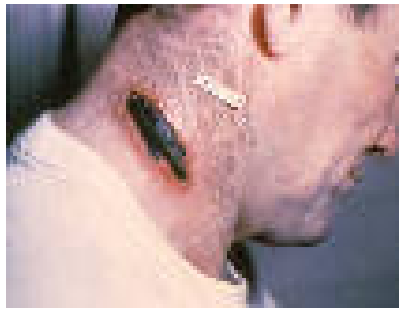


Anthrax Pathogenesis

- Meningitis and subarachnoid hemorrhage can also occur

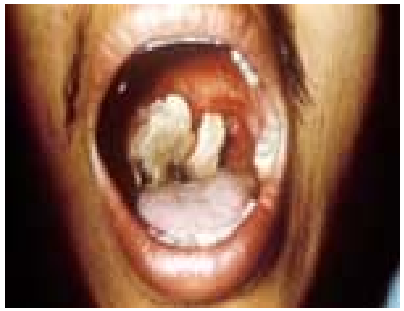


Epidemiology of Transmission



Cutaneous anthrax

Direct contact and fly bites



Oropharyngeal and gastrointestinal anthrax

Ingestion

Pulmonary/mediastinal anthrax

Inhalation

Infected herbivores and soil are reservoir

Clinical Syndromes of Anthrax

- Clinical presentation varies by route of infection
  - Cutaneous
  - Gastrointestinal
  - Oropharyngeal
  - Inhalational or Pulmonary
- Can also see combinations of these

Cutaneous Anthrax

- “Malignant pustule”
- 95 % of all naturally-acquired anthrax infections
- Caused by inoculation of spores (or bacilli) into compromised skin.
- Mechanical transmission by biting arthropods
- Low number of spores required for transmission

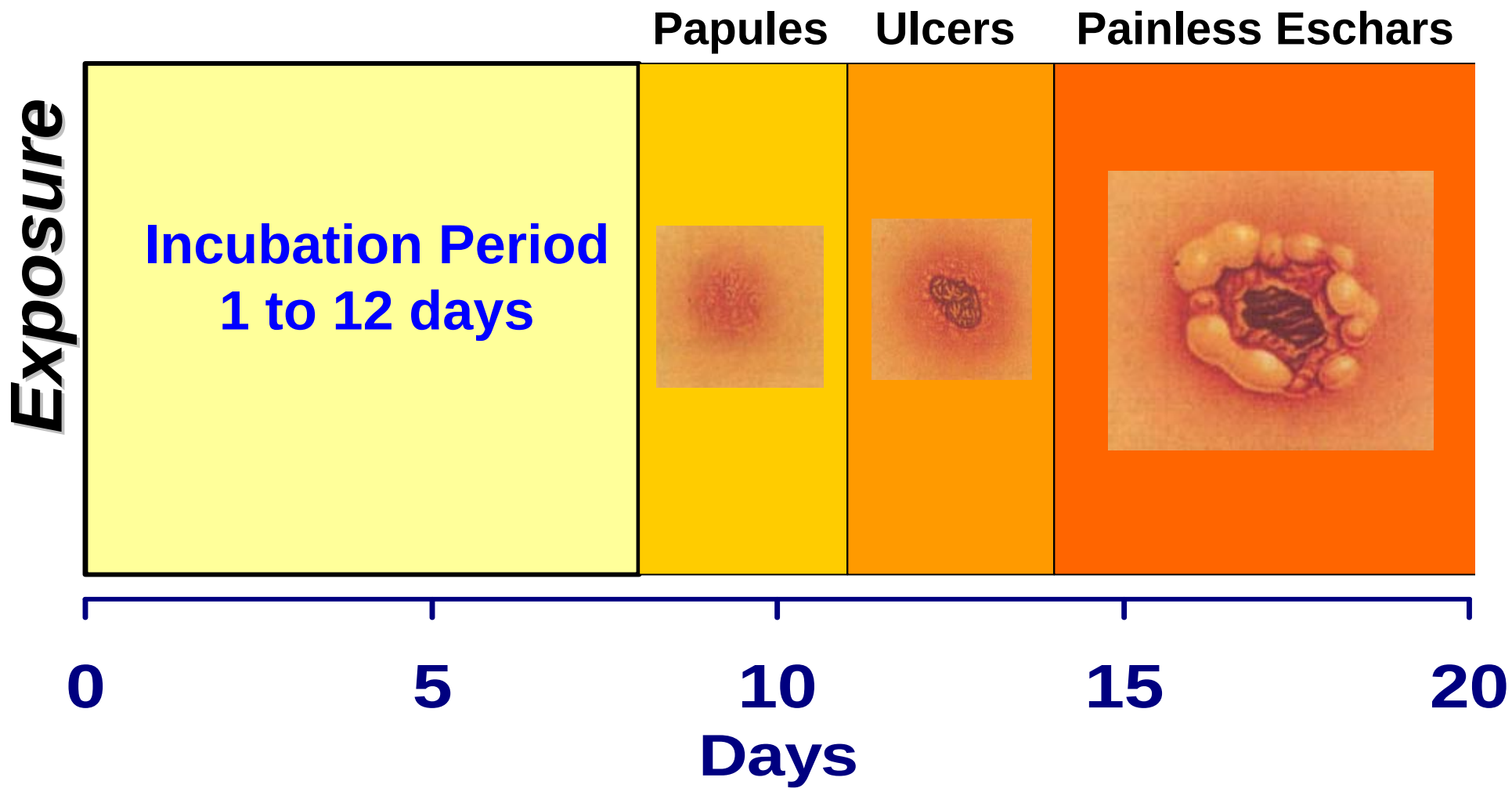
Cutaneous Anthrax

- Incubation period – 1 to 7 days (usually 2 to 5 days)
- Local symptoms- painless or pruritic papule or pustule becoming vesicular or ulcerative then becomes a black eschar
- Varying degree of edema
- May have satellite vesicles

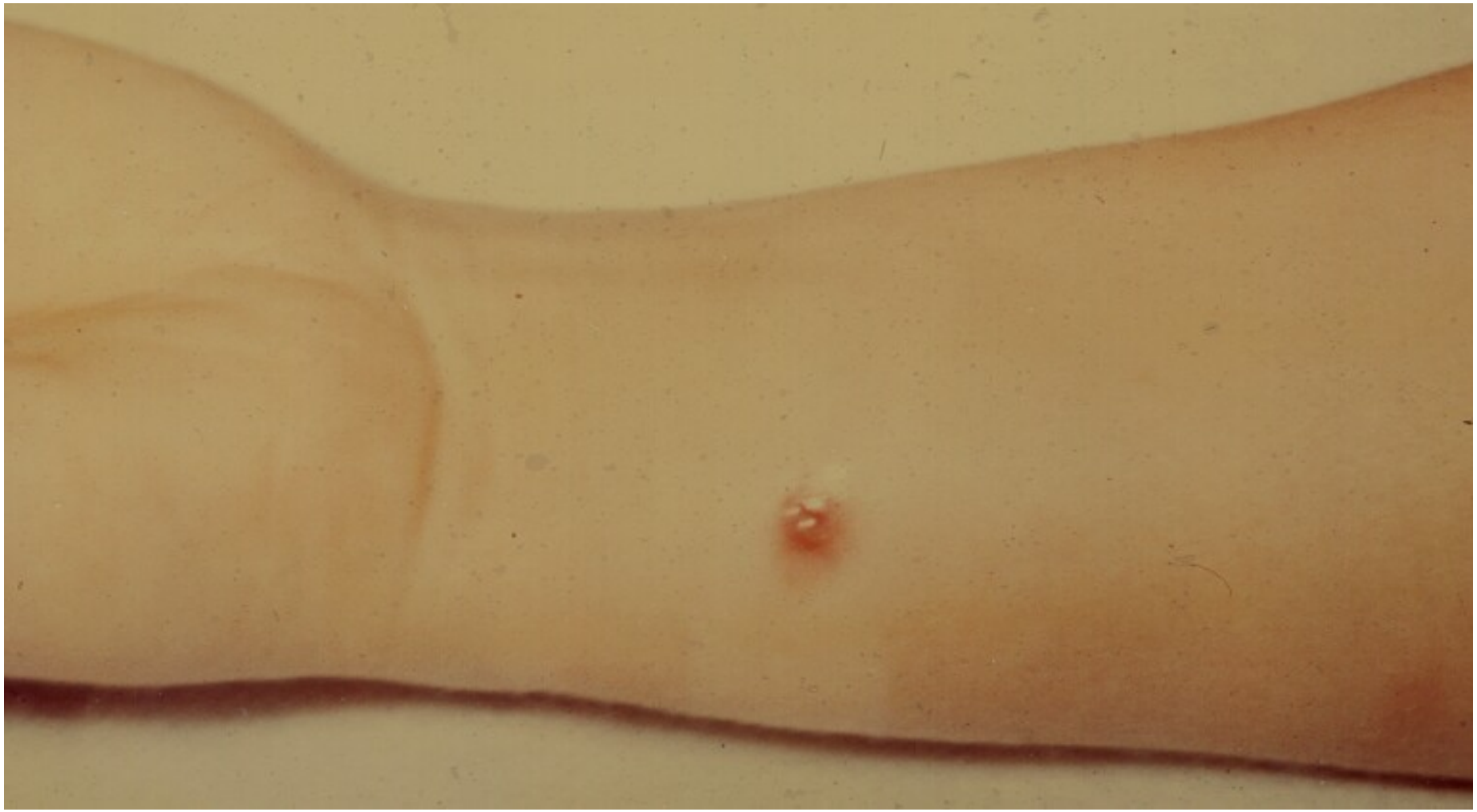
Cutaneous Anthrax

- Systemic symptoms- fever malaise, headache, regional lymphadenopathy
- Lesion- eschar develops over 2-3 weeks and separates and leaves a scar
- Septicemia rare, but can occur
- Mortality if untreated~20%
- Mortality if treated-<1%

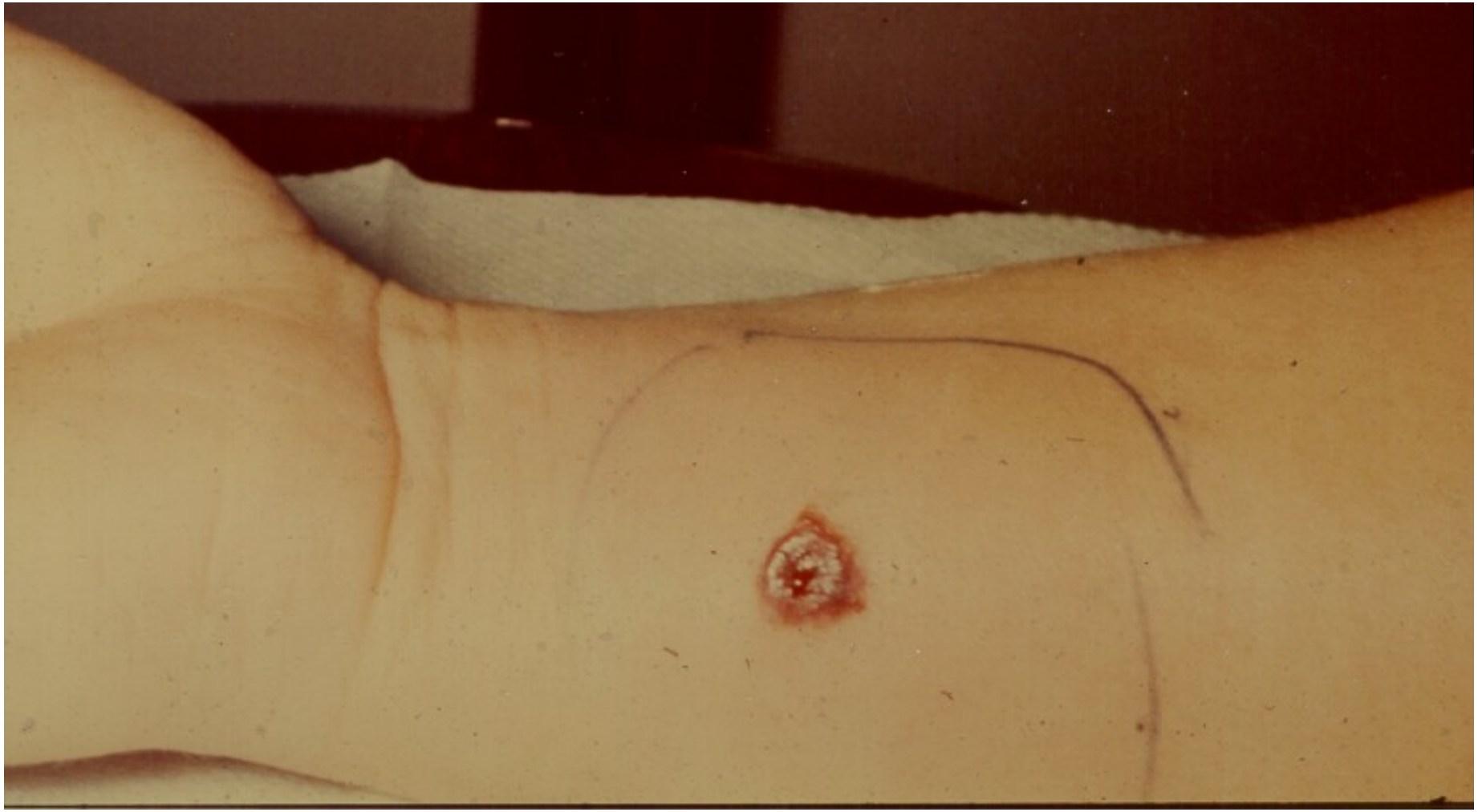
Clinical Timeline for Cutaneous Anthrax



Day 4



Day 5



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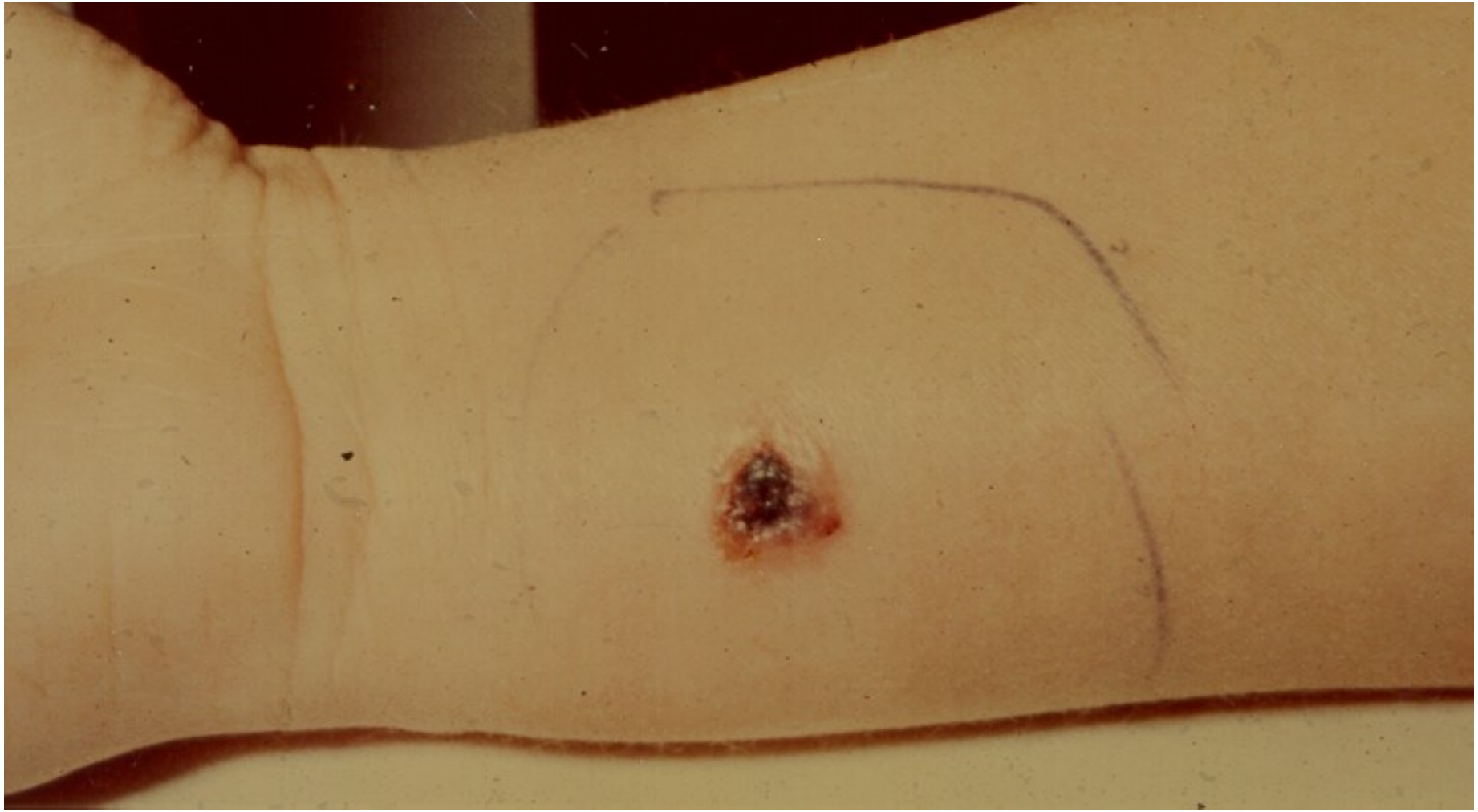
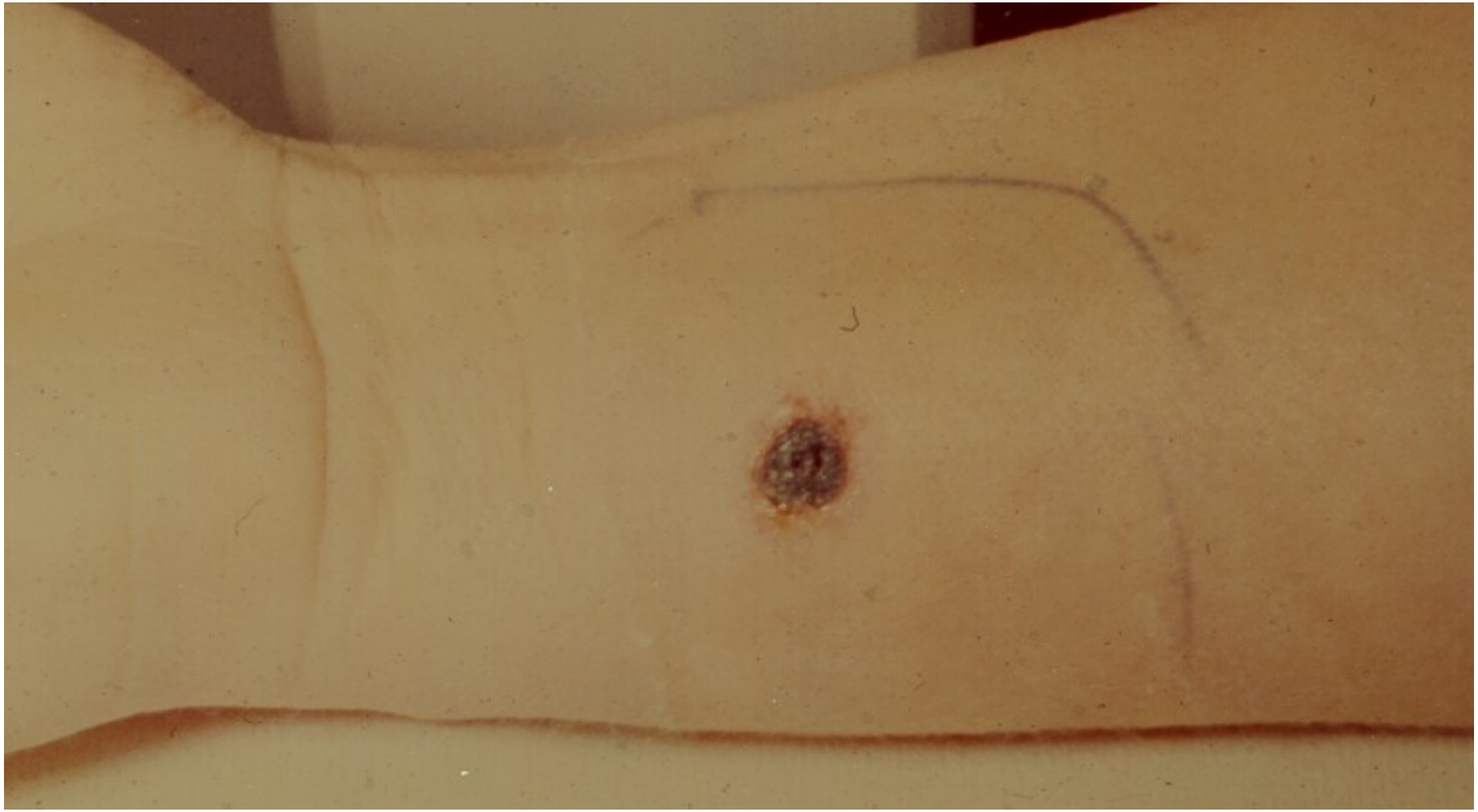
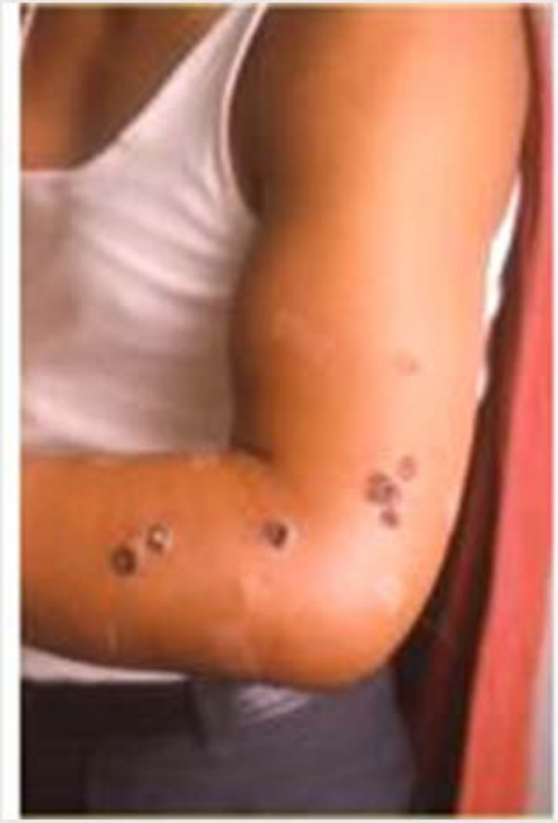
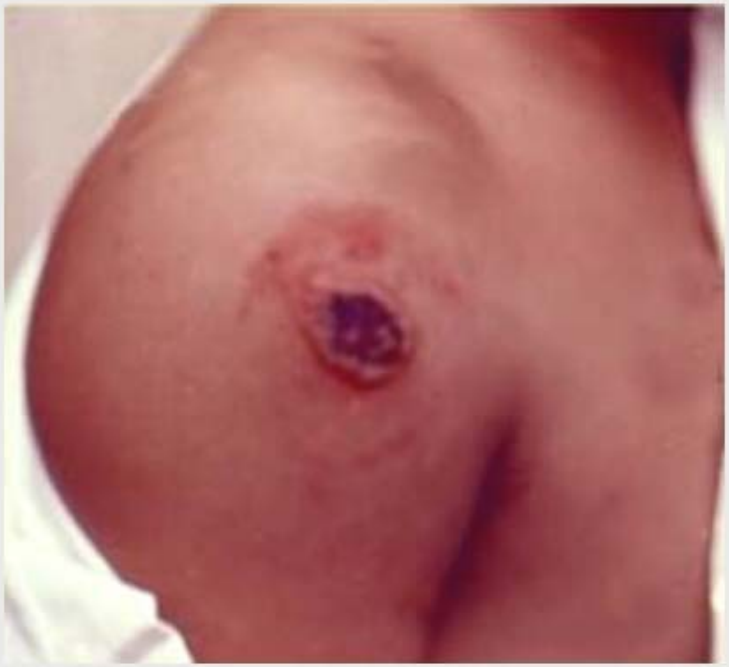


Day 10



Cutaneous Anthrax

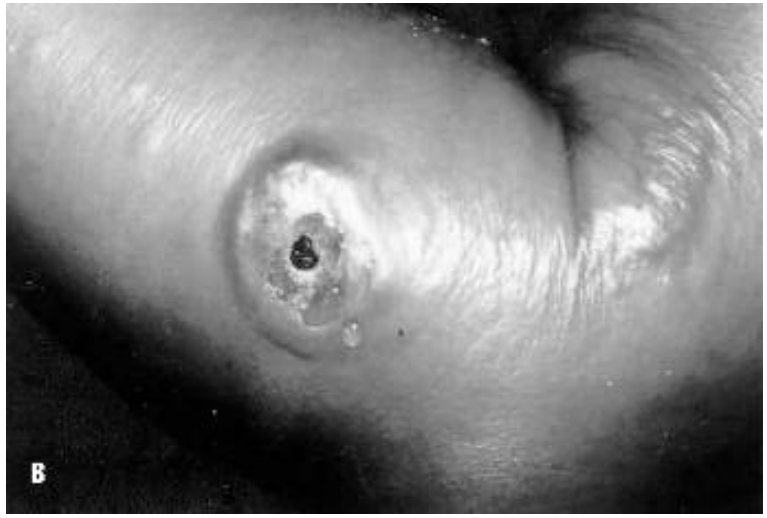




Images from US CDC

http://www.bt.cdc.gov/agent/anthrax/anthrax-images/cutaneous.asp


A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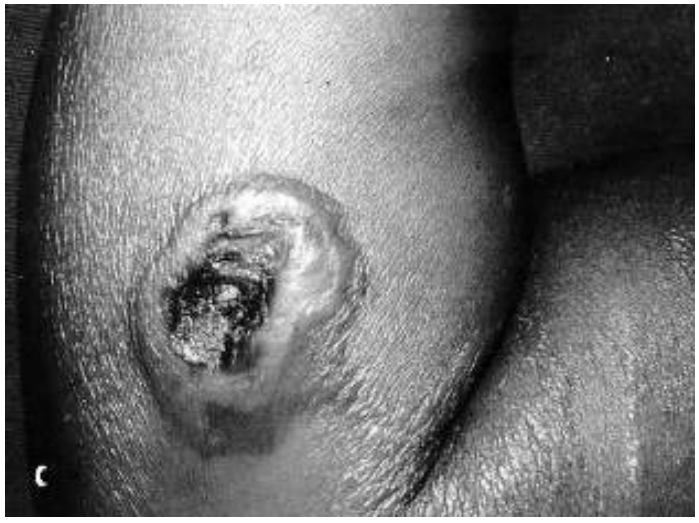
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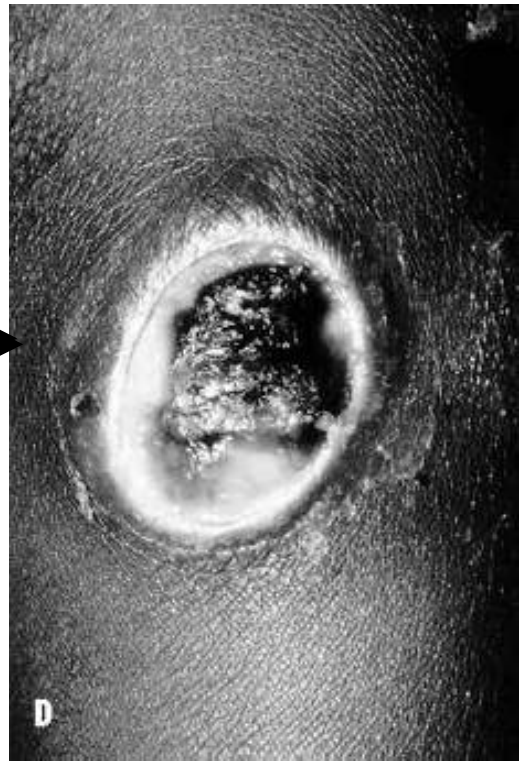
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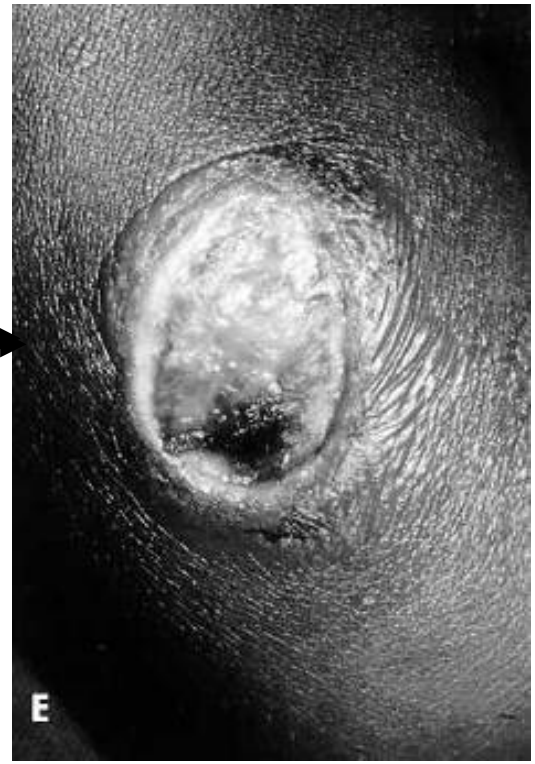
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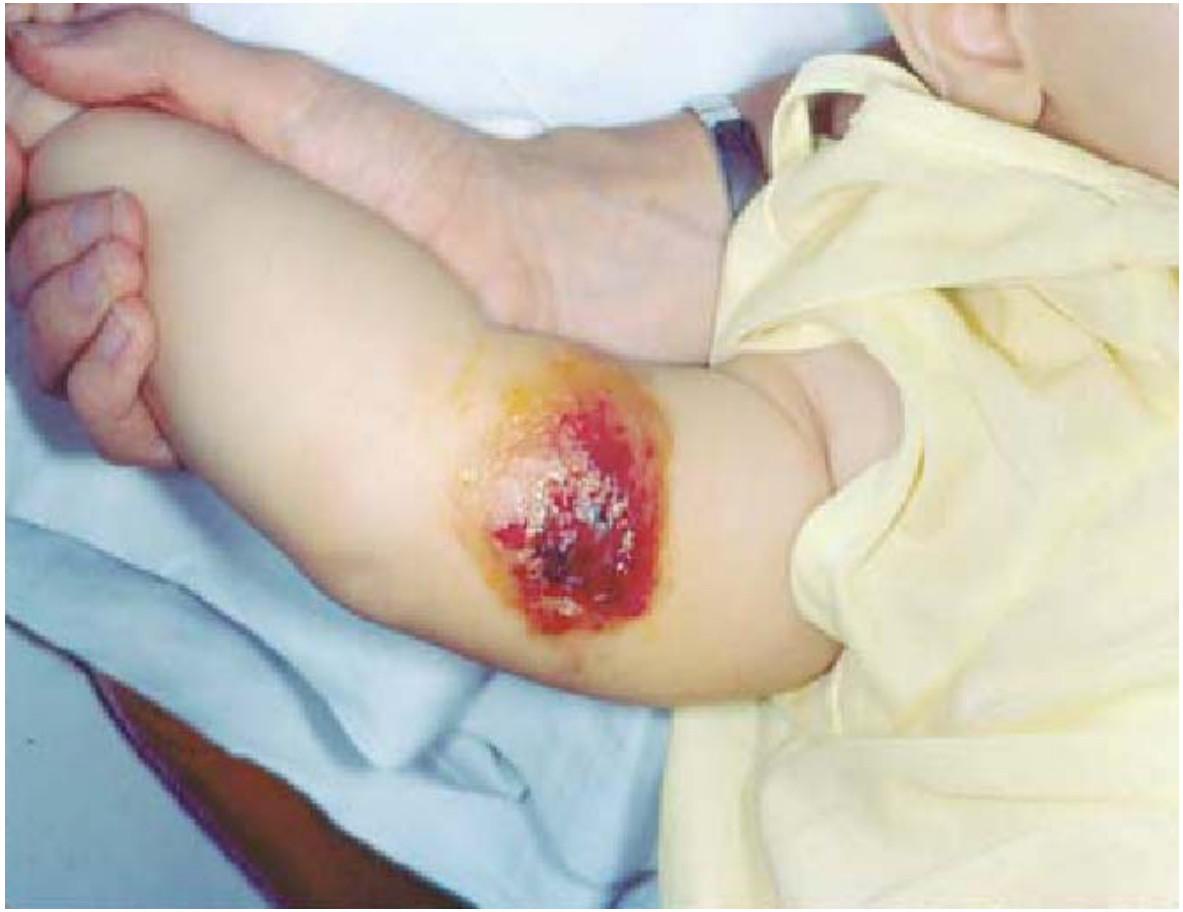

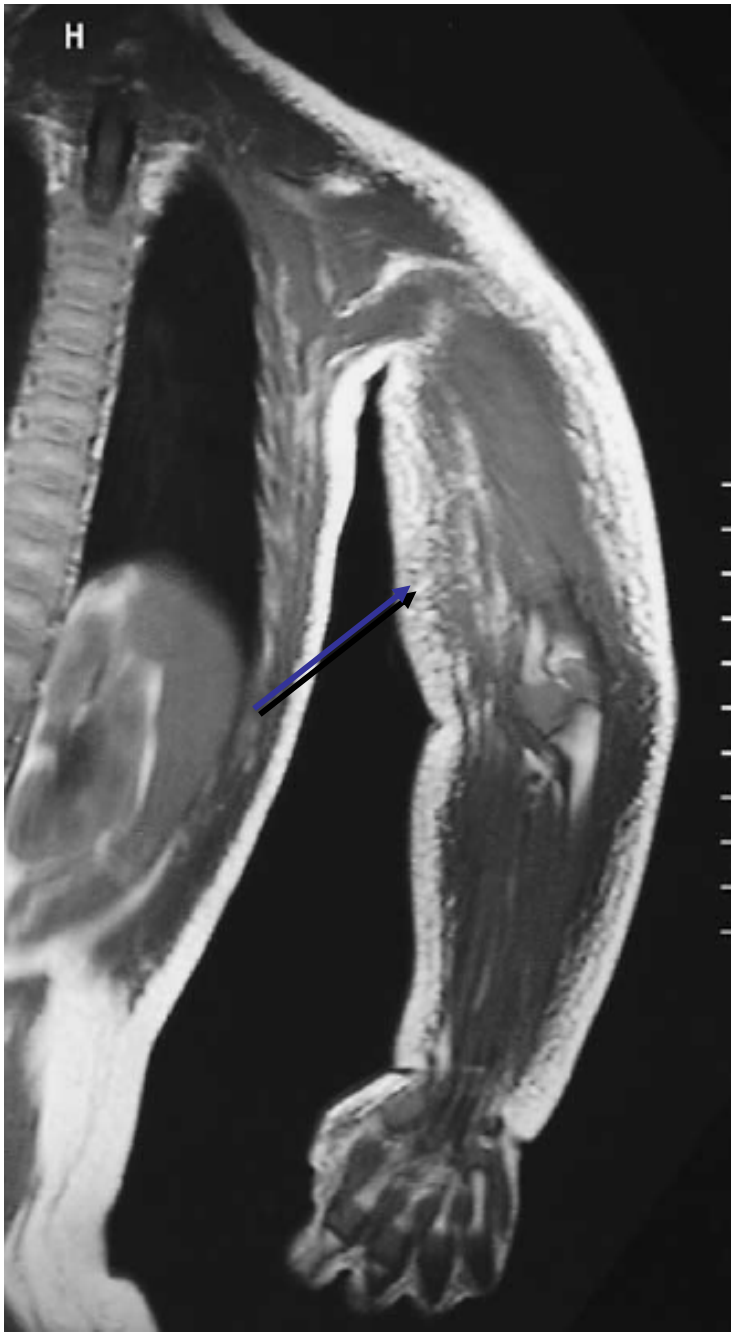
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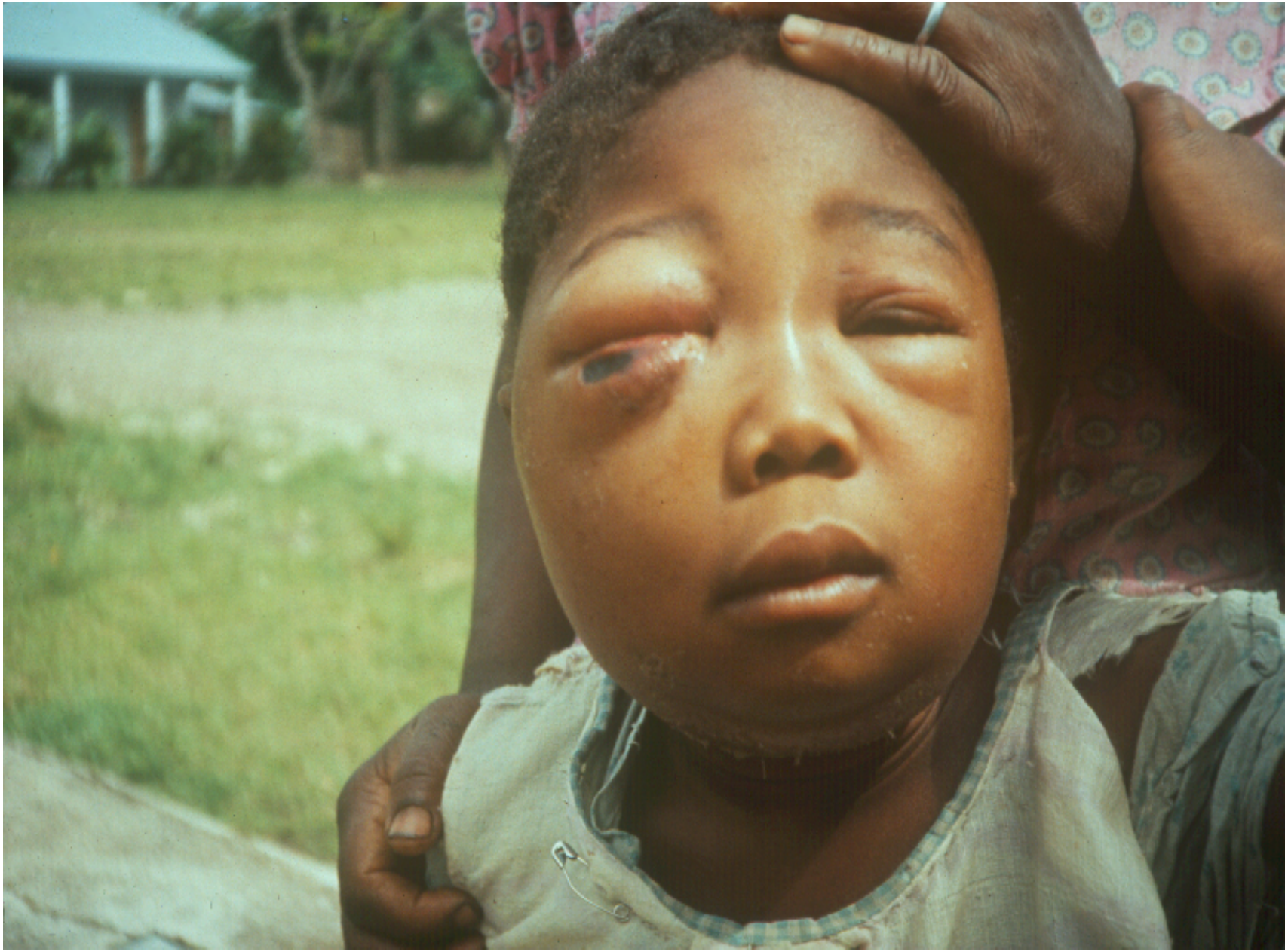
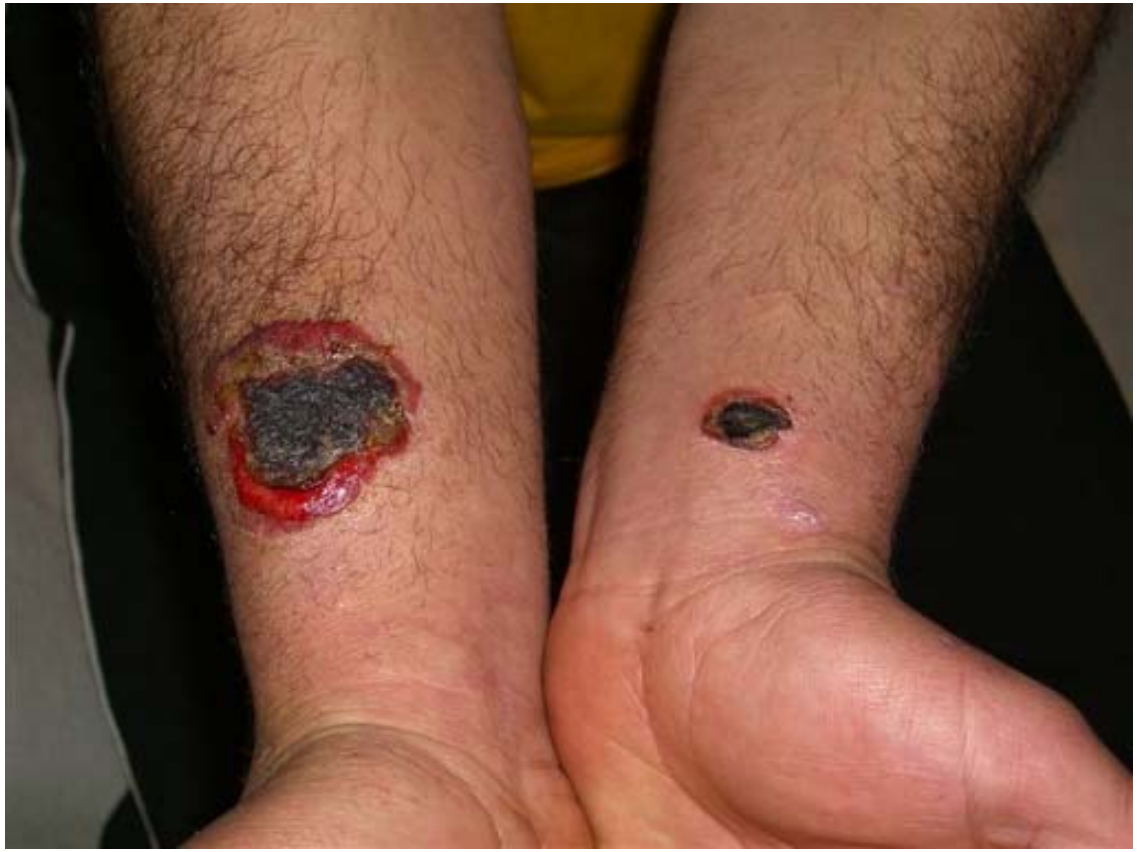
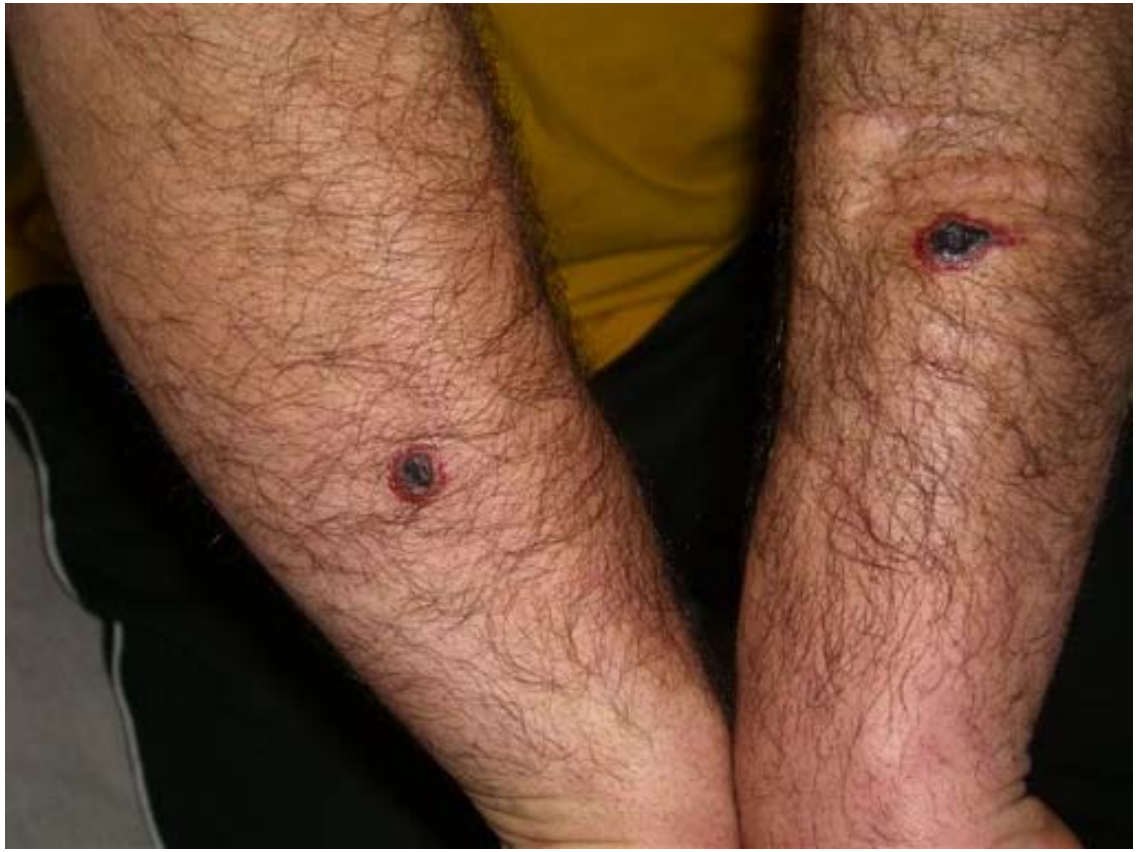
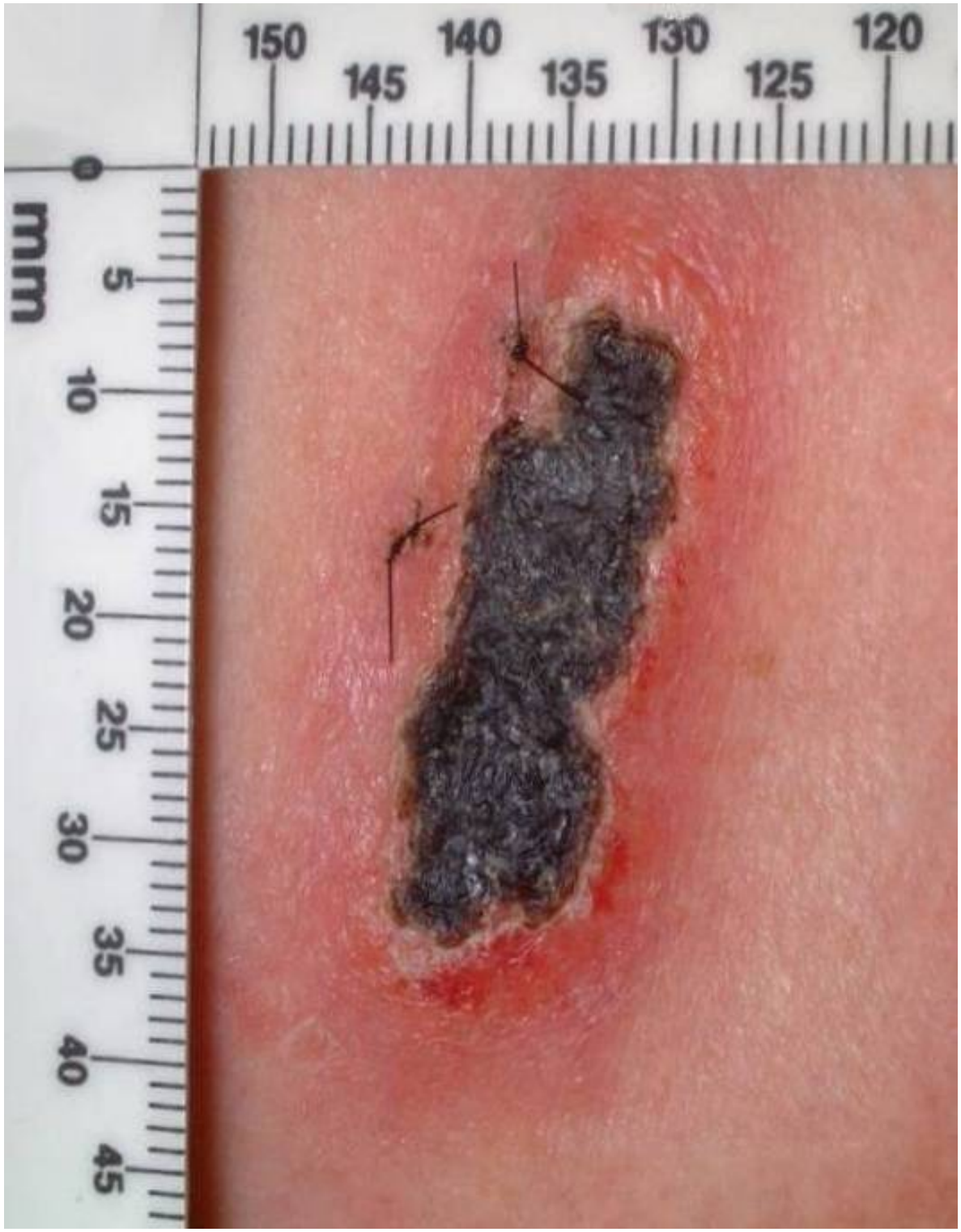




Courtesy of Robert Rivard MD



Courtesy of Robert Rivard MD


150
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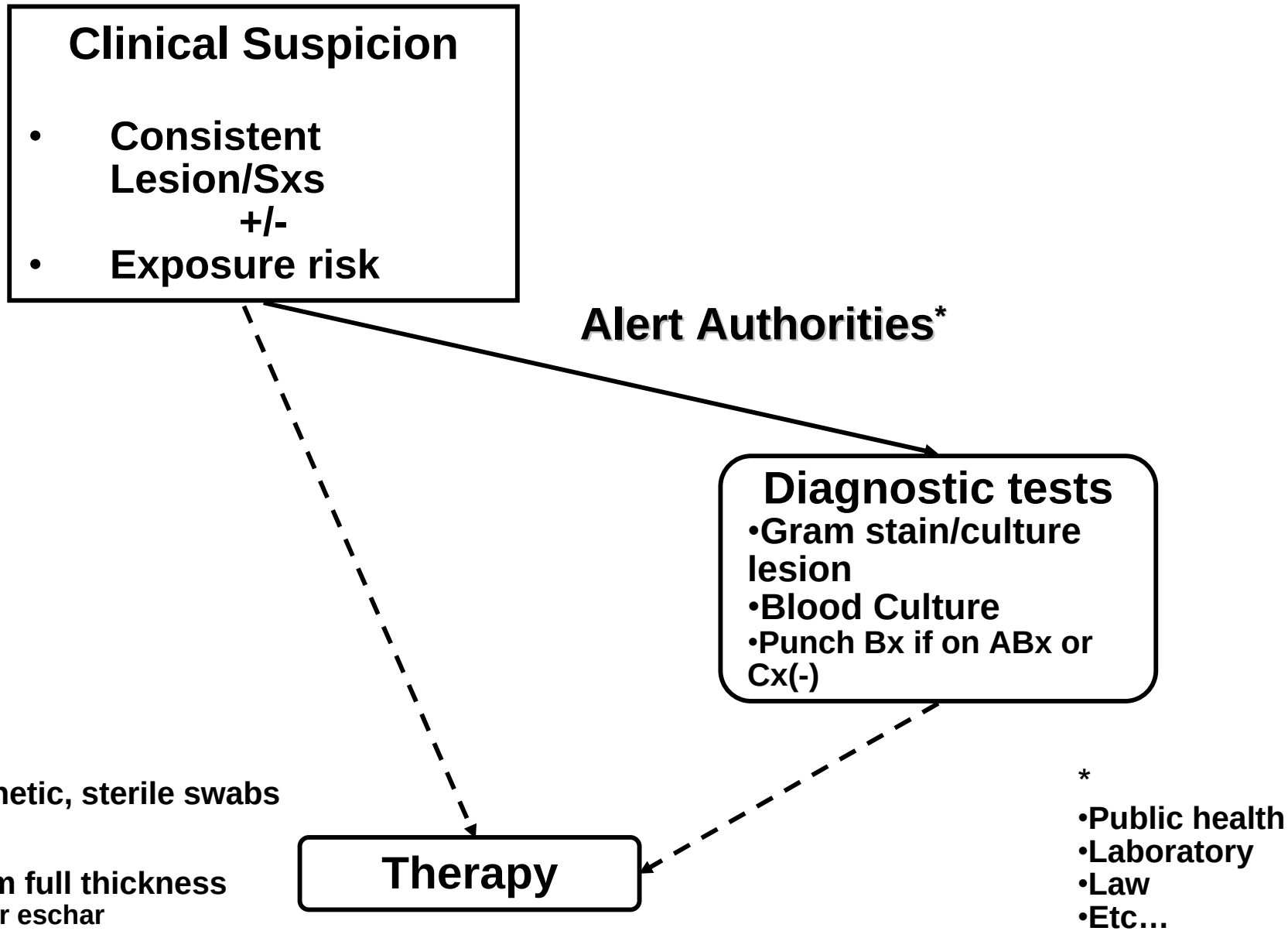
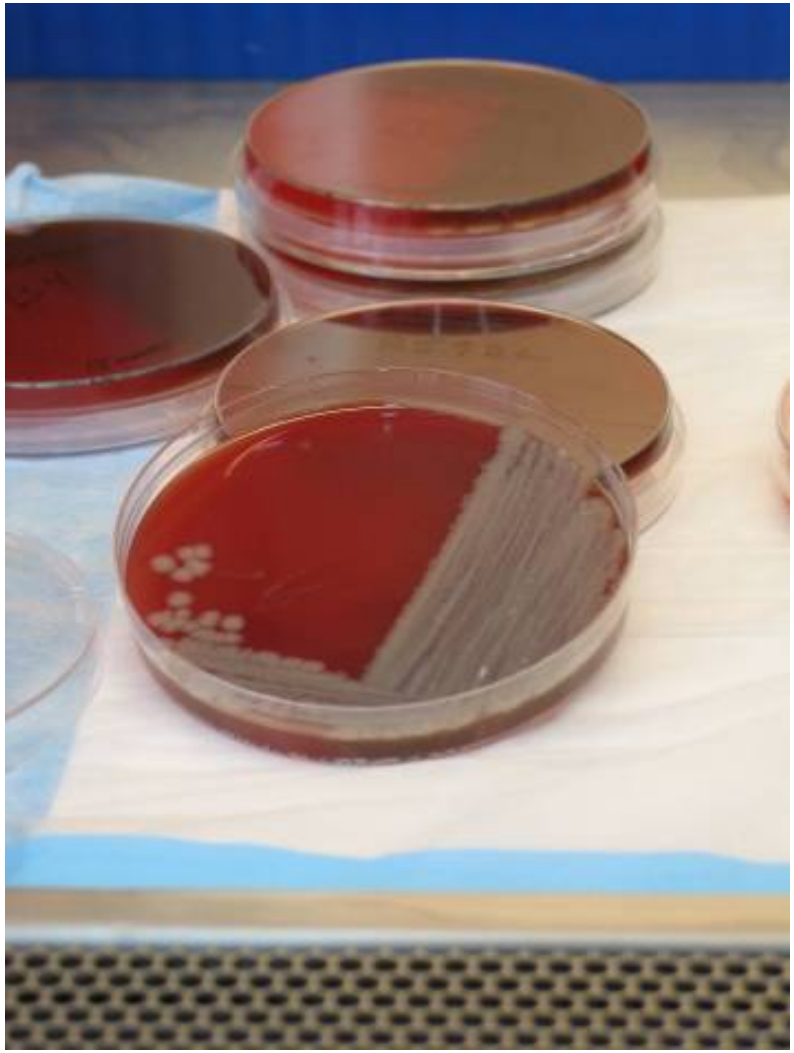
Diagnosis: Cutaneous Anthrax



Anthrax Diagnostics

- Presumptive diagnosis
  - Colony morphology
  - Gram stain
  - Biochemical testing
- Achievable in most hospital micro labs

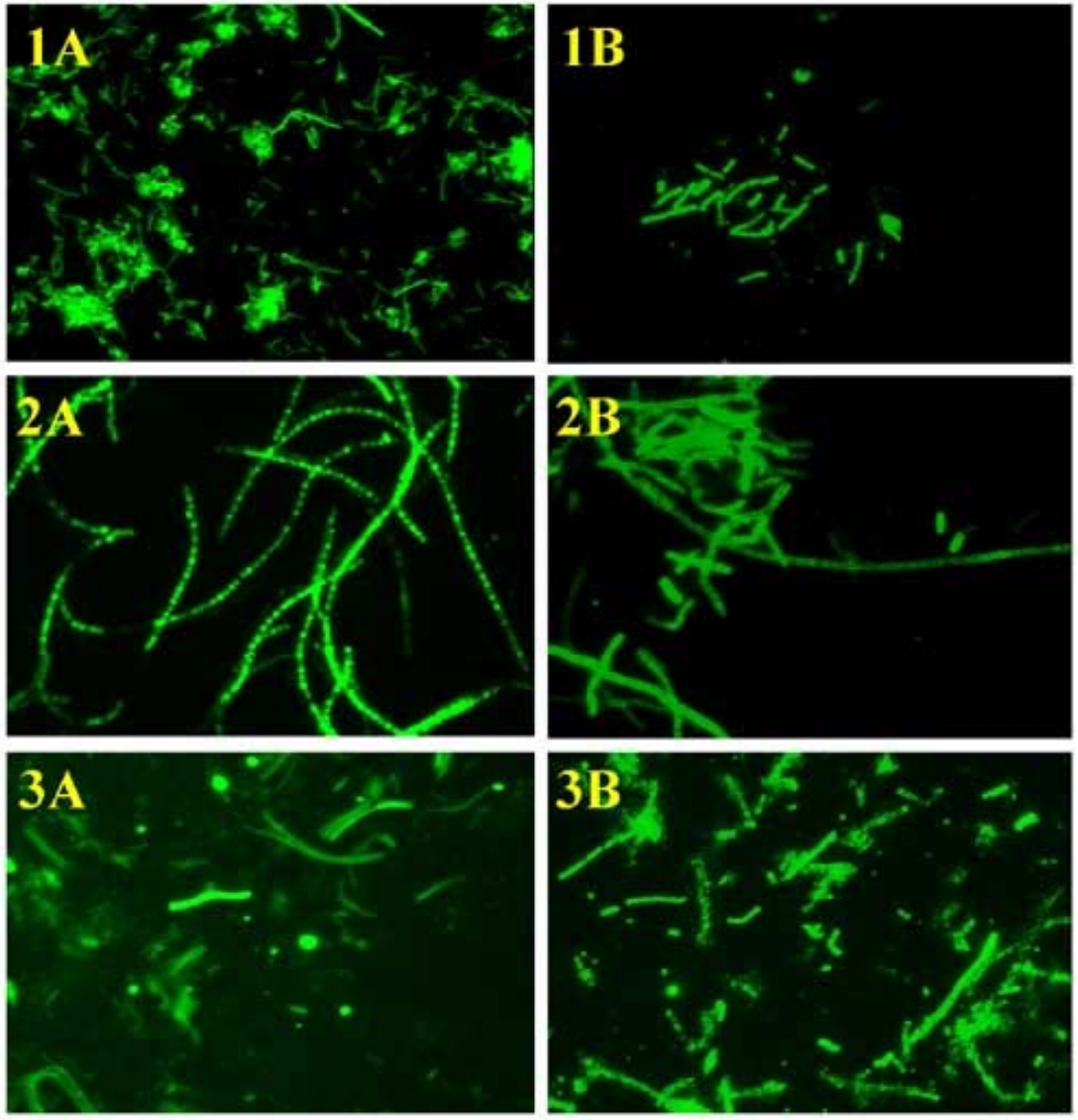


***B. anthracis* on SBA. Courtesy of Jason Paragas, PhD**

Anthrax Confirmatory Diagnostics

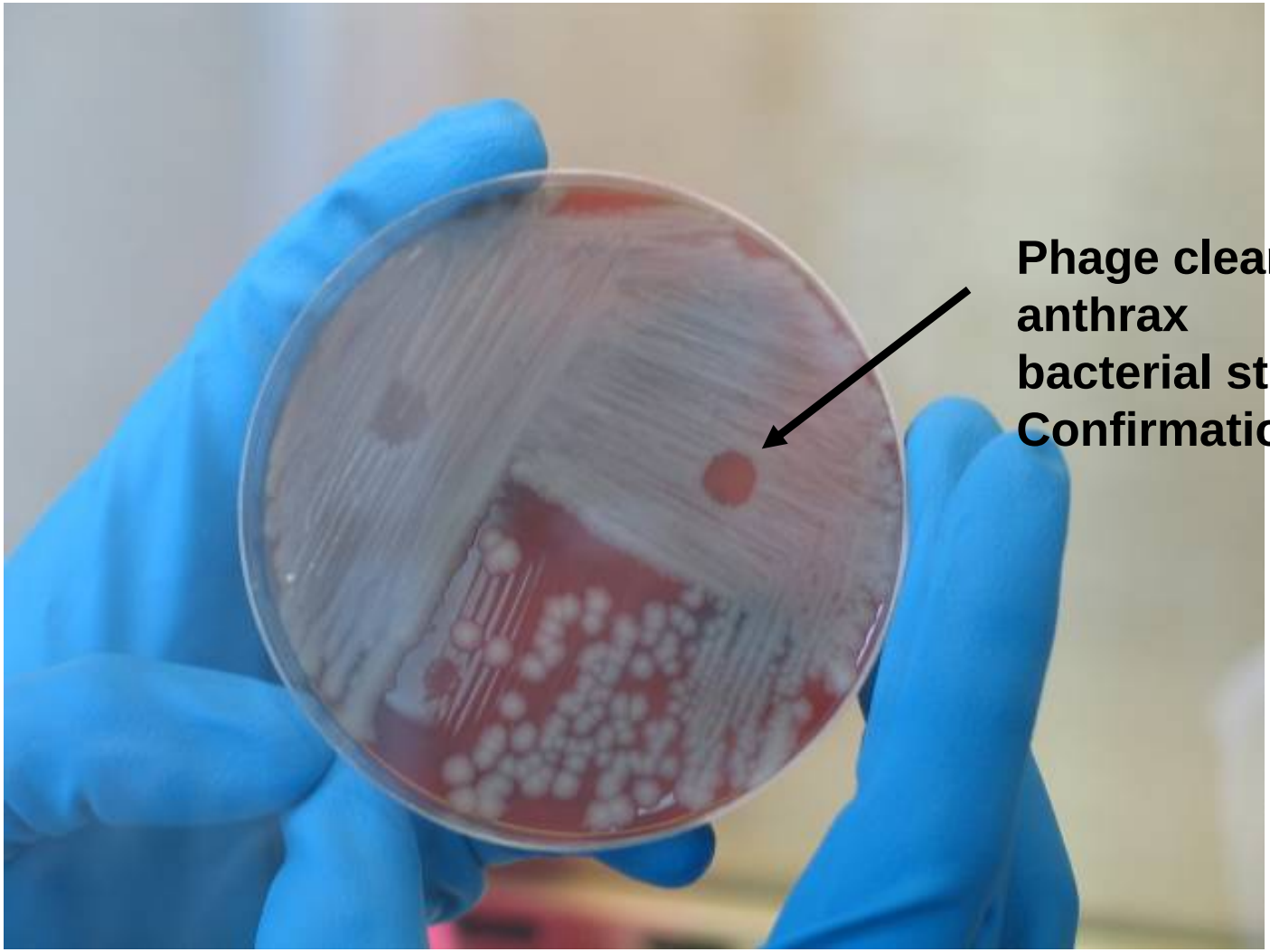
- DFA for
  - Cell wall
  - Capsule
- γ phage lysis test
- PCR*
  - Real-time assays approved
  - Probably not adequate by themselves

Anthrax DFA



De et al.
Emerging Infectious Diseases, 8;10; Oct 2002

Gamma-phage lysis



Phage clearing of anthrax bacterial streak. Confirmation of anthrax

Medical Management cutaneous anthrax

(without systemic symptoms)

1. PO Antibiotics (adult doses)
    1. Associated with potential BW aerosol attack:
        - Ciprofloxacin 500mg PO q12hr for 60 days, or
        - Doxycycline 100mg PO q12hr for 60 days*
    2. Natural exposure:
        - 7-10 days PO antibiotics
2. NSAIDS/Steroids for severe edema?
3. Infection control:
    - Contact precautions
    - Do not debride lesions

*Until susceptibilities known.
- May switch to Amoxicillin po
- Avoid DOXY in pregnancy and in children <8yr

Cutaneous anthrax

- PCN- used most extensively for natural cases
- Organism rapidly cleared following antibiotic therapy
- 25 patients with cutaneous anthrax and positive initial cultures of blister fluid-given 2 M units of crystalline PNC q 6hrs, all cultures negative w/in 5 hrs of first dose

Ronaghy et al. Curr.Ther. Res. Clin. Exp. 1972;14:721

Gastrointestinal Anthrax

- RARE, naturally-occurring disease
- Ingestion of insufficiently cooked, contaminated meat (vegetative bacilli?)
- Probably requires a large inoculum of organisms
- Incubation period 1-6 days

Gastrointestinal Anthrax

- Symptoms- nausea, vomiting, fever, abdominal pain -> hematemesis, bloody diarrhea or melena and massive serosanguinous ascites
- Pathology- ulcerative lesions of terminal ileum, cecum, with hemorrhagic mesenteric adenopathy

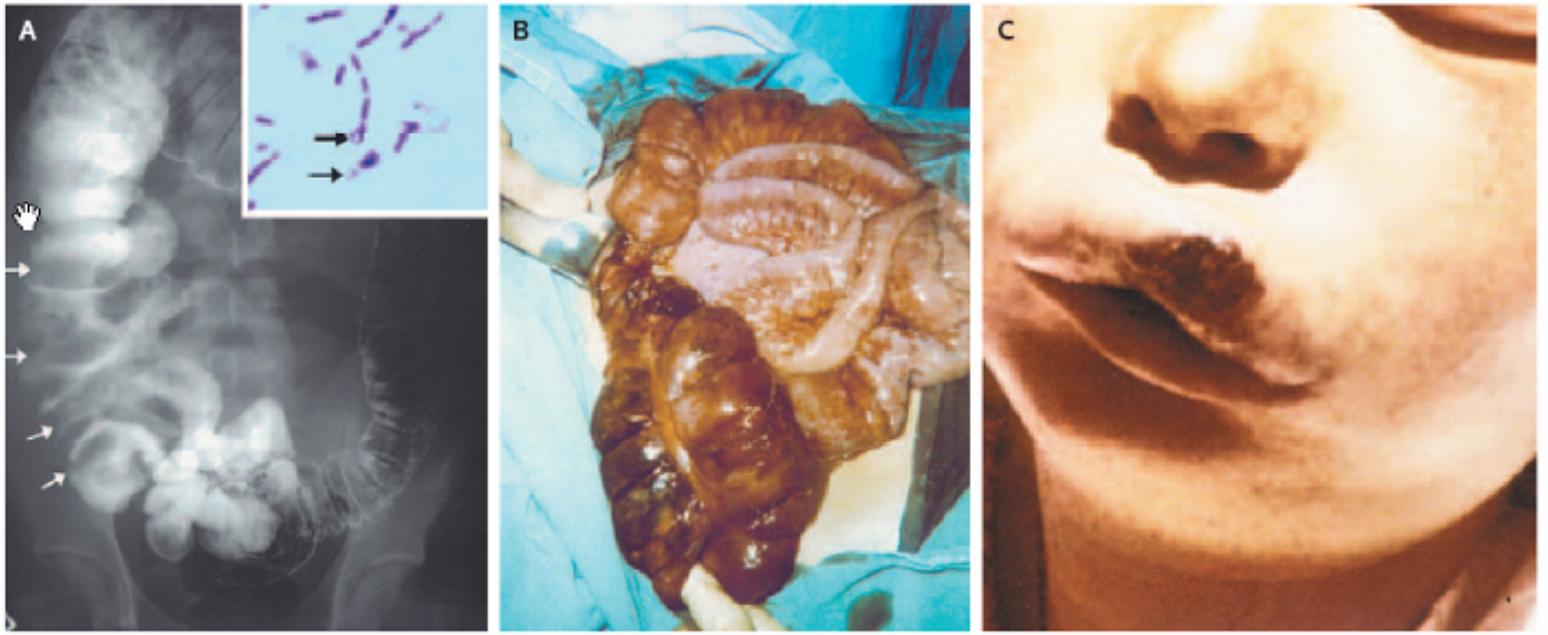
Gastrointestinal Anthrax

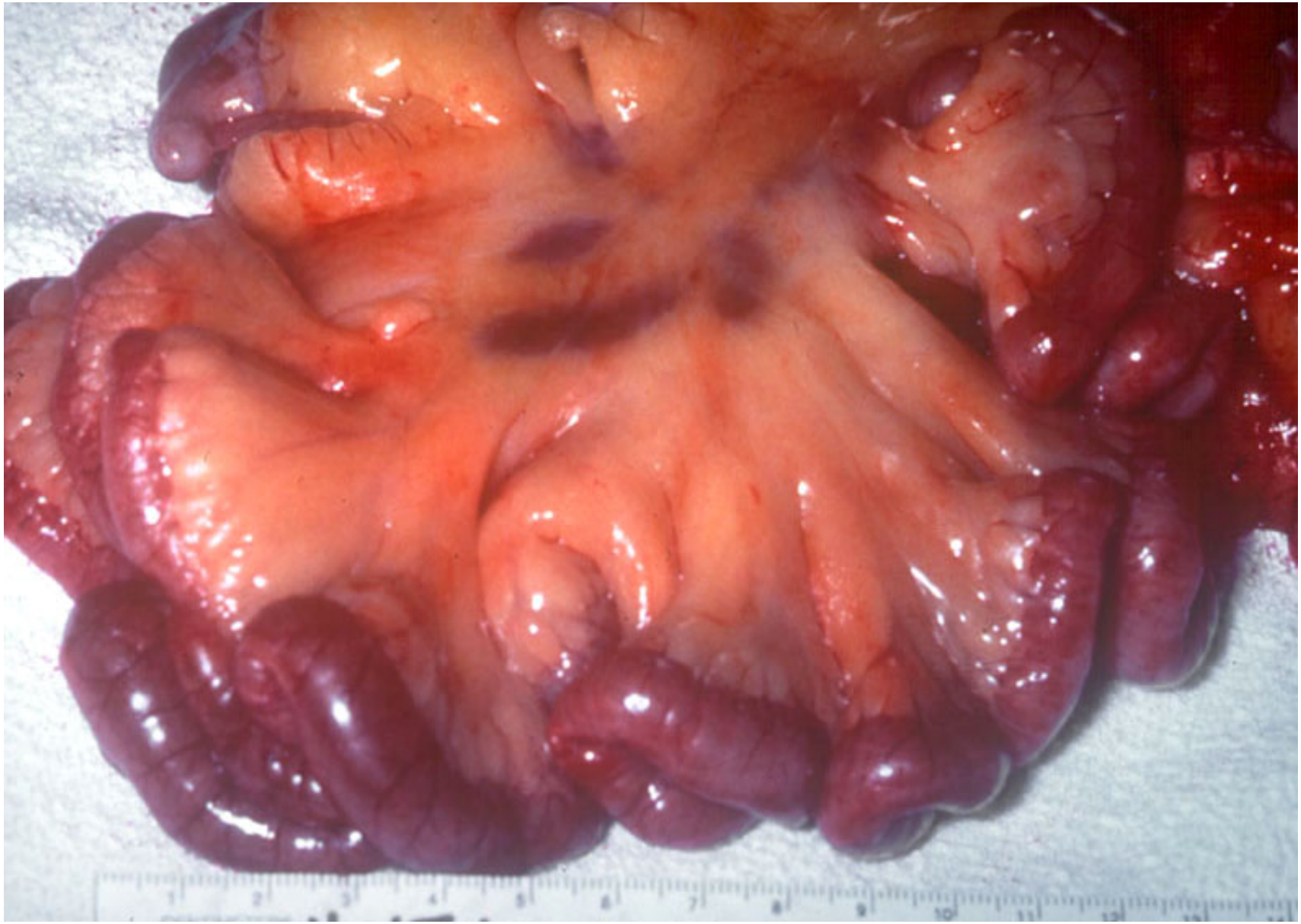
- Hematogenous spread via direct extension from GI lumen leading to bacteremia and septicemia
- Mortality~50%

The NEW ENGLAND JOURNAL of MEDICINE

IMAGES IN CLINICAL MEDICINE

Anthrax of the Cecum





Diagnosis: Gastrointestinal Anthrax (Suggested)

Clinical Suspicion

- Consistent Symptoms
  +/-
- Exposure risk

Alert Authorities*

Diagnostic tests

- **Stool Culture (variably +), Blood Culture**
- **Acute Abdominal w/u to r/o other causes (CBC/electrolytes/Abdom films/LAEs, etc…)**
- **Consider: Serology (anti-PA),Blood sample for PCR?**
- **Ascites: GS/Cx/IHC/PCR**

Therapy

Same as inhalational Dz

IHC = immunohistochemical stain
GS = gram stain
Cx = culture
PCR = polymerase chain reaction
w/u = workup
r/o = rule-out
LAE = liver-associated enzymes

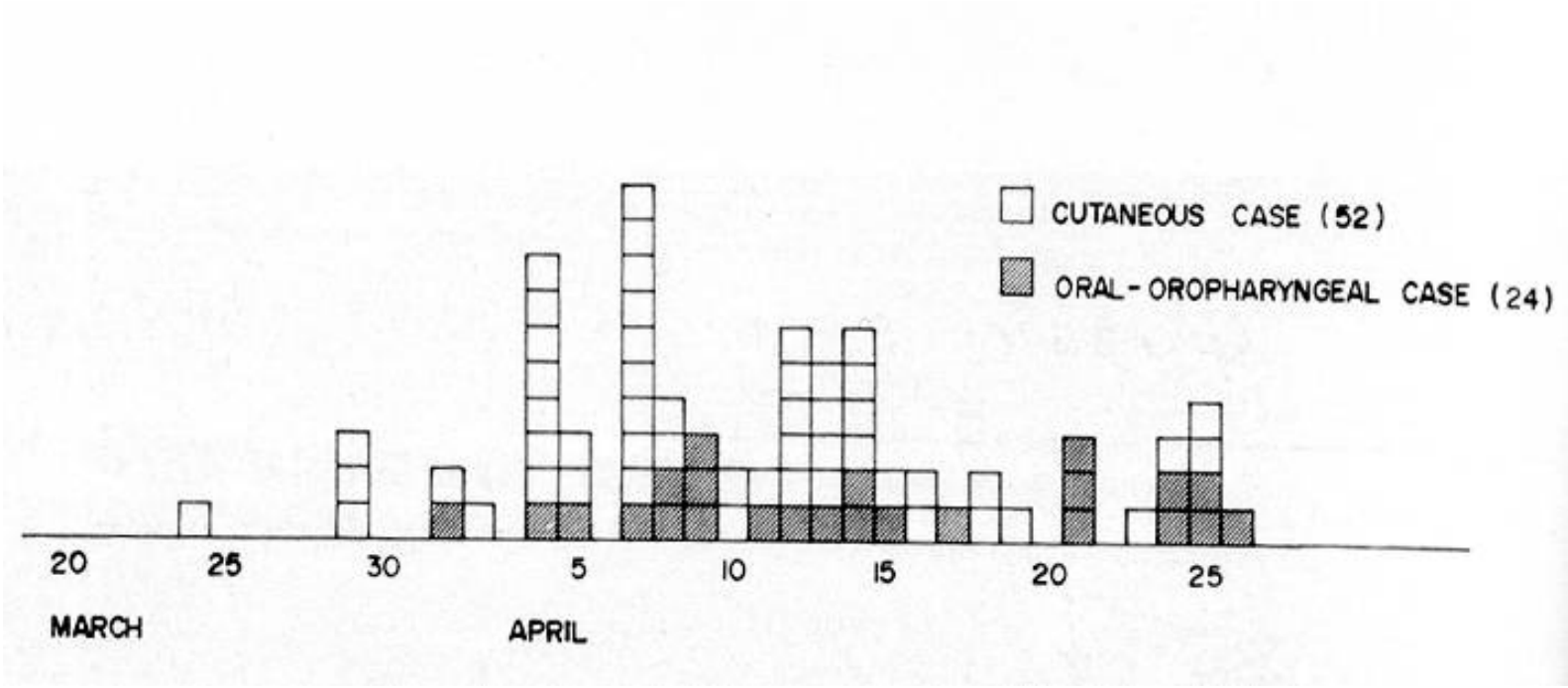
*

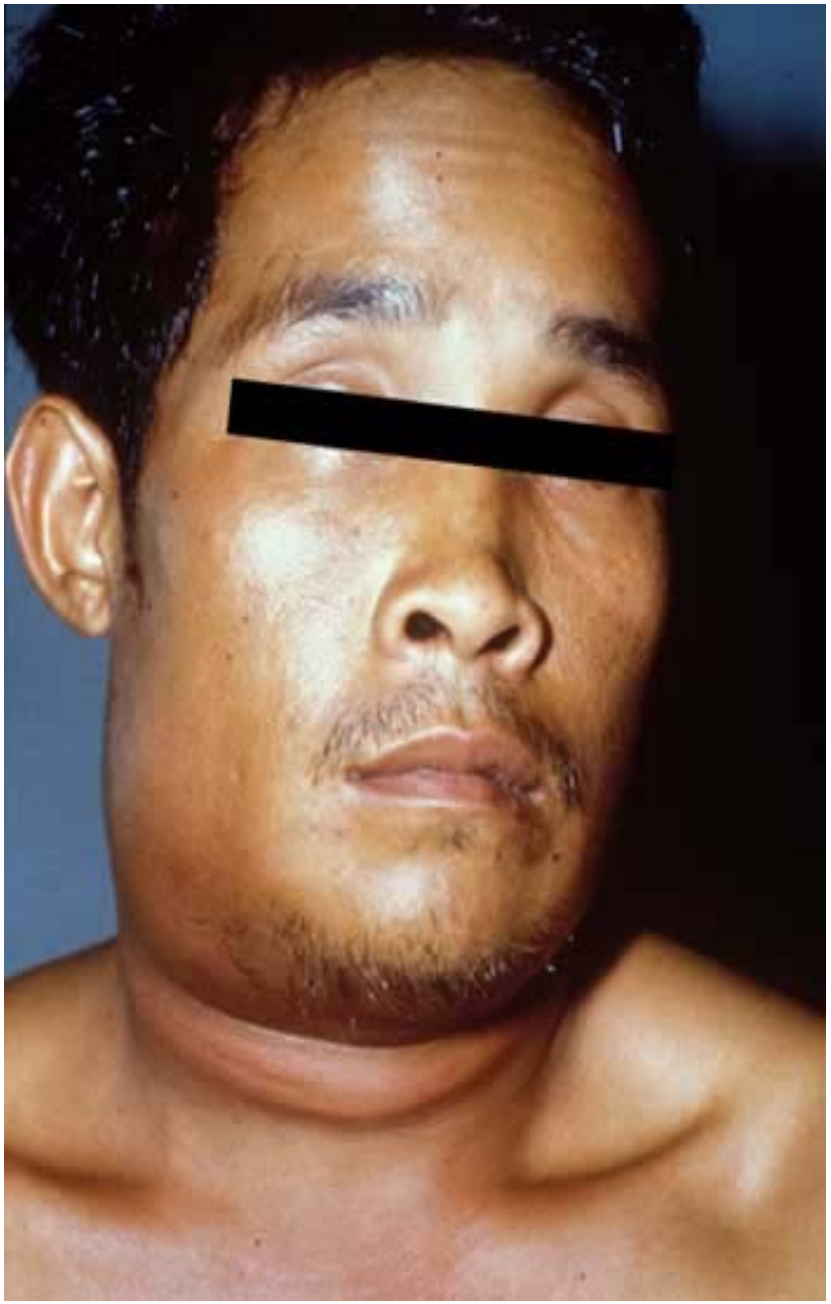
- Public health
- Laboratory
- Law
- Etc…

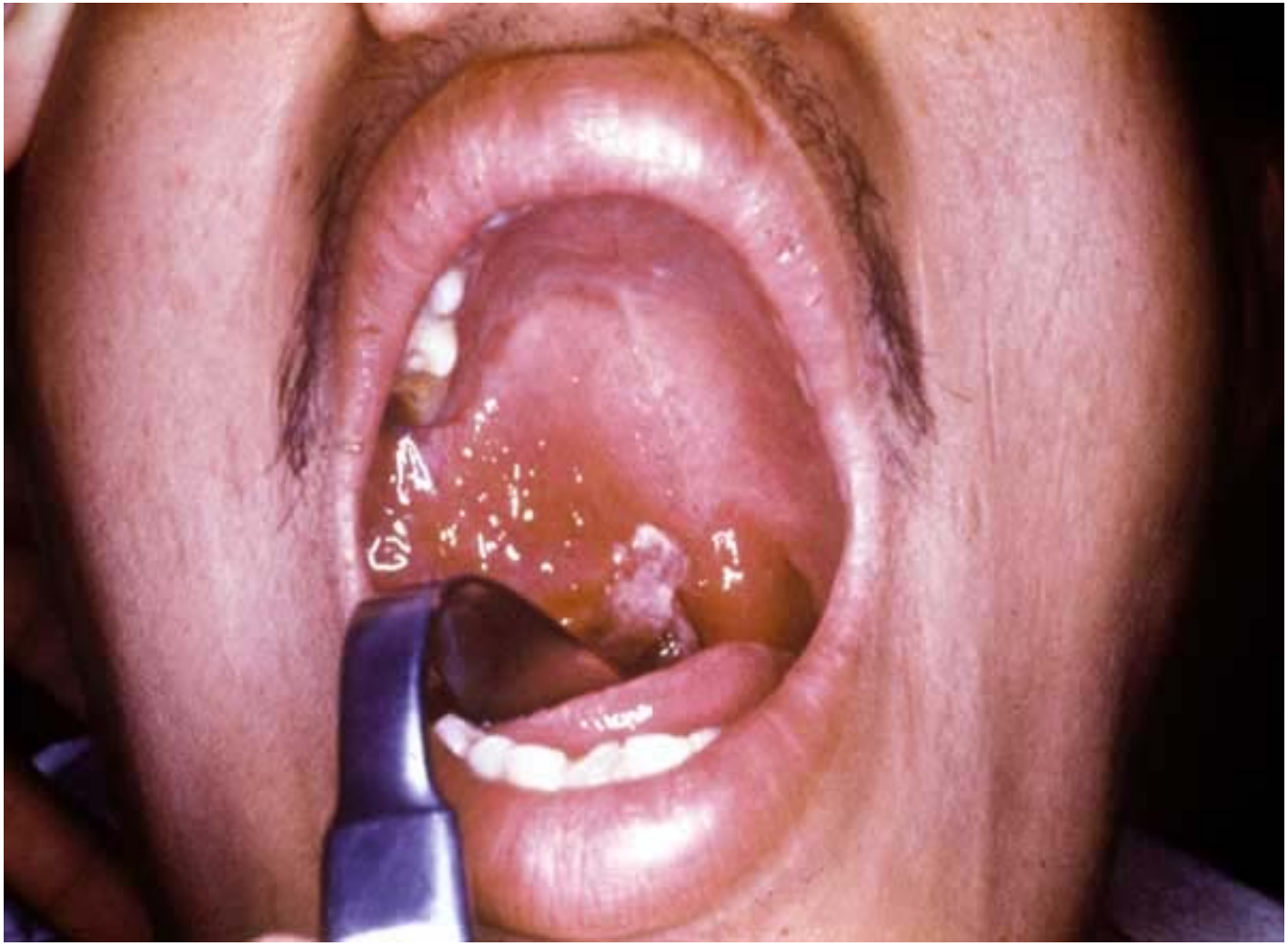
Oropharyngeal Anthrax

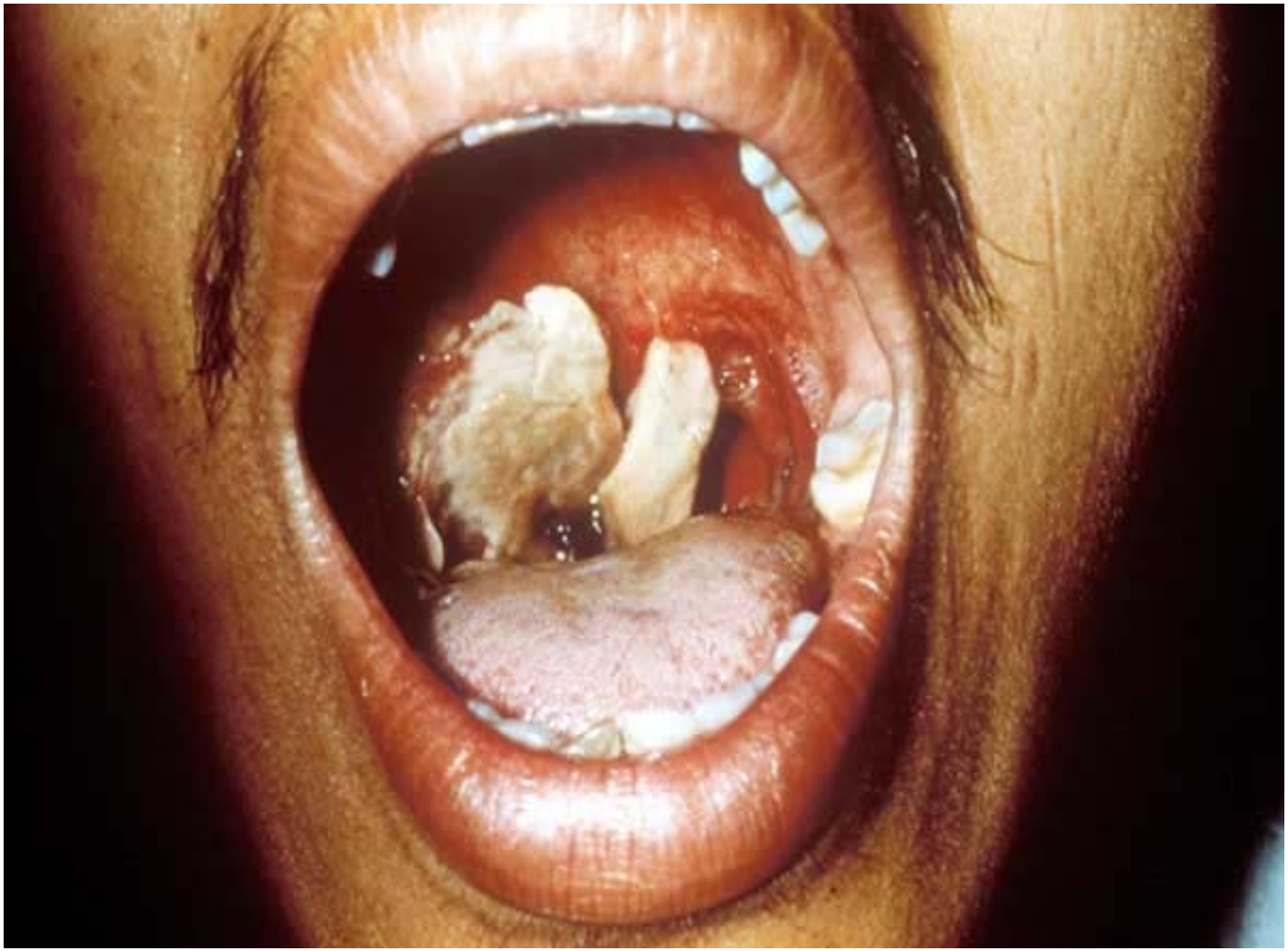
- Rare form of anthrax
- “cutaneous anthrax of the oropharynx”
- Fever, severe pharyngitis with oral ulcers, dysphagia, regional lymphadenopathy, severe neck swelling
- Risk of airway compromise
- Mortality~25%

Thailand, Water buffalo exposure

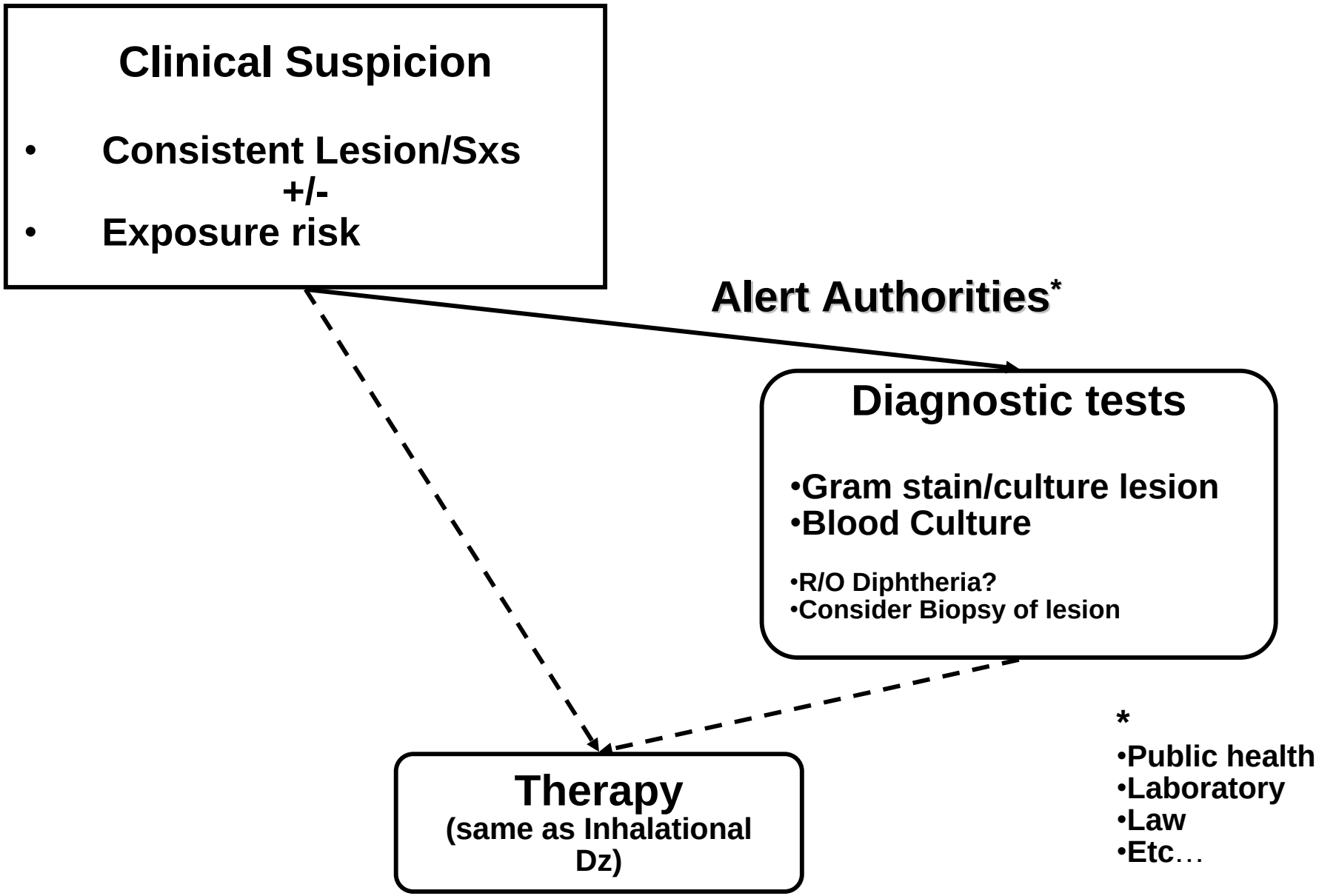




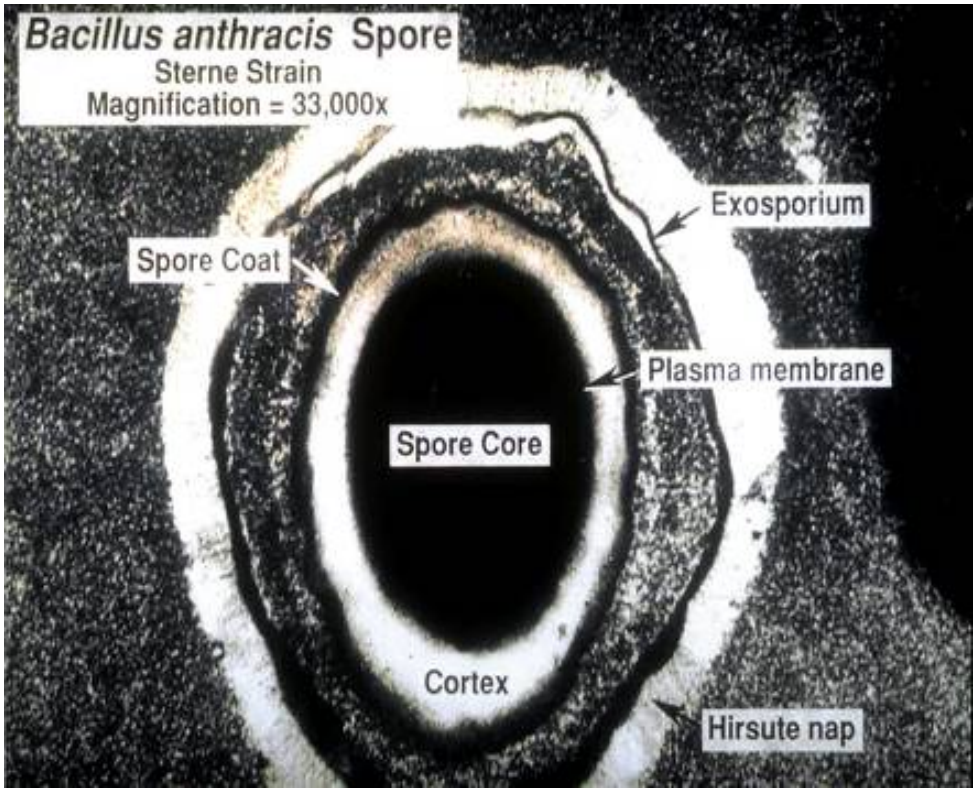


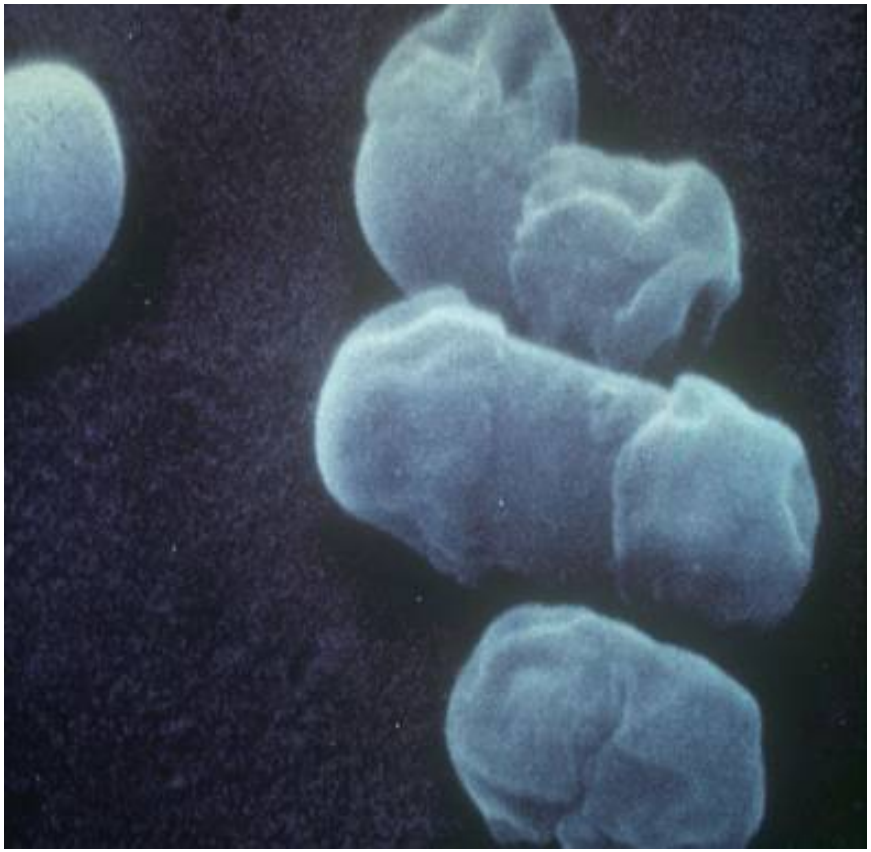


Diagnosis: Oropharyngeal Anthrax (Suggested)



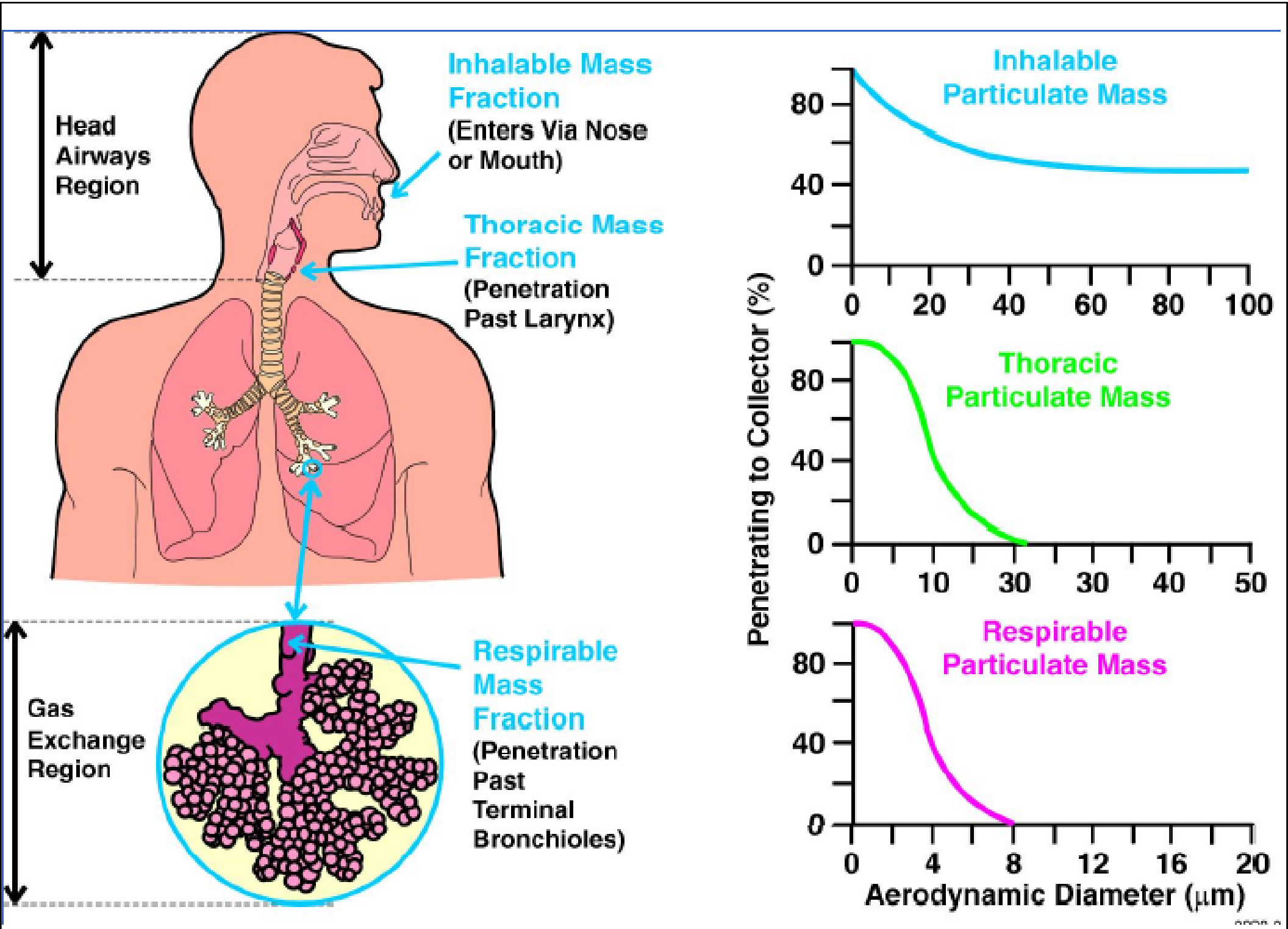
Inhalational Anthrax





BW Threat

- B. anthracis was weaponized by the US, USSR, Iraq, etc.
- Inhalational anthrax results from the inhalation of aerosolized spores
- 8,000-55,000 spores =human LD_{50}
- Inhalational anthrax mortality up to 100%
- Efficient downwind spread compared to chemical agents


Head Airways Region
Inhalable Mass Fraction (Enters Via Nose or Mouth)
Thoracic Mass Fraction (Penetration Past Larynx)
Gas Exchange Region
Respirable Mass Fraction (Penetration Past Terminal Bronchioles)
Inhalable Particulate Mass
Thoracic Particulate Mass
Respirable Particulate Mass
Penetrating to Collector (%)
80
40
0
0 20 40 60 80 100
80
40
0
0 10 30 30 40 50
80
40
0
0 4 8 12 16 20
Aerodynamic Diameter (μm)

How Dangerous are Spores in a Letter?

Defence R&D Suffield, Canada

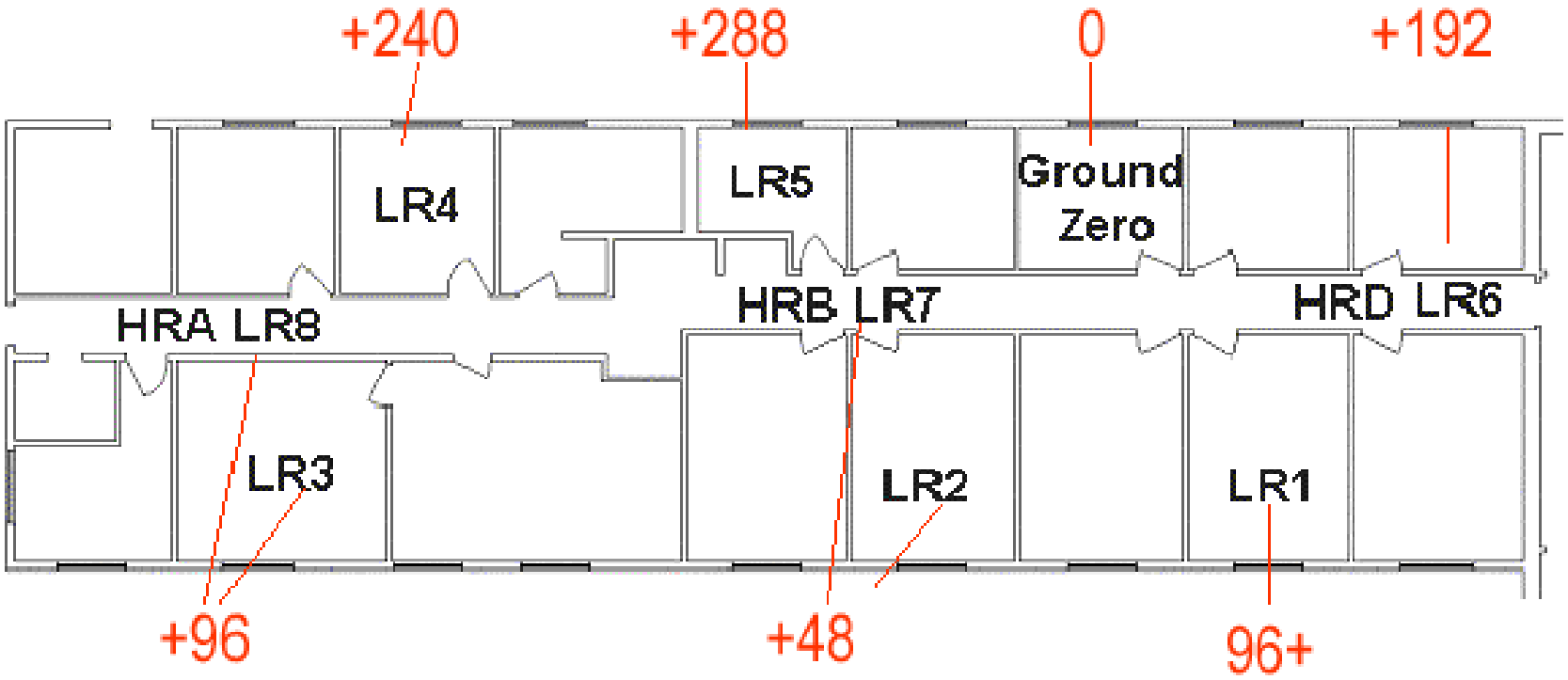
How large an inoculum?

Canadian Defence Research Establishment Suffield

- Risk of transmission of spores from an envelope
- 0.1 or 1.0 g of *Bacillus globigii* spores released in a 10x18x10' room
- Significant numbers of spores aerosolized within seconds
  - >99% 2.5 to 10 µm size
  - estimate of 480 to 3080 LD_{50s} potentially inhaled in 10 min.
  - "the aerosol would quickly spread throughout the room so that other workers….would likely inhale lethal doses"

Kournikakis B, Armour SJ, Boulet CA, et al. Risk Assessment of Anthrax Threat Letters. Defence Research Establishment Suffield 2001; Technical Report TR-2001-048

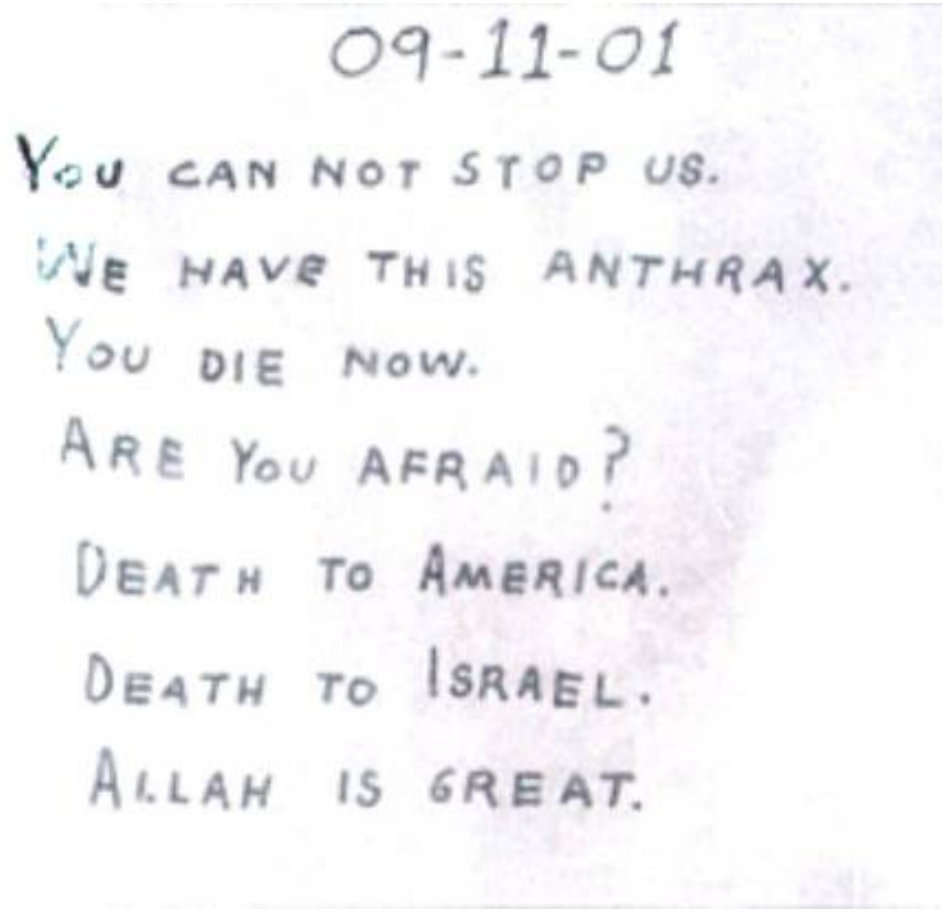
How Fast Do Anthrax Spores Spread in a Room?



+ XX = # of seconds to peak following peak at Ground Zero

Objective Assessment of the Hazard from Anthrax Terrorist Attacks in an Office Environment
Bill Kournikakis and Jim Ho
Defence R&D Canada - Suffield

Hart Senate Office Bldg, Oct 2001



09-11-01

YOU CAN NOT STOP US.

WE HAVE THIS ANTHRAX.

YOU DIE NOW.

ARE YOU AFRAID?

DEATH TO AMERICA.

DEATH TO ISRAEL.

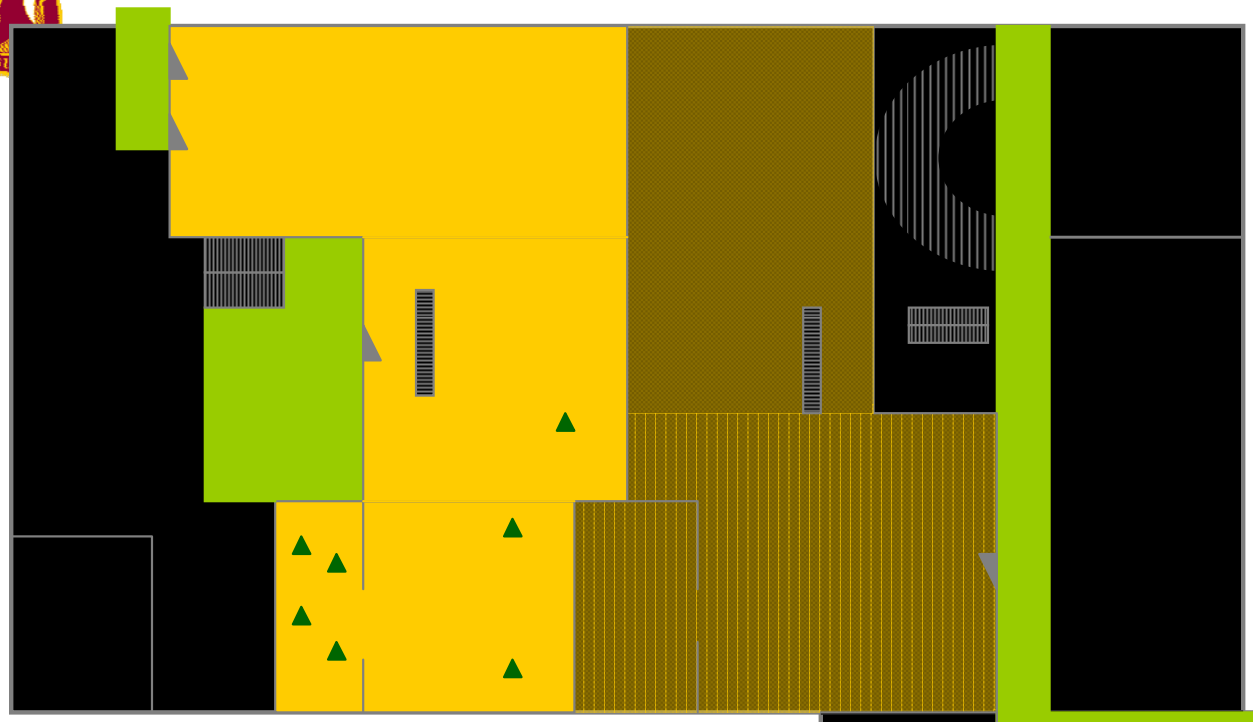
ALLAH IS GREAT.

SE 6th Fl. Hart Bldg

- Daschle office
- Feingold office
- Envelope opened
- Doorway
- Staircase

▲ 6th Fl. Positive NS

Group	No. pos.	Tot. No.	% pos.
Daschle	13	13	100
Feingold	2	15	13
Responders	6	59	10

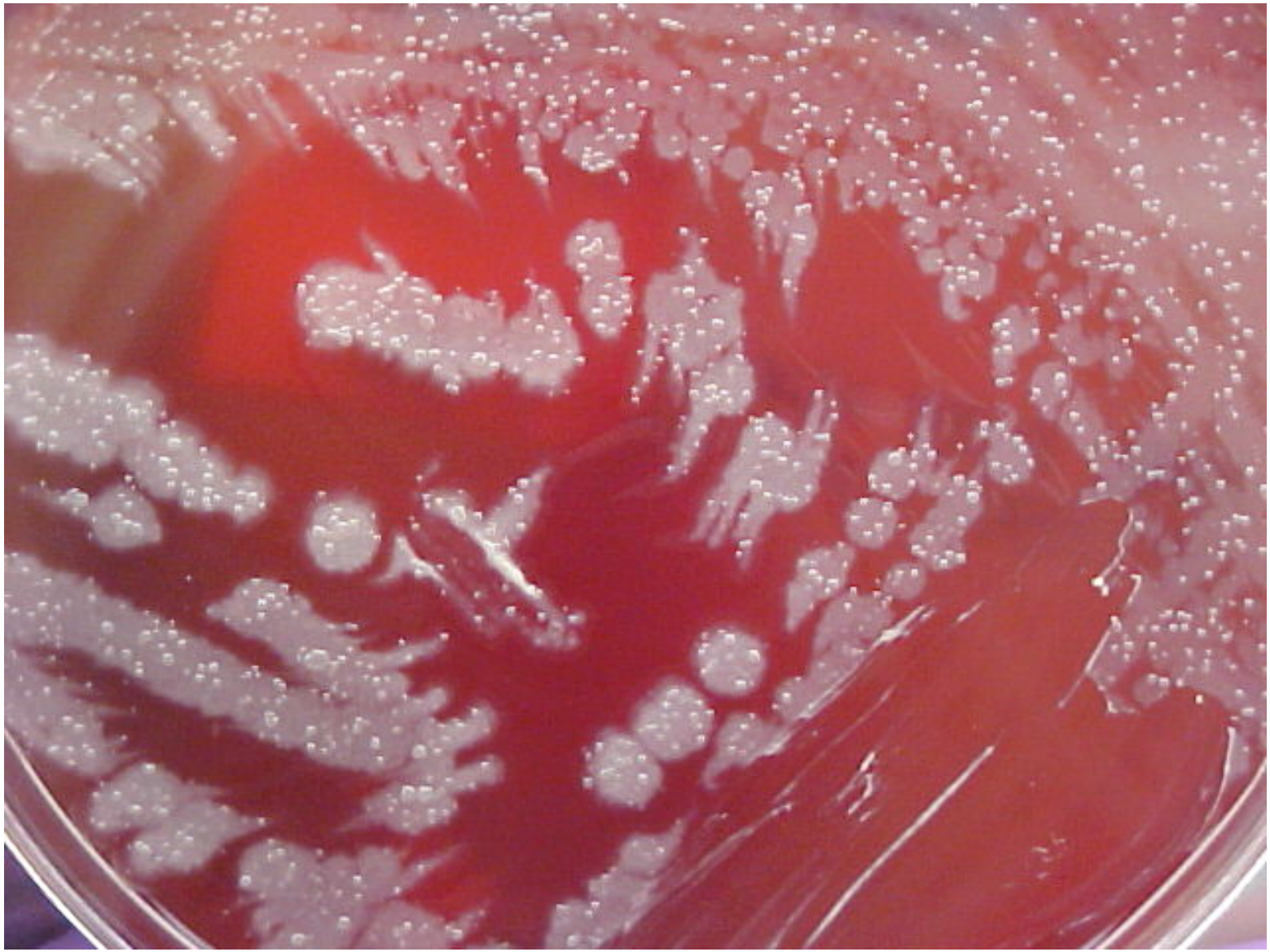


SE 5th Fl. Hart Bldg

Daschle office
Feingold office
Doorway
Staircase

5th Fl. Positive NS

Group	No. pos.	Tot. No.	% pos.
Daschle	7	25	28
Feingold	0	15	0



Overnight, Sheep Blood Agar

G. Martin, MD
R. Paolucci

Inhalational (Pulmonary/Mediastinal) Anthrax

Exposure

Incubation Period

1- 6 days
(range 1- 43 days)

Early Phase

(2 - 4 days)

~Fever, chills
~Fatigue, malaise
~Nonproductive cough
~Nausea/vomiting
~Dyspnea
~Drenching sweats
~Pleuritic pain

Possible Clinical Improvement

(lasts hours to days)

Late Phase

~Sudden fever
~Tachycardia
~Diaphoresis
~Subcutaneous chest/neck edema
~Respiratory distress
~Shock
~Death

(death often occurs within hours to days)

Inhalational Anthrax

- CBC with mild WBC elevation/left shift, mild increase in AST/ALT, hypoalbuminemia, hemoconcentration common
- Typically no lung findings on physical exam. CXR or Chest CT may show effusions, mediastinal widening
- Hemorrhagic meningitis seen in up to 50% of cases, GI hemorrhage common
- Mortality >85% historically, 45% in 2001 Amerithrax

Sverdlovsk Autopsy Findings

- Inhalation Anthrax
  - Hemorrhagic necrosis of thoracic lymph nodes 42/42
  - Hemorrhagic mediastinitis 42/42
  - Focal hemorrhagic necrotizing pneumonia 11/42
- Metastatic infection
  - Multiple gastrointestinal submucosal lesions 39/42
  - Hemorrhagic meningitis 21/42
- Microbiology
  - *B. anthracis* identified by tissue culture in 20/42
  - *B. anthracis* identified by histology 35/42
  - PCR analysis of tissue from 11 victims demonstrated DNA from vaccine and at least 4 different virulent *B. anthracis* strains

Amerithrax 2001

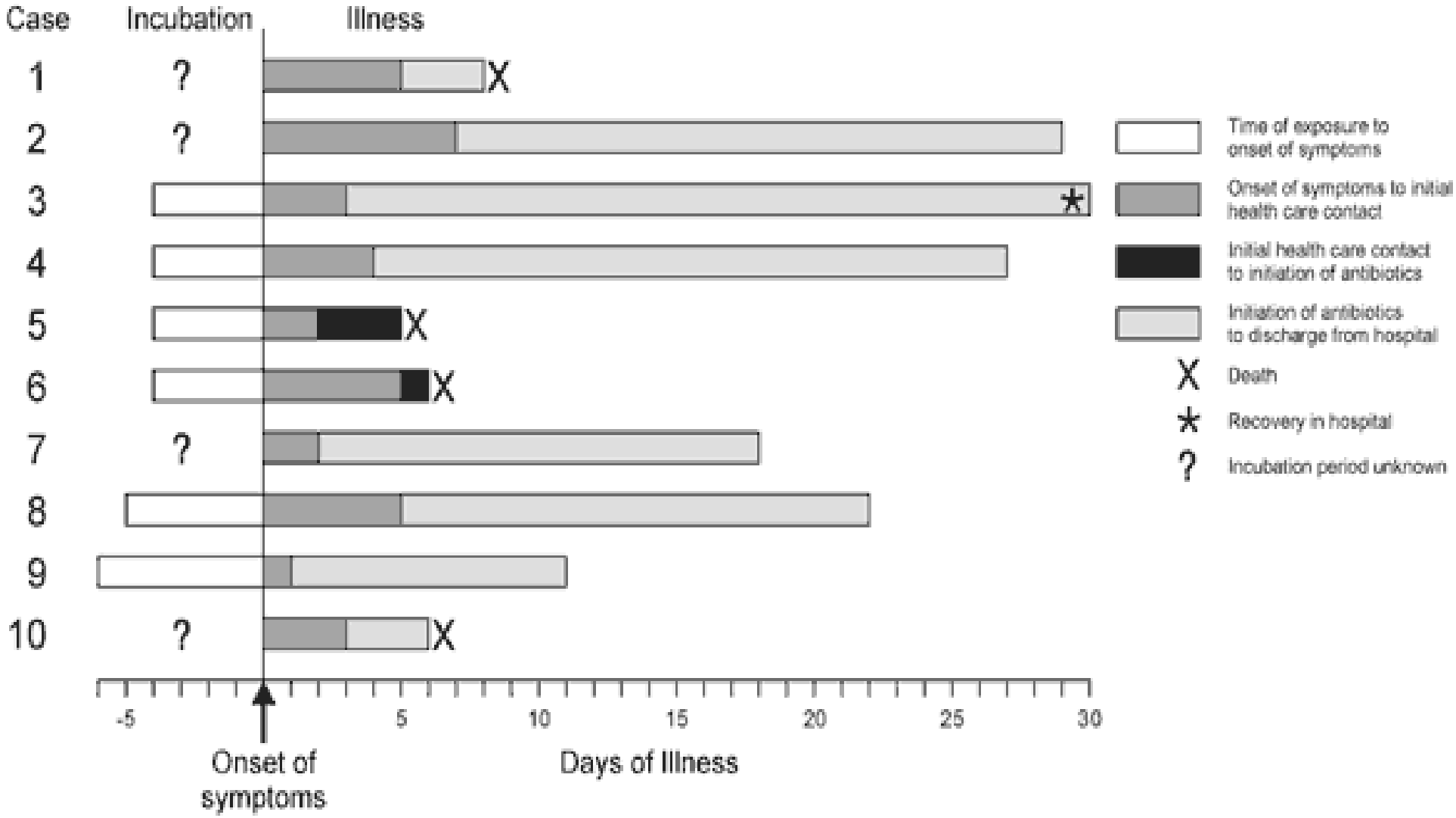
- 18 total (confirmed) cases:
  - 11 inhalational
  - 12 cutaneous (7 confirmed, 5 suspected)
  - 5 deaths among inhalational (45% mort.)
  - Inhalational:
    - Median age ~56 (range 43-94) 64% male
    - All except 2 known to handle mail
    - Median incubation period (N=6) 4-6 days
    - 5 pts – cardio / cerebro vascular dz; 1 type 2 DM; 1 hx asthma
    - No hx smoking except 94y/o

Amerithrax 2001

Table 2. Symptoms for 10 patients with bioterrorism-related inhalational anthrax, October-November 2001

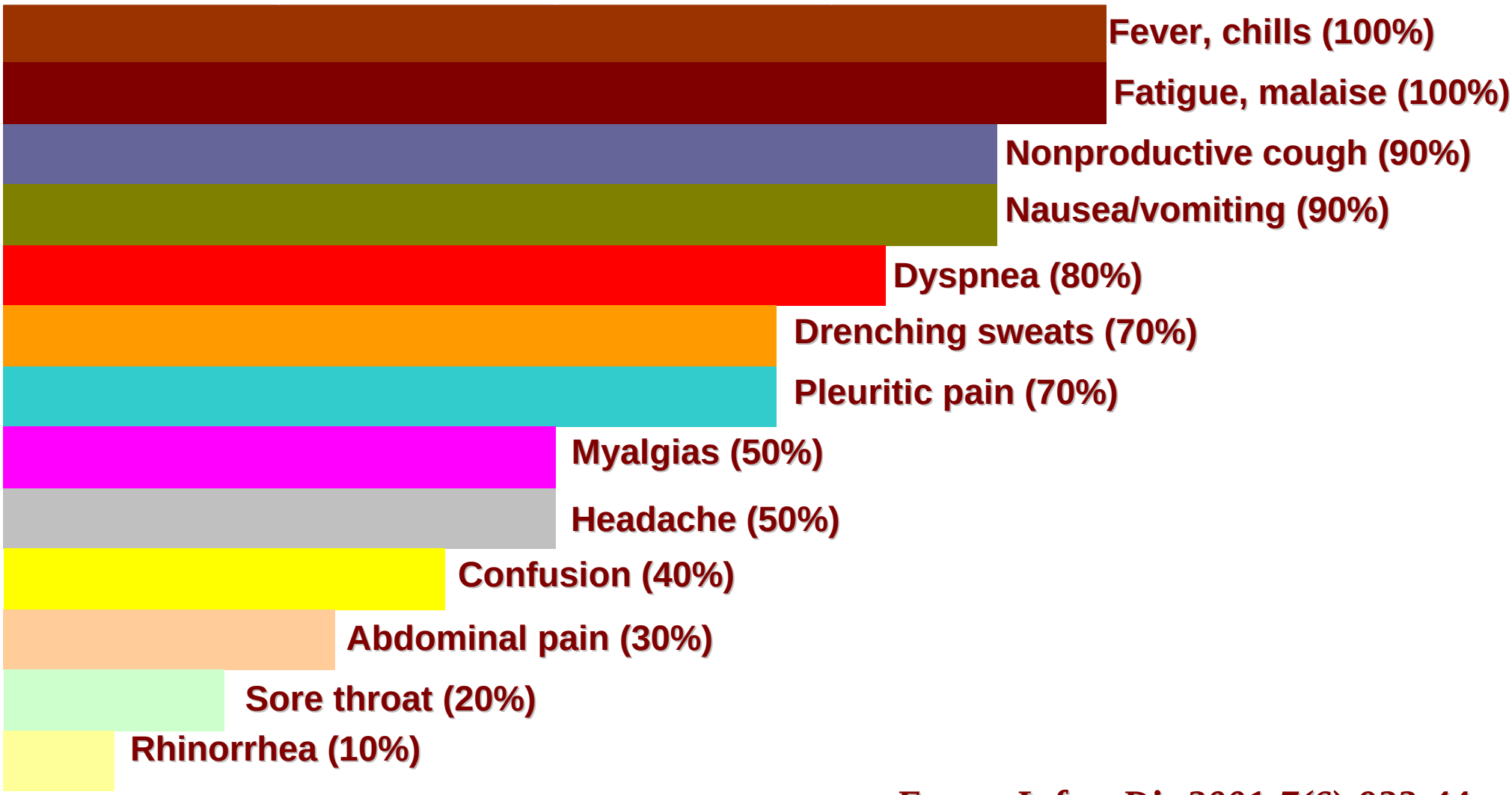
Symptoms	n=10
Fever, chills	10
Fatigue, malaise, lethargy	10
Cough (minimally or nonproductive)	9
Nausea or vomiting	9
Dyspnea	8
Sweats, often drenching	7
Chest discomfort or pleuritic pain	7
Myalgias	6
Headache	5
Confusion	4
Abdominal pain	3
Sore throat	2
Rhinorrhea	1

Amerithrax 2001

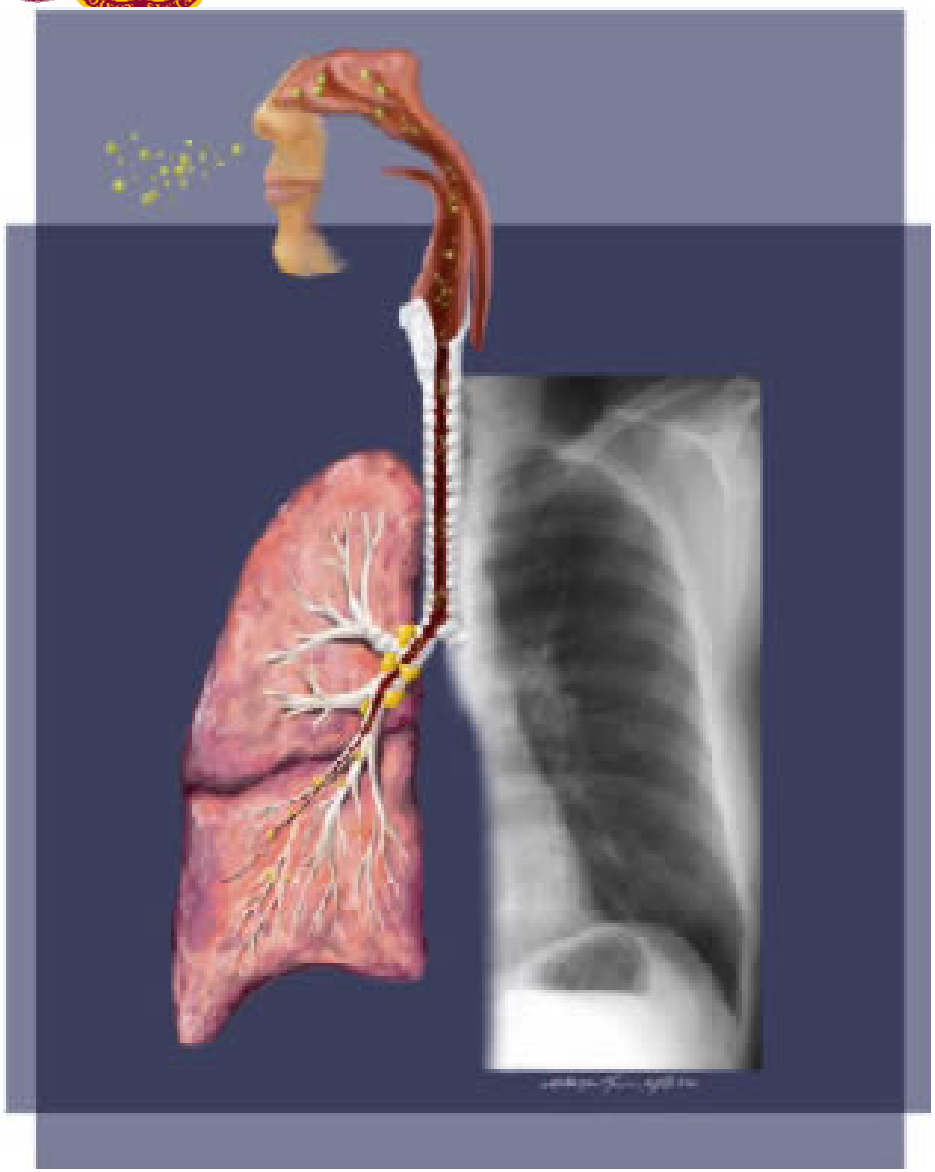


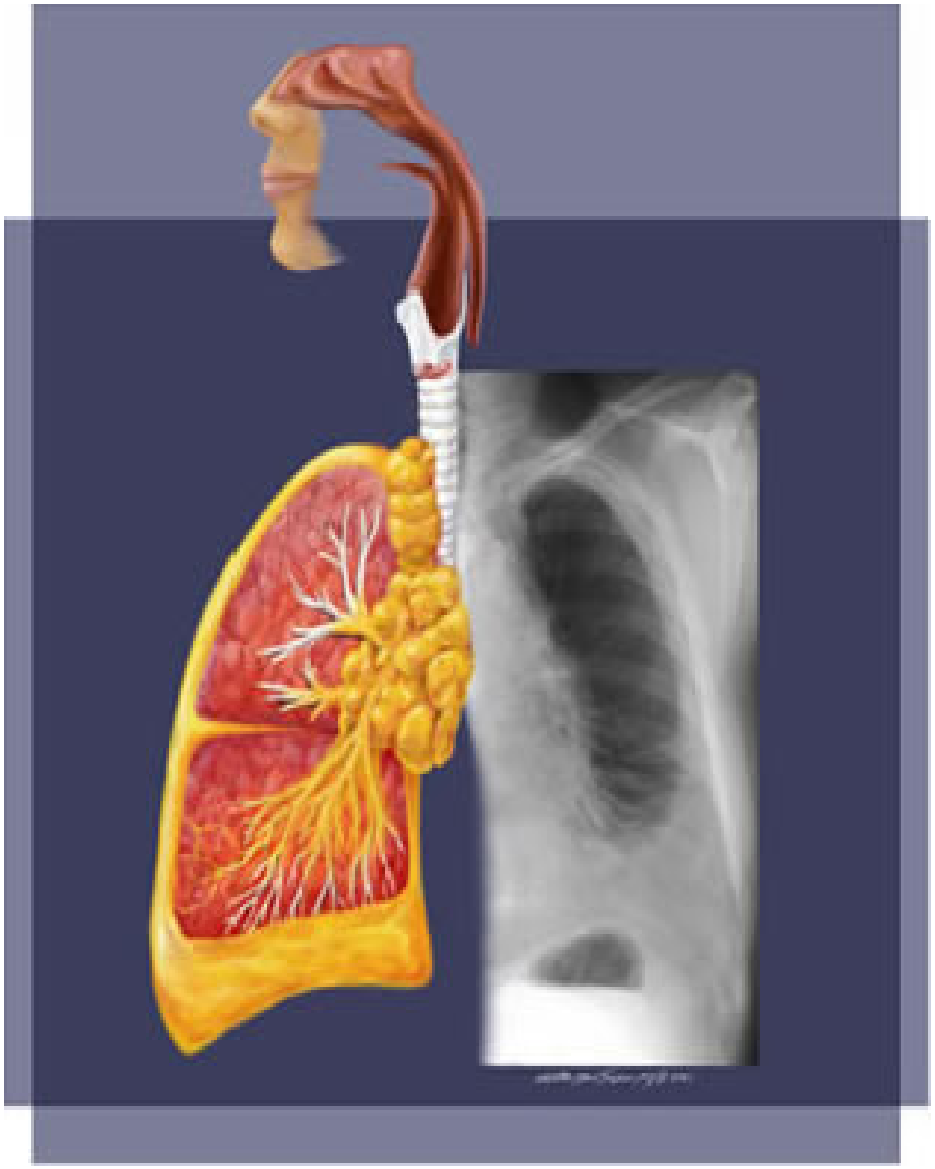
Clinical Symptoms, Pulmonary/Mediastinal Anthrax Cases, United States, 2001 (N=10)

Percent of Cases with Sign/Symptom

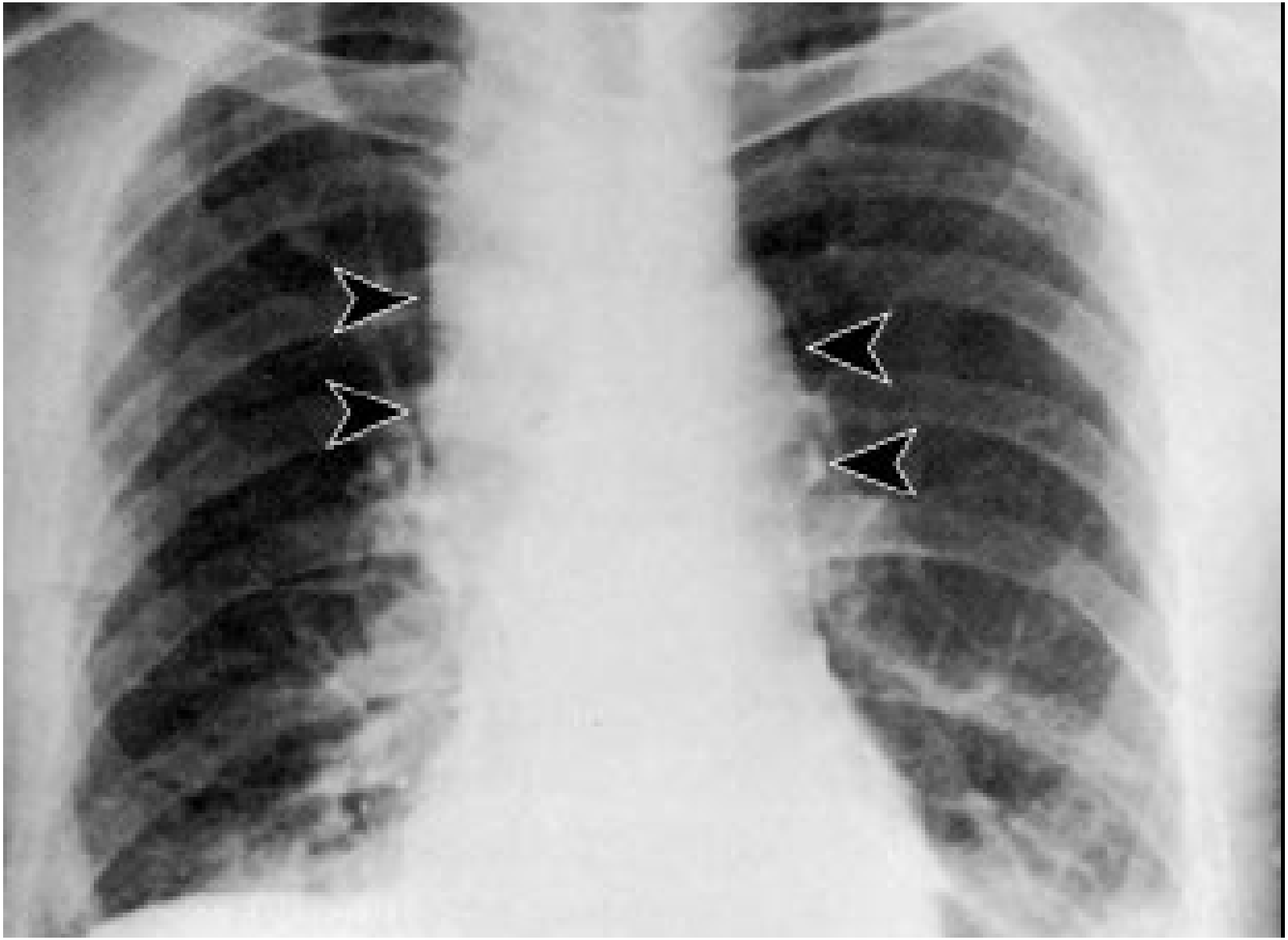


Emerg Infect Dis 2001;7(6):933-44



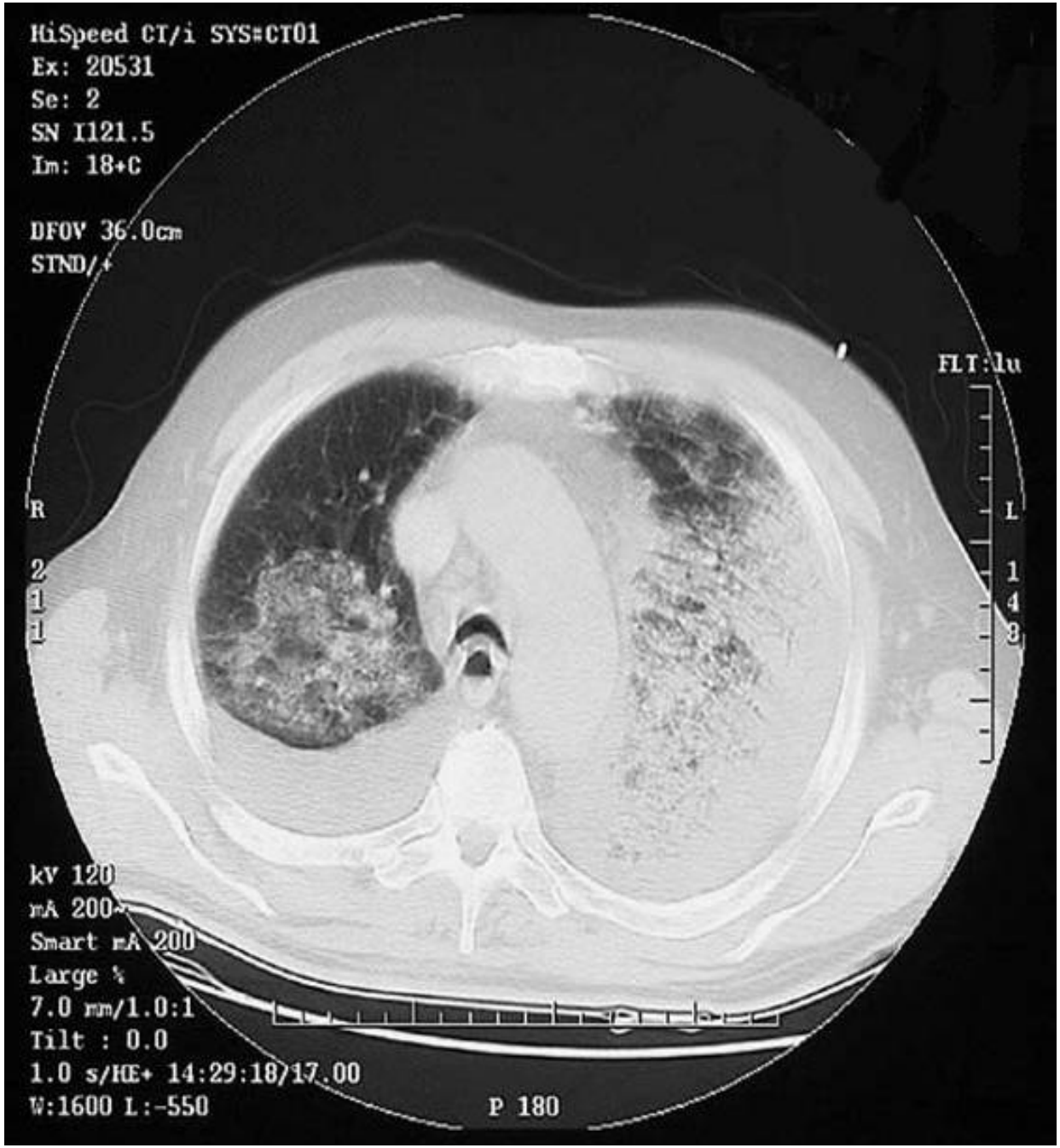


AFIP



CXR or CT

Normal, +/- hilar adenopathy (early)
Widened mediastinum, pleural effusions (may be late)
Usually no infiltrates (ARDS: late)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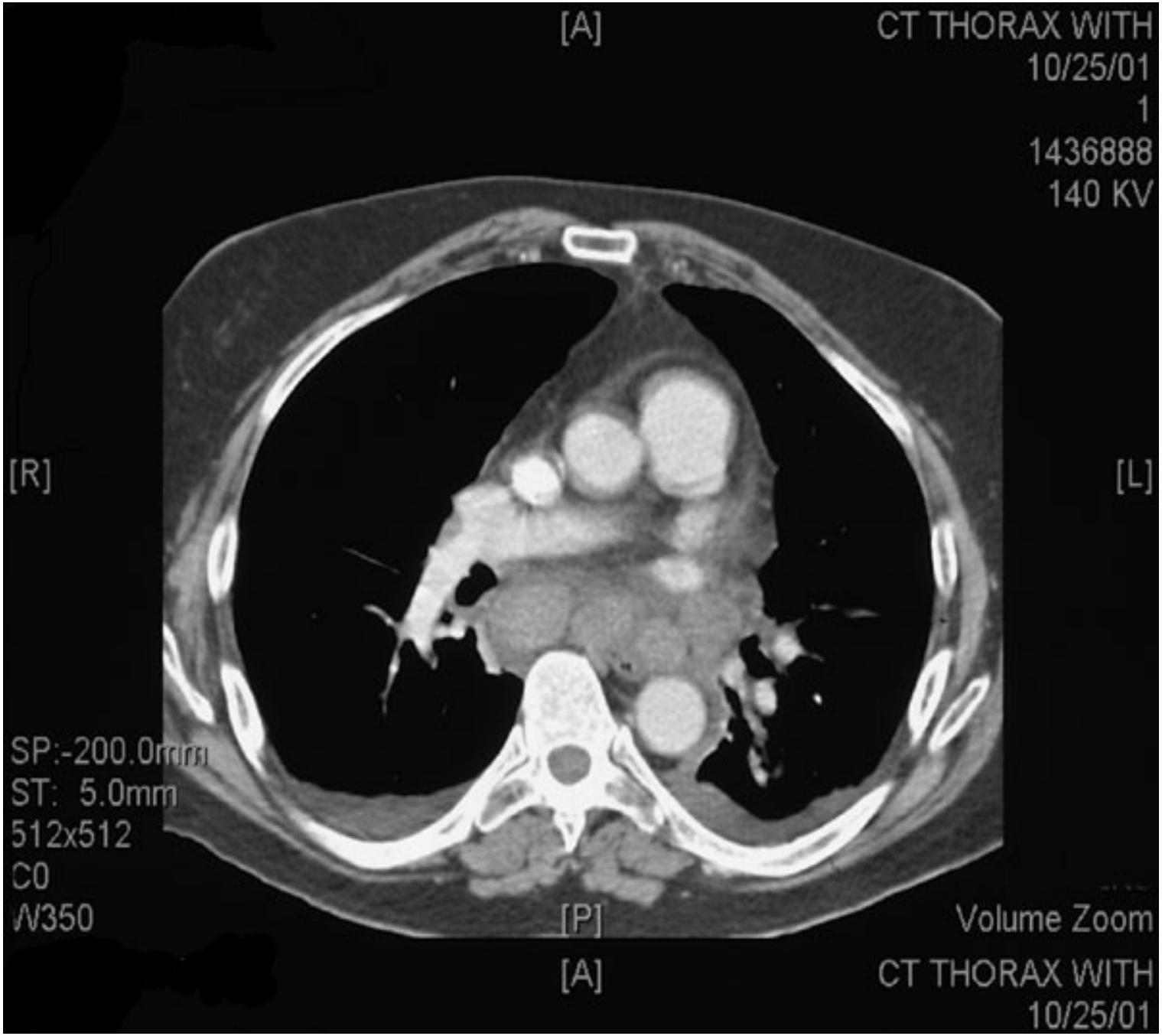
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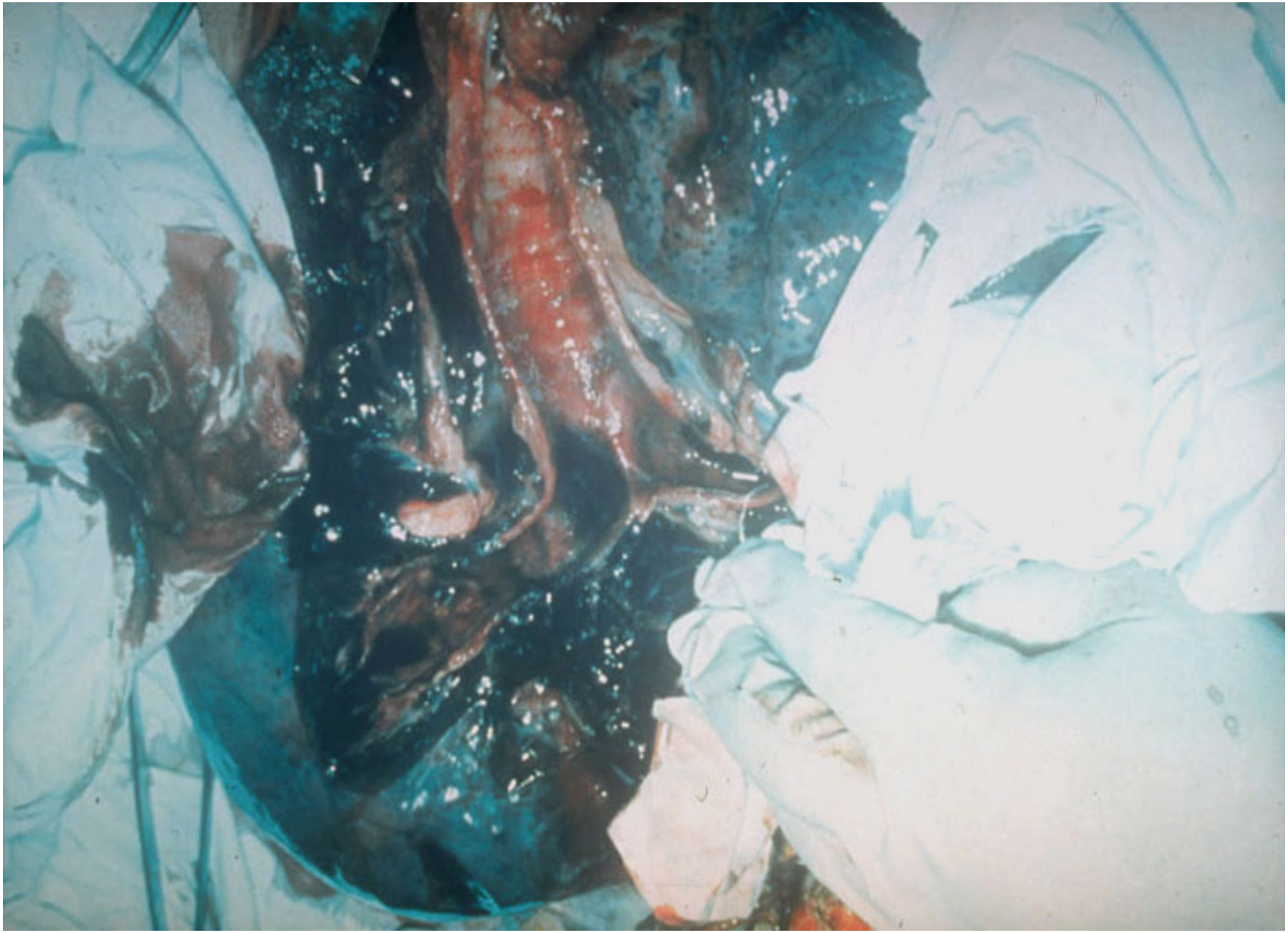
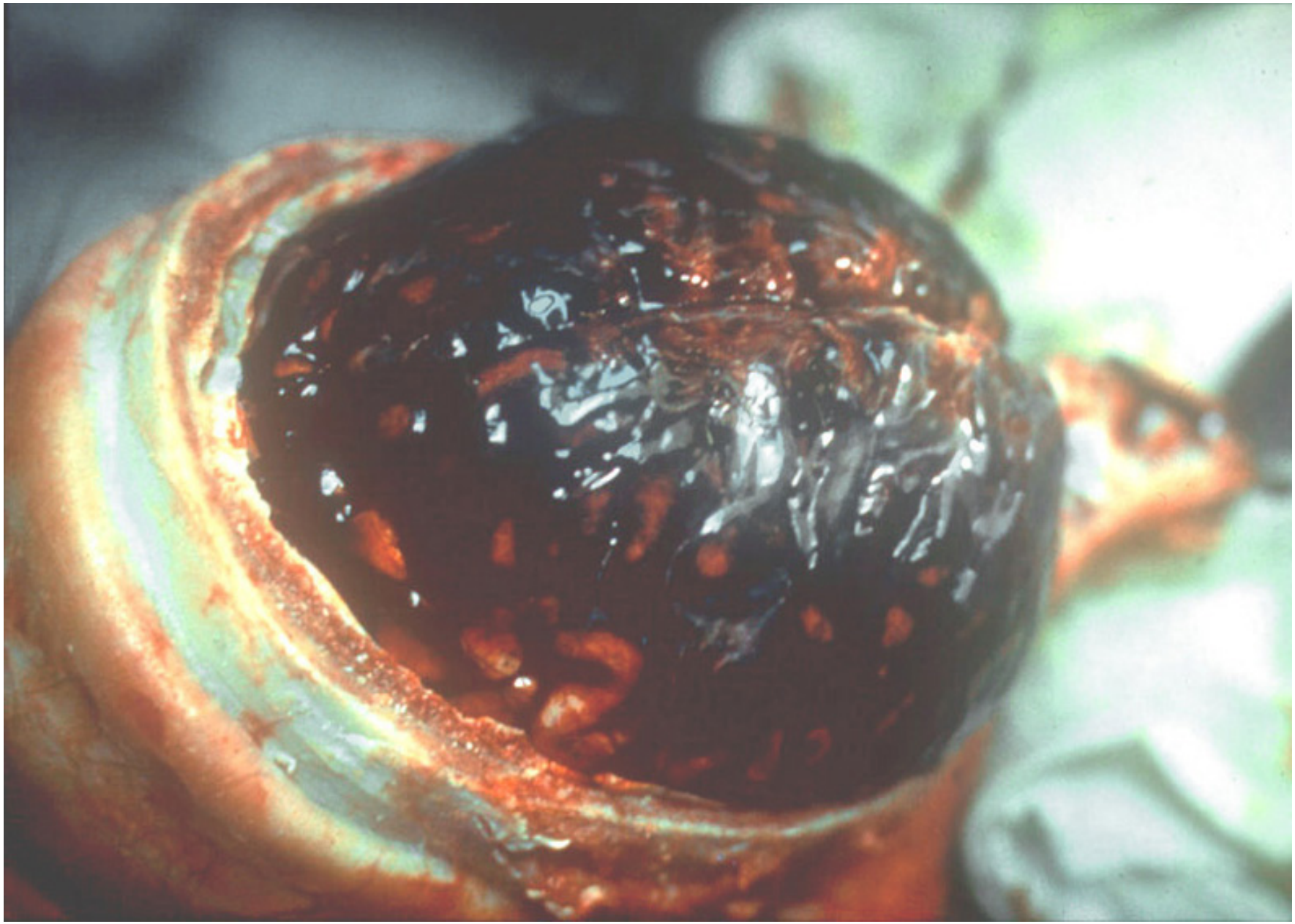
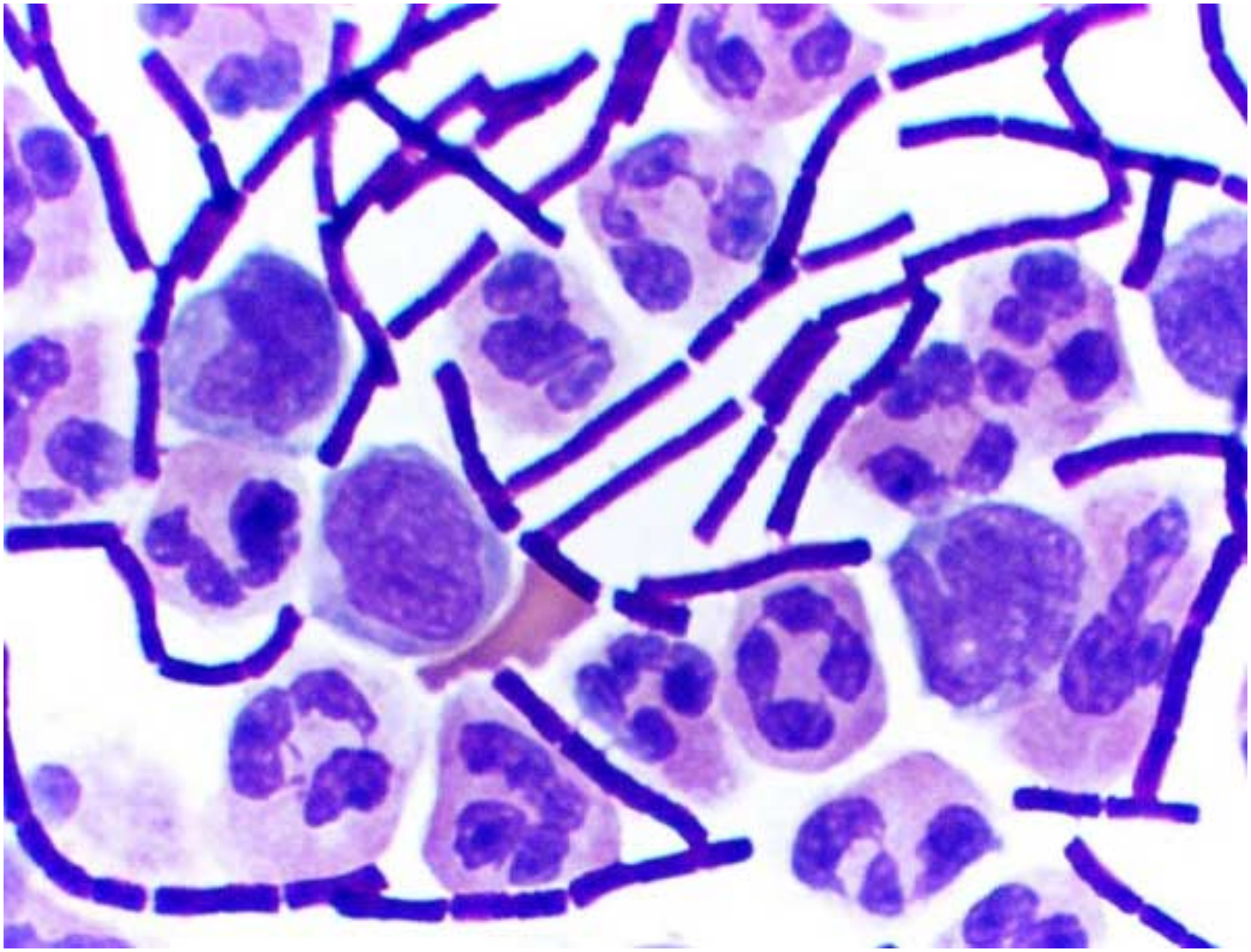
CT THORAX WITH
10/25/01







Diagnosis: Inhalational Anthrax

Clinical Suspicion

- **Consistent Symptoms**
  +/-
- **Exposure risk**

Alert Authorities*

Diagnostic tests

- **CBC/electrolytes/AST/ALT/Blood Cx/CXR**
  - **CT if CXR nml**
  - **consider GS of blood (Suggested…not standard)**
- **Serology (anti-PA), Blood for PCR?**
- **Neuro Sns: LP (GS/ Cell Cnt/Cx + IHC/PCR)**
- **Pleural Eff: Tap (GS/Cell Cnt/Cx + IHC/PCR)**
- **R/O infIluenza?**

Therapy

IHC = immunohistochemical stain
GS = gram stain
Cx = culture
PCR = polymerase chain reaction
CXR = chest radiograph
CT = chest computed tomography

*
- Public health
- Laboratory
- Law
- Etc…

Inhalational Anthrax Management

- Review of 82 Inhalational Anthrax (IA) cases between 1900 and 2005 showed the mortality rate during the 2001 anthrax attack was significantly lower then historically reported for IA (45 vs. 92%)
- The review identified the following statistical differences in the treatment of IA survivors and those who died

Inhalational Anthrax Management

- Therapy initiated during the prodromal phase (75 vs. 10%)
- Multidrug antibiotic regimen (67 vs. 21%)
- Pleural fluid drainage (83 vs. 9%)
- Anthrax antiserum therapy (among cases prior to 2001 (25 vs. 3%)

Holty JE et al. Ann Intern Med 2006;144:83

Antibiotics for Inhalational Anthrax

- Ciprofloxacin or other fluroquinolones with a similar spectrum of activity and CNS penetration are recommended over doxycycline
- One or two additional antimicrobials with adequate CNS penetration and expected *in vitro* activity such as rifampin, vancomycin, penicillin, ampicillin, meropenem
- Clindamycin recommended for inclusion because of its ability to inhibit protein synthesis
- Switch to single PO med upon improvement, to complete at least 60 day course of antibiotics (?)
- May have to use PO antibiotics in mass casualty situation

•Avoid Doxy in pregnancy, children under 8yr old
•Same antibiotic regimen for **GI anthrax** or **septic cutaneous anthrax**

Combination antibiotic therapy

- Multi drug regimens used in the 2001 case in patients who survived included
  - Ciprofloxacin, rifampin and vancomycin OR
  - Ciprofloxacin, rifampin, and clindamycin
- PCN not recommended based on the presence of an inducible beta-lactamase in the *B. anthracis* isolates

Antibiotics With Activity vs. *B. anthracis*

First-line Agents

- Fluoroquinolones (cipro)*†
- Tetracyclines (doxycycline)*†
- Penicillins*†
- Clindamycin*†
- Rifampin*†
- Vancomycin†
- Imepenem
- Macrolides (erythromycin)*
- Chloramphenicol

Second-line Agents

- Aminoglycosides
- Cefazolin
- † Quinupristin/Dalfopris-tin (Synercid)*†
- Linezolid†
- Daptomycin

***well-documented inherent or inducible resistance**

†animal model efficacy data

Postexposure prophylaxis

- CDC currently recommends 60 days of oral antibiotics in combination with a 3 dose series of anthrax vaccine adsorbed (AVA) at 0, 2 and 4 weeks.
- AVA not currently approved by FDA for PEP (so need a Investigational New Drug protocol or an Emergency Use Authorization).
- Antibiotics approved for PEP include Ciprofloxacin, Doxycycline, Penicillin G procaine and Levofloxacin*
- PCN should not be used initially for PEP as β-lactam resistance has been identified among naturally occurring isolates.

Postexposure prophylaxis

- PCN should not be used initially for PEP as *β*-lactam resistance has been identified among naturally occurring isolates.
- Amoxicillin can be used for PEP once the *B. anthracis* strain has been proven penicillin susceptible and when other antimicrobial agents are not considered safe to use such as for pediatric patients, nursing and pregnant women.
- However Amoxicillin is not FDA approved for this indication and is thus considered “off label.”

Postexposure prophylaxis in persons immunized* against anthrax

- * Immunized = completed 6 doses and boosters up-to-date, or minimum of 3 doses w/in 6 months.
- Ciprofloxacin, Levofloxacin or Doxycycline for 4 weeks.
- Carefully monitor patients after stopping antibiotics

Duration of PEP?

Earlier Primate Studies and Spore Kinetics

- **Persistence of viable spores in 50 animals with PCN and vaccine prophylaxis**
  - **42 days: 15-20% of initial retained spores**
  - **50 days: 2%**
  - **75 days: 0.5-1.0%**
  - **100 days: Traces**
- **Death of one animal from anthrax 98 days after spore inhalation**
- **Viable spores in the lungs of all apparently healthy monkeys sacrificed 55-84 days post exposure**

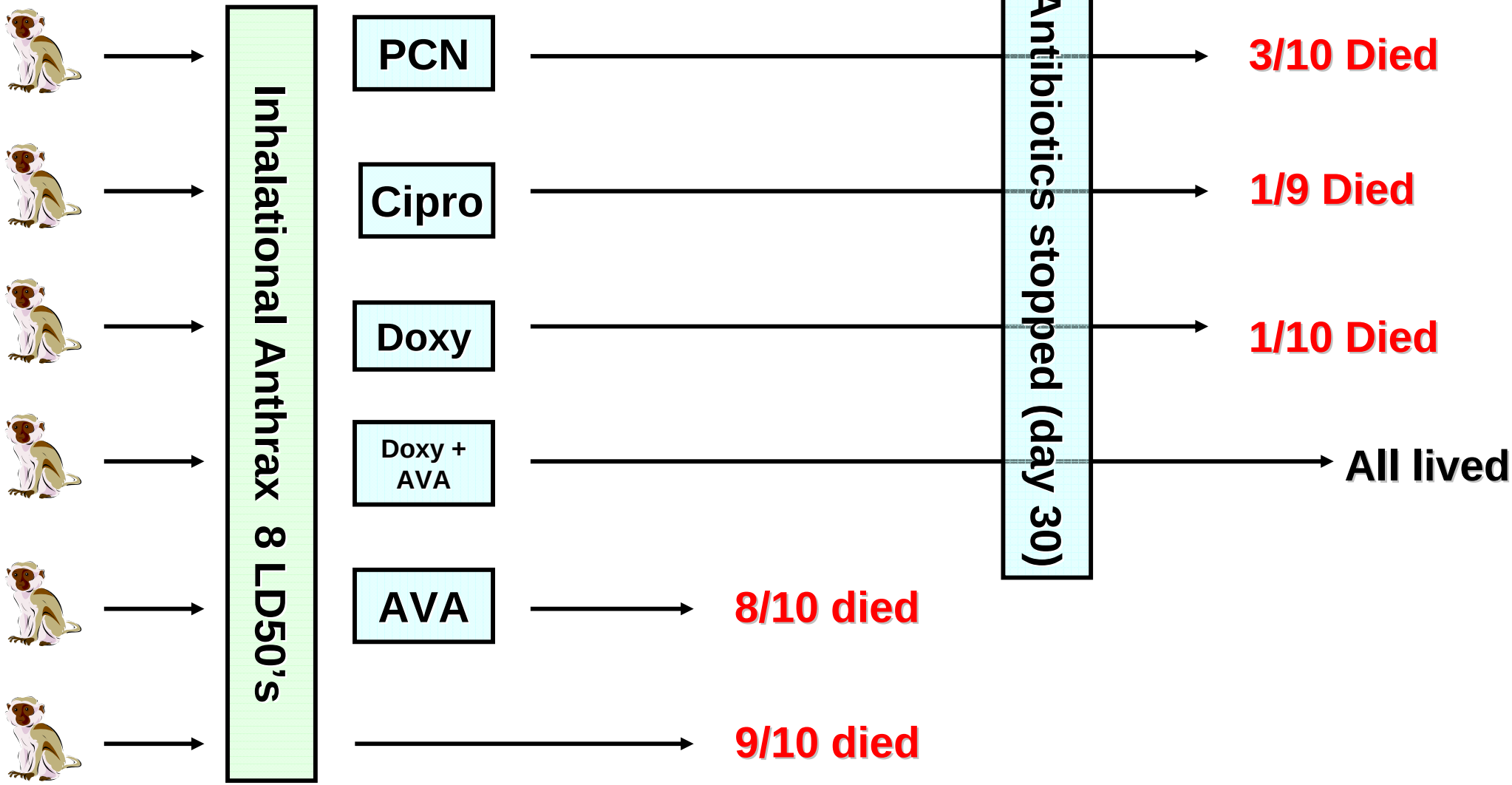
Henderson DW, Peacock S, Belton FC. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. J Hyg 1956; 54:28-36

Glassman HN. Discussion - Industrial Inhalation Anthrax. Bacteriol Rev 1966; 30:657-659

Gochenour WS, Sawyer WD, Henderson JE, et al. On the recognition and therapy of Simian woolsorter's disease. J. Hyg 1963; 61:317-322

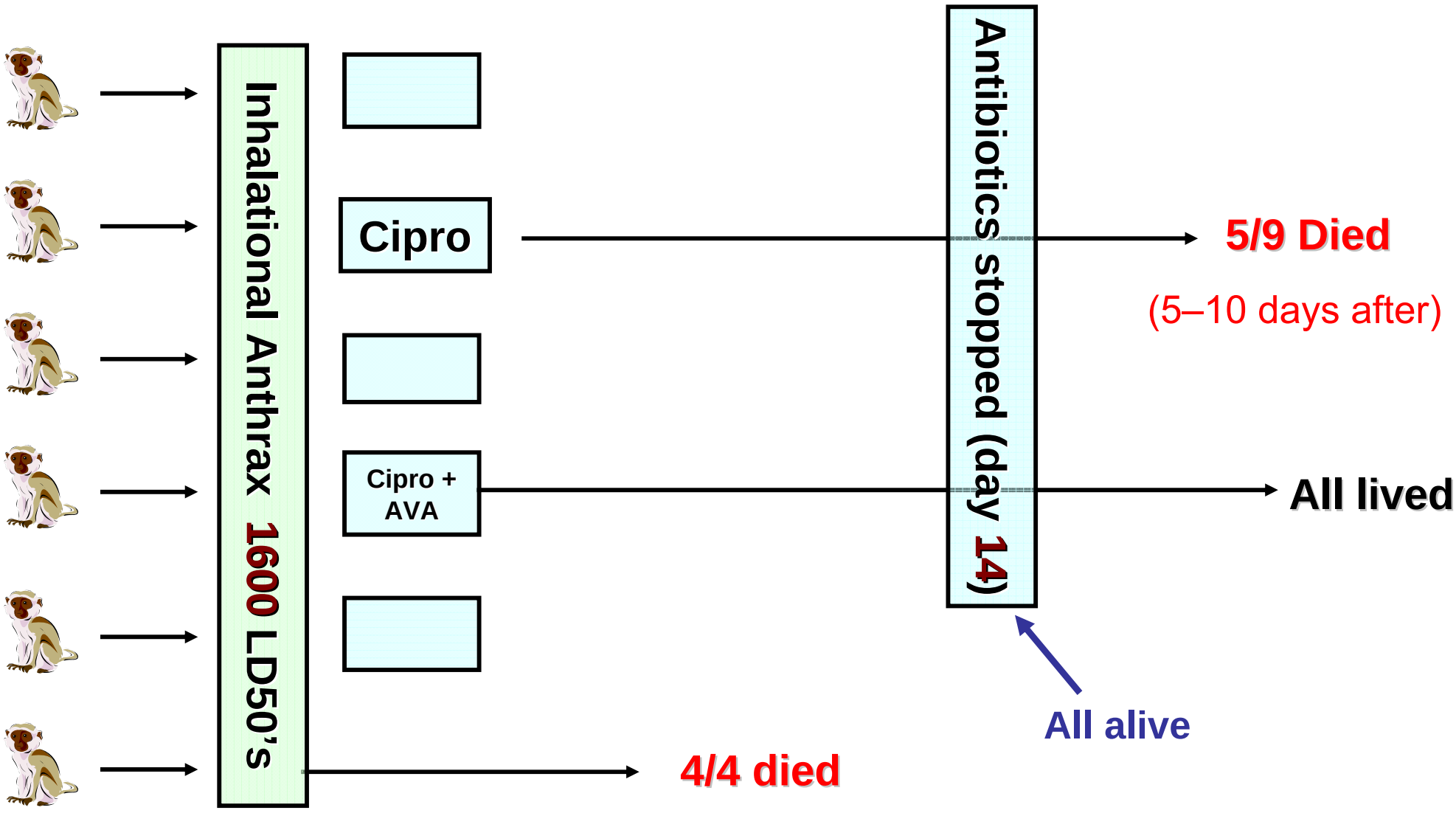
Inhalational Anthrax Chemoprophylaxis

Friedlander, et al JID, 1993;167:1239-42



Inhalational Anthrax Chemoprophylaxis

Vietri, et al. PNAS, 2006;103:7813-7816



Anthrax: Infection Control

- Inhalational anthrax not transmissible from person to person
- Autopsy may incur risk
- Cutaneous anthrax RARELY transmitted

USAMRIID

ANTHRAX VACCINE

Anthrax Vaccine History

- Anthrax-one of the first bacterial vaccines developed (1881)
- Live attenuated Sterne vaccine to protect animals (1930's)
- Live vaccines for human use-former Soviet Union (1940's)
- Chemical (non-living) vaccine US, UK (1950's)

Anthrax Immunity

- Antibodies are the main mechanism of vaccine-induced immunity
- Protection can be transferred with serum from animals vaccinated with spores, culture filtrates, or protective antigen (PA) alone
- Exact mechanism of antibody induced protection not completely understood

Friedlander et.al. 2002. Curr. Tops. Microbiol. Immuno 271:33-60

U.S. Anthrax Vaccine

- Anthrax Vaccine Adsorbed (AVA-Biothrax) (MDPH, MDPH-PA, AVA)
- Licensed by the Food and Drug Administration (FDA) since 1970
- Noninfectious sterile filtrate from a culture of an attenuated strain of *B. anthracis*
- Adsorbed to the adjuvant aluminum hydroxide (Alhydrogel)

Anthrax Vaccine

- AVA given subcutaneously at 0, 2, and 4 weeks and 6, 12, and 18 months.
- Vaccine can be administered to healthy individuals aged 18 to 65 years
- Contraindications include any active infection, acute illness, pregnancy or temporary use of immune-suppressing drugs.

Anthrax Vaccine

- Vaccination with AVA induces an immune response to PA (AVA-40 µg/mL of PA)
- >1/3 develop detectable anti-PA IgG after an single inoculation
- 95% after the 2nd injection
- 100% after three doses
- The peak IgG response occurs after the 4th(6 month) dose

Pitman R.R et. al. 2006 Vaccine (in press)

AVA Efficacy

- Brachman Study (1954-9): 4 wool-sorting mills (379 vacc, 414 controls)
  - 23 cases in control (5 inhalation)
  - 3 cases in vaccinated (0 inhalation)
  - 92.5% efficacy

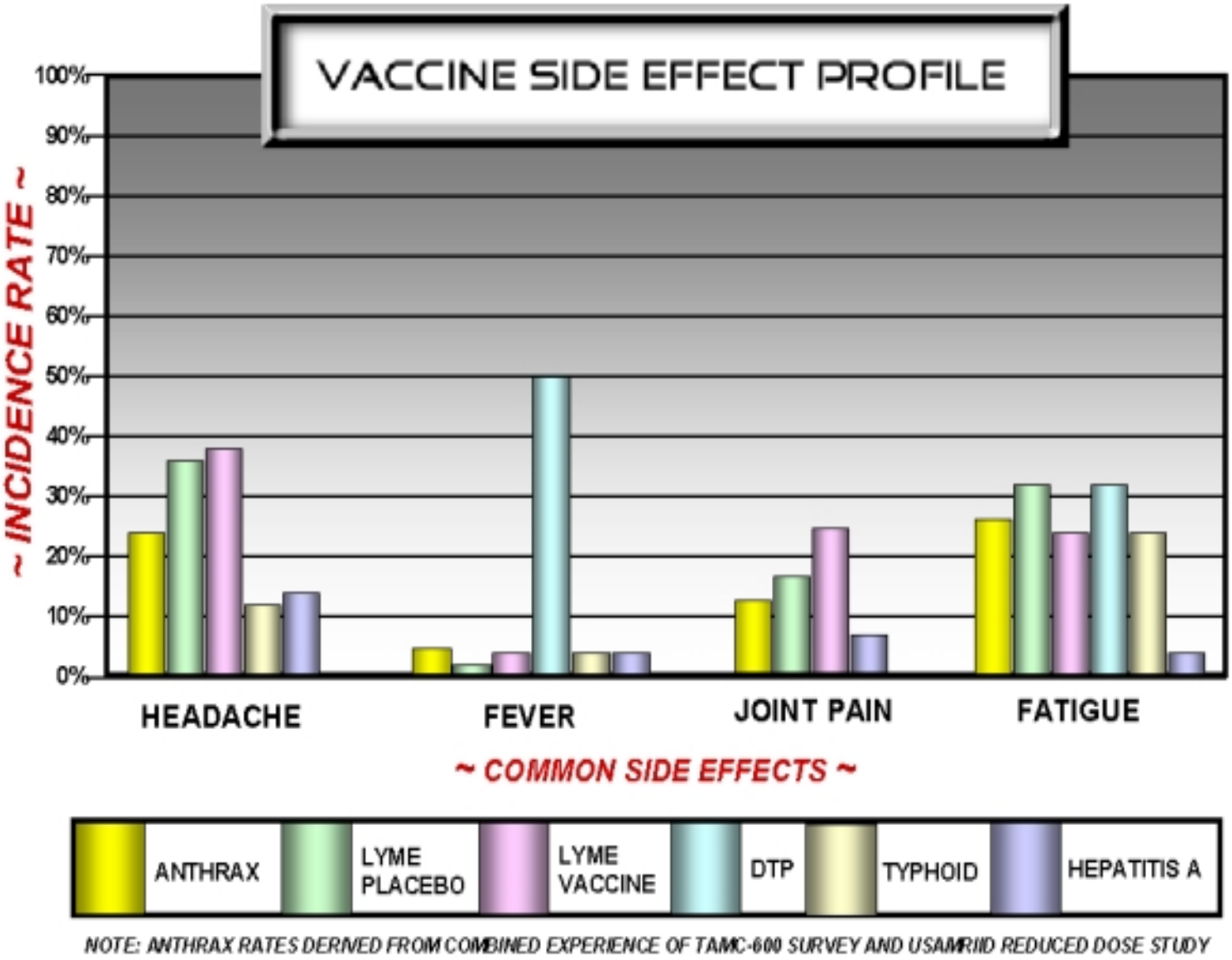
Brachman P.S. et al. 1962 Am. J. Pub. Health 56:632-645

AVA Efficacy

- In nonhuman primates, AVA provides close to 100% protection against an aerosol challenge with the Ames strain.
- 20/21 (95%) animals vaccinated at 0 and 2 weeks survived
- In another study, a single dose of AVA protected 10/10 (100%) animal from a lethal aerosol challenge at 6 weeks

Ivins B.E. et. al. 1995. Salisbury Med. Bull. 87:125-126

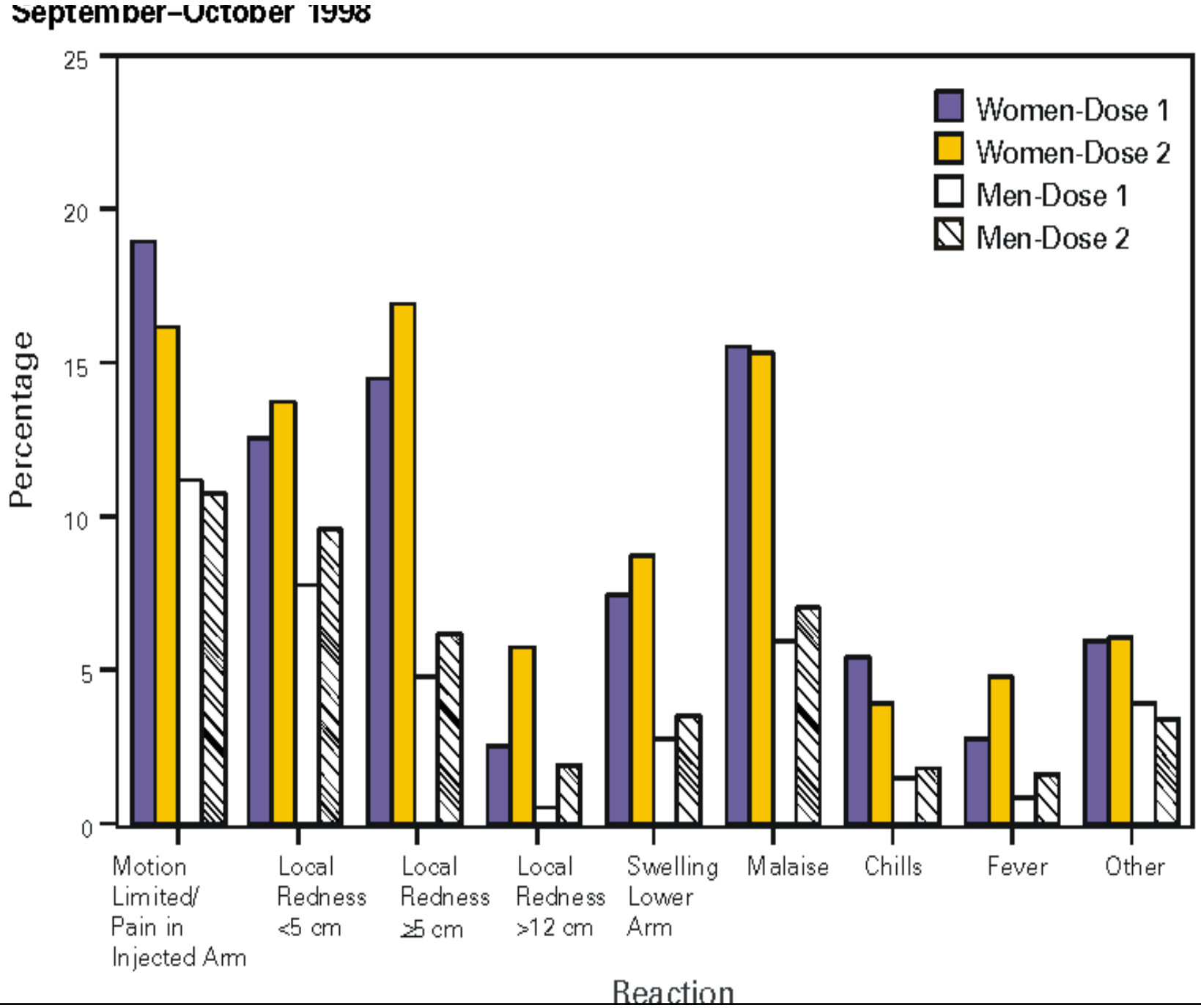
Ivins B.E. et. al. 1998. Vaccine. 16:1141-1148



-SQ nodules common…self-limited
-Some have significant local edema

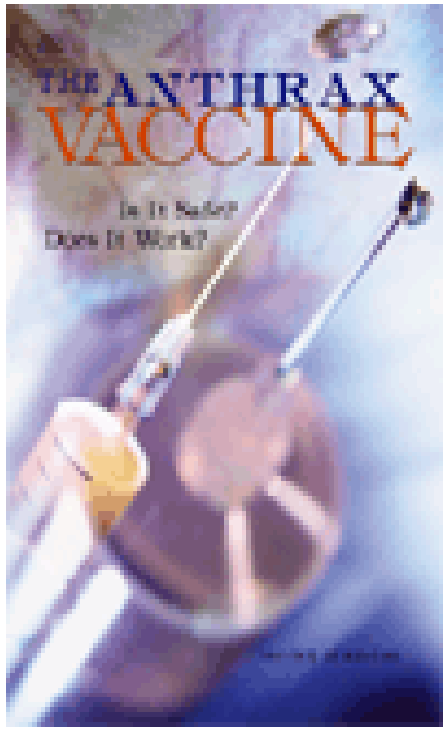
-Side effects worse in woman than men
-2nd and 3rd doses worse than others

Self-reported reactions to AVA – US Forces in Korea, Sep-Oct 1998



Institute of Medicine Study

March 2002



Anthrax vaccine is “safe and efficacious” for pre-exposure prevention of inhalational anthrax

http://www.nap.edu/html/anthrax/index.html

FDA Final Rule & Order 15 Dec 2005

- AVA is effective in prevention of anthrax "regardless of route of exposure"
- DoD issues directive from OSD on 22 DEC 05 to resume AVIP
  - Voluntary while policy was reviewed
  - Mandatory anthrax vaccine to be resumed

Mandatory Anthrax Vaccine Immunization Program (AVIP)

- DoD announced resumption of in October 2006
- Military personnel, emergency-essential DoD civilians and contractors
- Based on defined geographic areas or roles
- Allows personnel no longer deployed to higher threat areas to receive follow-up vaccine doses and booster shots on a voluntary basis

THE ASSISTANT SECRETARY OF DEFENSE

1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

HEALTH AFFAIRS

FEB 8 2007

MEMORANDUM FOR ASSISTANT SECRETARY OF THE ARMY (M&RA)

SUBJECT: Approval of U.S. Army Anthrax Vaccine Immunization Program Implementation Plan

In accordance with the Under Secretary of Defense for Personnel and Readiness memorandum, dated December 6, 2006, Subject: Implementation of the Anthrax Vaccine Immunization Program (AVIP), each Service is required to submit and receive approval of their AVIP plan before mandatory vaccinations can commence.

The U.S. Army AVIP implementation plan meets the requirements established in the memorandum above. As such, I approve the U.S. Army AVIP implementation plan for service wide distribution and education.

This policy is effective immediately and should be communicated to the appropriate Army organizations involved in the implementation of the AVIP. Specific questions regarding implementation may be directed to the Military Vaccine Agency at (703) 681-5101.

William Winkenwerder, Jr., MD

cc:
Surgeon General of the Army

United States Army
Medical Research Institute
of Infectious Diseases

Brucellosis, Q Fever, Glanders & Melioidosis

COL Mark Kortepeter, MC

Epi Triangle: Brucellosis

Organism

Host/Envt

Envt/Vehicle

Brucellosis: History

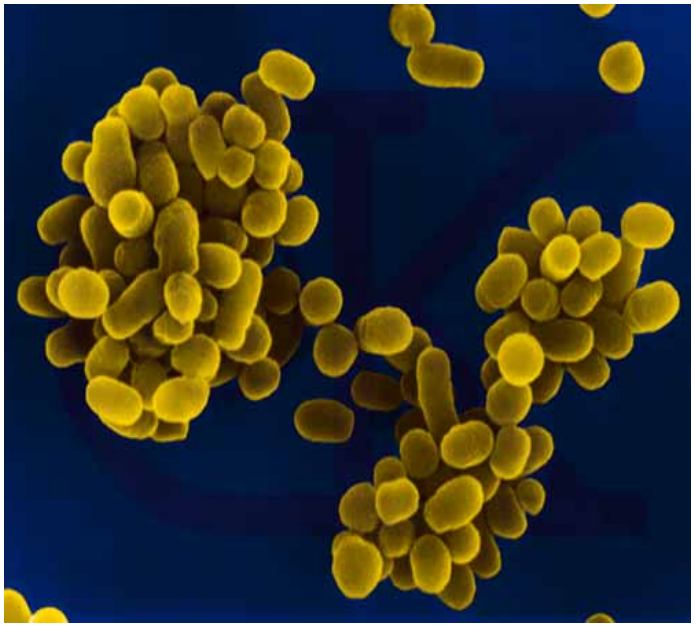
- 1850s: "Mediterranean fever" (*B. melitensis*)
  - 1st described among British soldiers in Malta, Crimean War
  - Reservoir: native goats
- 1886: 1st isolated by Bruce ("*Micrococcus melitensis*")
- 1897: Described/named (*Brucella*) by Bang & Stribolt
- Synonyms for human disease:
  - Undulant fever
  - Malta fever
  - Rock fever
  - Gibraltar fever
  - *Melitoccie* goat fever
  - Texas fever
  - Rio Grande fever
  - Bang fever
  - Brucella fever

Brucellosis as a Bioweapon

- *Brucella suis:*
  - First agent weaponized (aerosol) in former U.S. offensive program, 1954
  - Pine Bluff Arsenal, Arkansas, 1950s & 60s
- Easy to acquire & maintain
  - In Iraq: 20% of goats & 10% of sheep infected
  - Relatively tolerant of dessication
- Very low infectious dose
- Potential for animal & human disease
- Definitive dx takes time
- Incapacitating disease with potential for chronic debilitating disease

Brucellosis: Microbiology

- Gram-neg, non-motile aerobic coccobacilli
- Slow growing (doubling time 2 hours)
- BSL-3 (under-hood) precautions in lab
  - #1 lab-acquired infection



Brucellosis

7 currently recognized *Brucella* species,

4 cause human disease:

***Brucella* spp**	**Reservoirs**		**Pathogenicity to Humans**
	Primary	**Secondary**	
melitensis	sheep, goat	dog, camels	highest
suis	pig (wild & domestic)	dog, cattle, reindeer, caribou	high
abortus	cattle, bison, deer	goat, sheep, dog, camels	moderate
canis	dog, coyote	human (rare)	moderate

Brucellosis: Epidemiology

- Distribution is worldwide, species vary by location

ProMED Digest Tuesday, February 27 2007 Volume 2007: Number 103

Brucellosis, human - China (Heilongjiang) 20070226.1755
Brucellosis, human - Kyrgyzstan (Osh) 20061120.3311
Brucellosis, human, bovine - Kyrgyzstan (02): background 20060924.2725
Brucellosis, human, bovine - Kyrgyzstan (Chuiskiy) 20060923.2715 2005-
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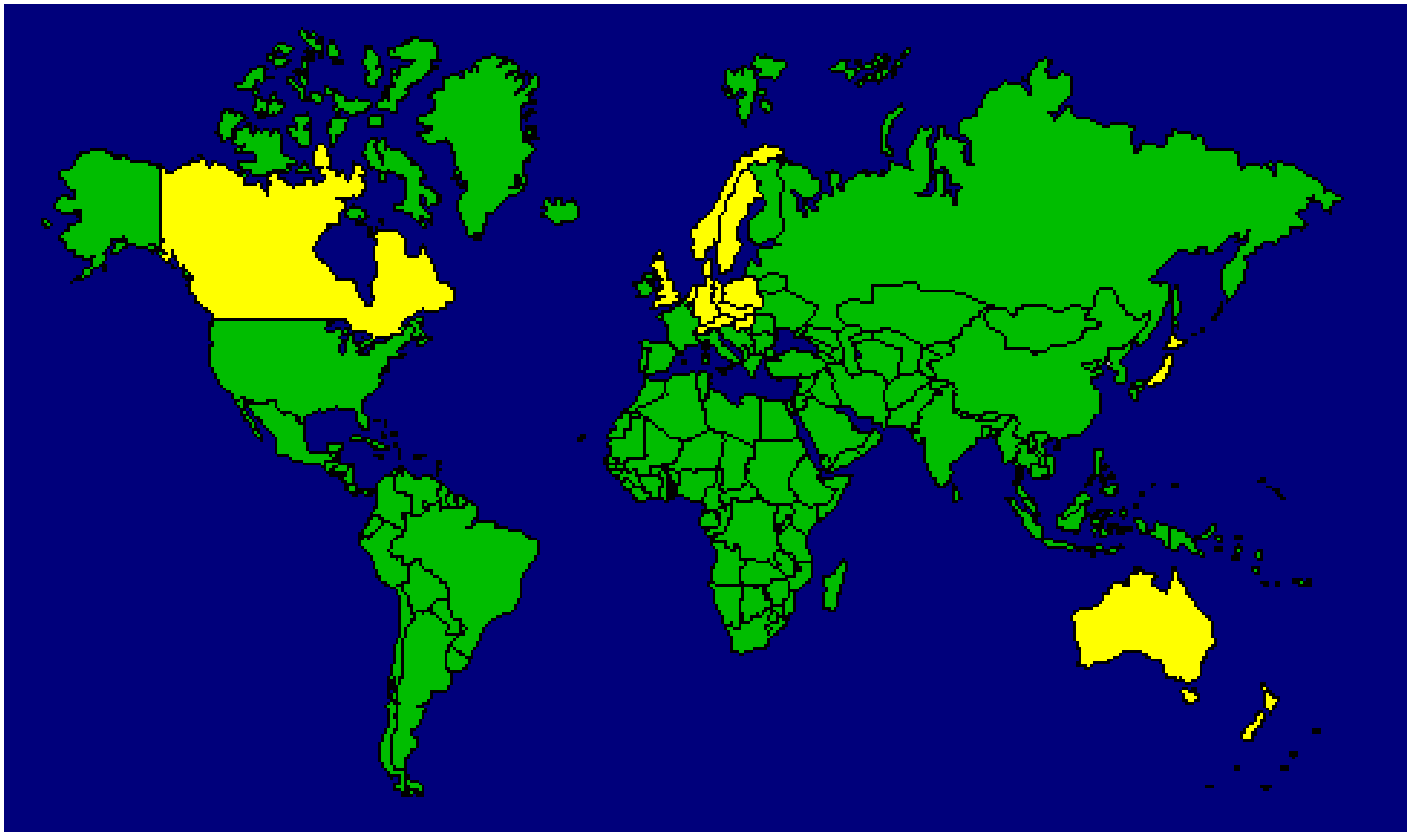
Brucellosis, human - Kyrgyzstan (Jelalabad) 20050712.1975
Brucellosis, human - Czech Republic ex Turkey (03) 20051024.3098
Brucellosis, human - Czech Republic ex Turkey 20051017.3030
Brucellosis, human, bovine - Venezuela 20051007.2932
Brucellosis, human, caprine - Thailand (Kanchanaburi) 20050907.2646
Brucellosis, human - Russia (Dagestan) 20050826.2523
Brucellosis, human - Kyrgyzstan (Jelalabad) 20050712.1975
Brucellosis, human - Bosnia & Herzegovina 20050614.1658
Brucellosis, human - Bulgaria ex Greece 20050328.0896 2004-

Brucellosis - China (02): Hong Kong 20041224.3396
Brucellosis - China 20041222.3371
Brucellosis, human & caprines - Saudi Arabia (Jizan) 20040716.1926
Brucellosis, humans & caprines - Lebanon 20040715.1907

Epidemiology: Distribution



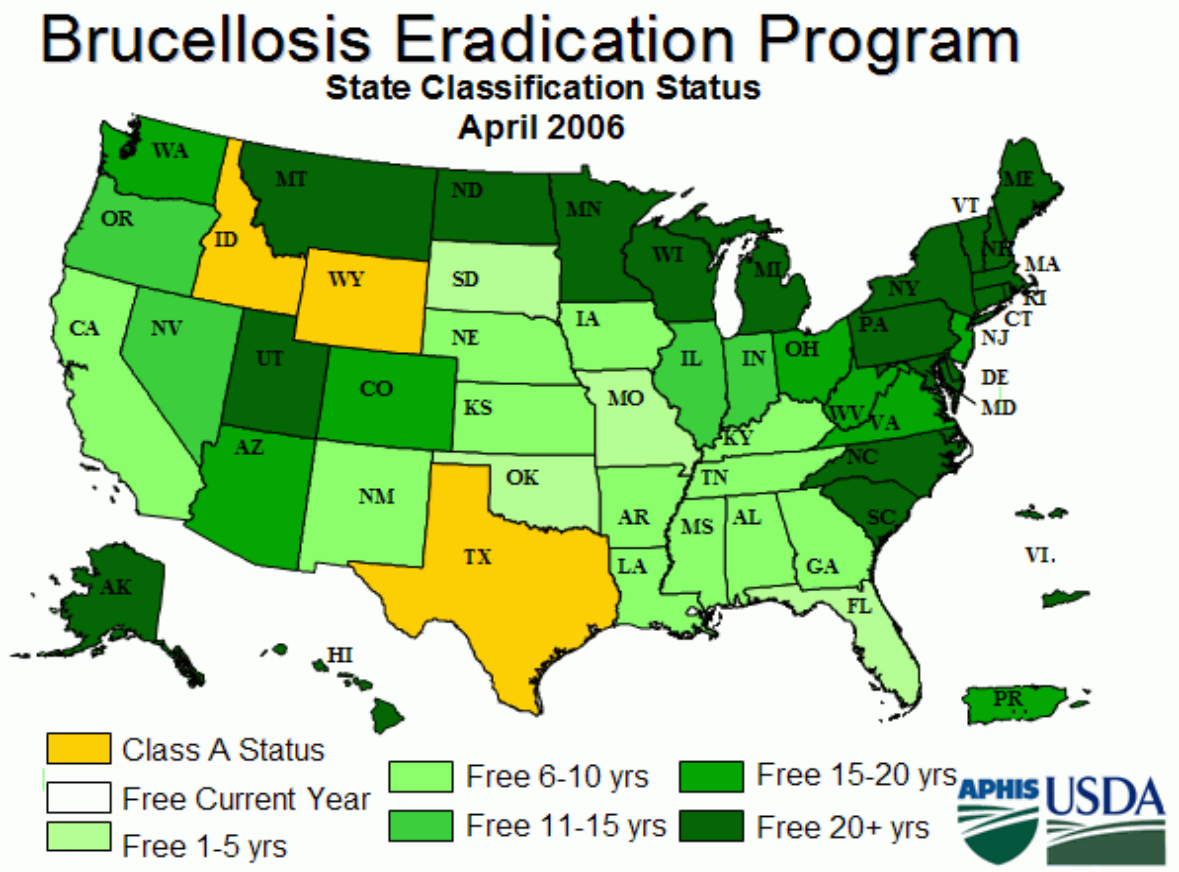
Free of *Brucella abortus* and *B. melitensis*

Sporadic or endemic

Highest prevalence: Mediterranean basin, Arabian peninsula, Central and South America

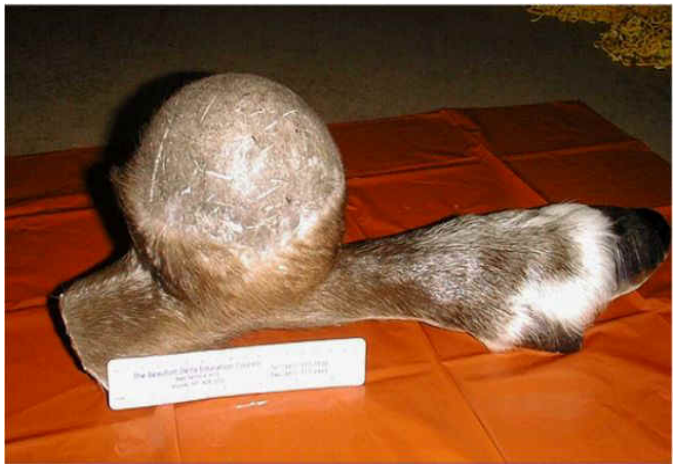
Brucellosis: Epidemiology

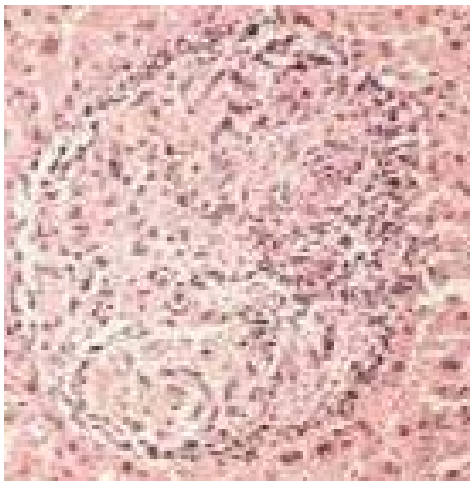
- Developed countries have widely eradicated in animals
  - U.S., 2006: Animal cases in 3 states; eradication ongoing
  - U.S. cases peaked in 1975 (>300)



Brucellosis in ***Animals***

- Granulomatous infections (similar to Tb)
  - Fascia, multiple internal organs & hides
- Primarily affects reproductive systems
  - Septic abortion, orchitis, infertility/sterility
- Musculoskeletal system
  - Osteomyelitis, arthritis
- Xmission enhanced by close quarters
  - Pens, stockyards





Brucellosis in ***Humans***

- Primarily transmitted by ingestion
  - Raw dairy products (milk, cheese) 70%
    - Imported, unpasteurized cheeses
  - Raw meat, liver, blood 29%
- But also
  - Animal contact 1%
  - Airborne infections (less frequent)
    - Bacteria survive well in aerosols & resist drying
  - Occupational transmission with animal handlers
    - Inoculation: Abraded skin, mucosal surfaces (genital secretions, placentas)
    - Inhalation of aerosols or dusts containing organisms

      (ID_{50} 10-100 organisms)

Pathogenesis

- Intracellular – survives within monocytes
- 1) infects local lymphocytes
- 2) goes to regional LNs
- 3) Enters circulation – seeds distant organs
- 4) Tropism for reticulo-endothelial system

Brucellosis: Pathogenicity

- Natural incidence: Unknown
  - <0.1 to >200/100,000;
  - May be grossly underreported
- Incubation periods: Variable
  - One week to several months; Commonly, 3 or 4 weeks
- Symptom onset: Variable
  - Sudden, over a few days (50%)
  - Gradual, over weeks to months (50%)
- Disease severity: Moderate
  - < 5% require hospitalization
  - Mortality occurs in < 5% of *untreated* cases
  - Endocarditis/meningitis prominent causes of death

Brucellosis: Acute Symptoms

- *Non-specific* – frequently an "FUO"

Fever	100%
Sweating	89%
Fatigue/weakness	75%
Chills	69%
Low back pain	58%
Arthralgia	55%
Anorexia	42%
Headache	39%

- Pulmonary sx's not prominent in acute disease

Ref: AR Lulu *et al*, "Human Brucellosis in Kuwait", QJM, 249:39, 1988.

Brucellosis: Acute Signs

- Multiple organ system involvement

Hepatosplenomegaly	39%
Arthritis	22%
Splenomegaly only	19%
Lymphadenopathy	9%
Hepatomegaly only	8%
Epididymoorchitis	6%

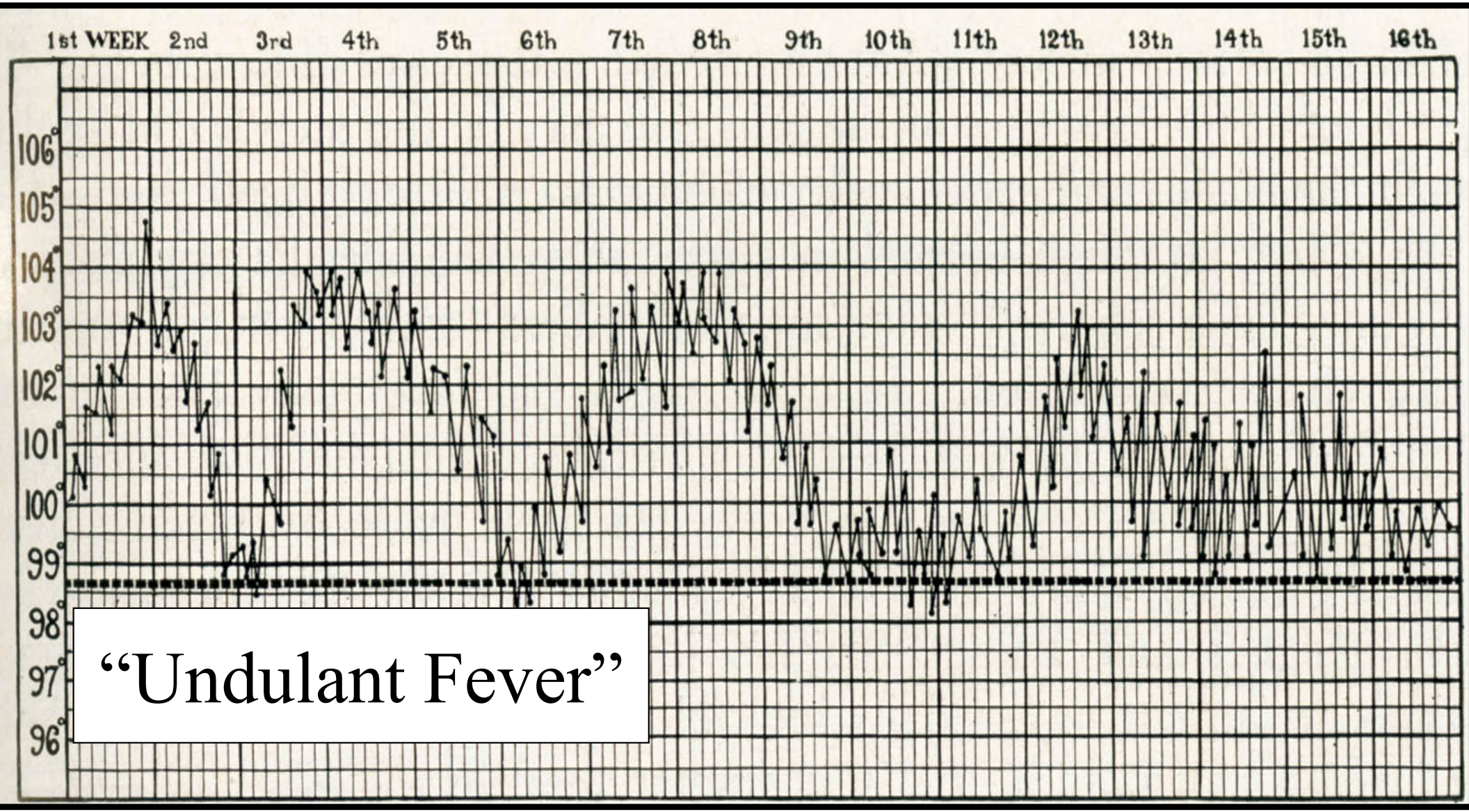
- Pulmonary signs not prominent in acute illness

Ref: AR Lulu *et al*, "Human Brucellosis in Kuwait", QJM, 249:39, 1988.

Brucellosis: Chronic Features

- "Undulant fever" > 90%
  - Unrecognized or untreated disease; intermittent fever
- "Focal" or "Localized when a specific organ system predominates:
- Osteoarticular disease ~ 40%
  - Septic arthritis, spondylitis, sacroiliitis, spinal osteomyelitis
- Pulmonary disease ~ 1 to 5%
  - Abscesses, nodules, bronchopneumonia, hilar adenopathy, & pleural effusion all reported
  - Does not correlate with known aerosol exposure
- Endocarditis < 2%

Brucellosis: Chronic Features



From RP Strong, *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*, 6th ed., Philadelphia, 1942.

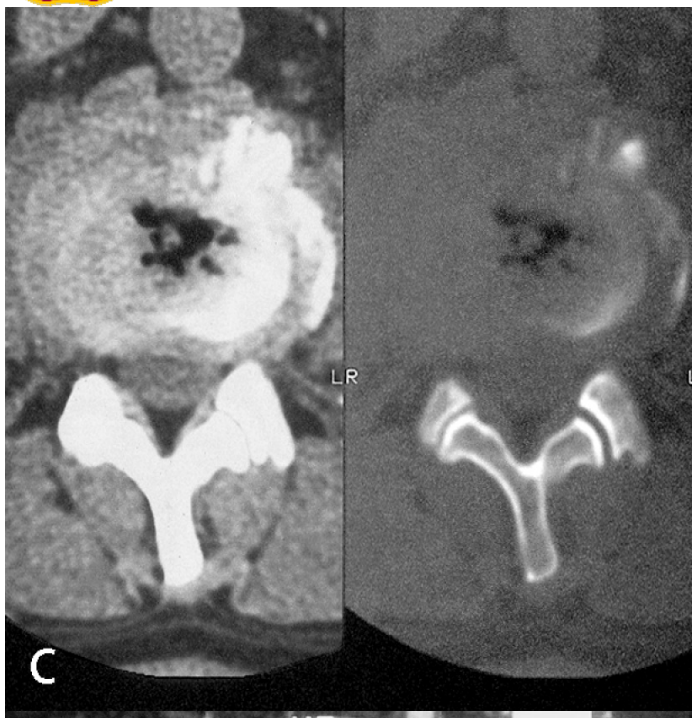
Brucellosis: Chronic Features

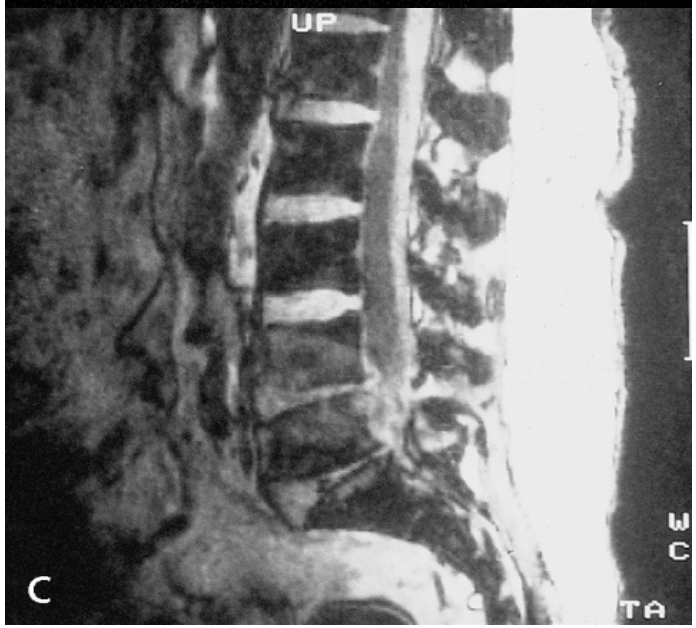
- Gastrointestinal disease
  - Ileitis, colitis, hepatitis
- Genito-urinary disease
  - Orchitis/epididymo-orchitis, intrauterine infection, renal abscess/granuloma
  - Spontaneous abortions 1st & 2nd trimester
- Neurological disease
  - Meningitis, encephalitis, peripheral neuropathy, brain/epidural abscess, radiculoneuropathies, meningovascular syndromes
  - Neuro-psychiatric
    - Common: Depression, headache, irritability
    - Neurotoxicologic process??
- Somatic complaints out of proportion to PE findings
  - "moldy" sweat

Brucellosis: Diagnosis

- Imaging
  - CT or MRI
    - Prolonged fever or M-S complaints
  - Technetium, gallium scans
    - Sacroiliitis, other axial skeletal infxn
  - Echocardiography
    - Endocarditis: Aortic > mitral valve lesions
  - Testicular U/S
    - Epididymoorchitis or abscess vs tumor
  - CXR
    - May be unremarkable even with respiratory sx's

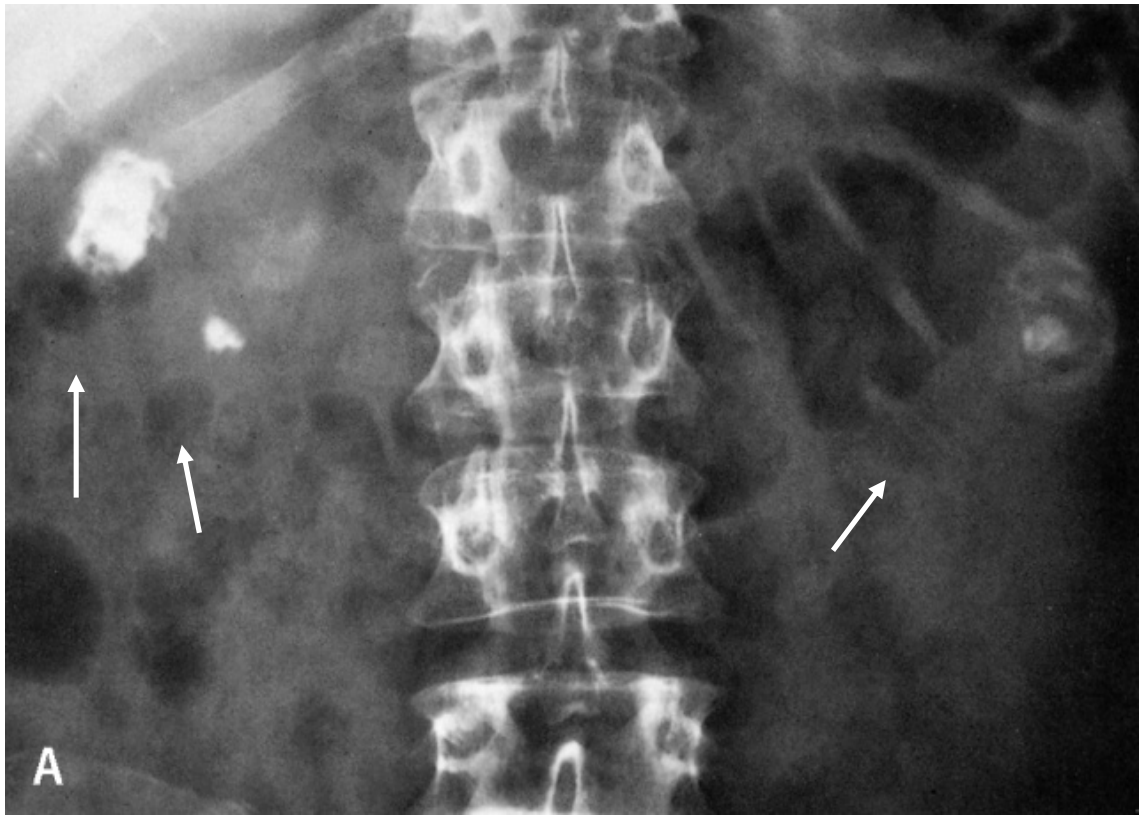
Brucellosis: X-ray Findings



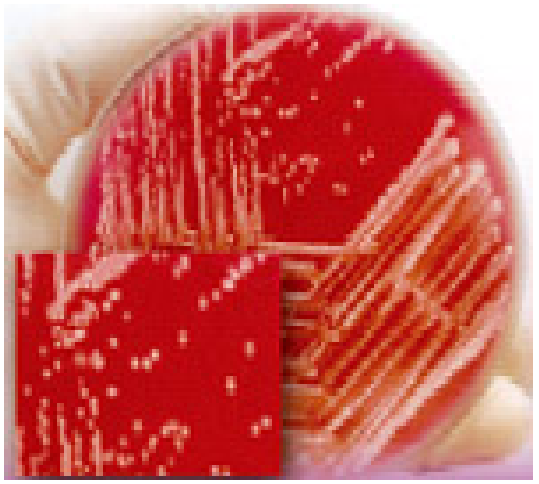


Osteoarticular

Renal



Brucellosis: Diagnosis



- Culture
  - *Best of several methods*
- Culture sites
  - Blood 14-50% sensitive in acute phase
  - Bone marrow > 90% sensitive in acute phase
  - CSF, urine, & joint aspirates also possible
- Culture methods
  - Traditional (non-automated) culture
    - Biphasic (Castaneda bottle method) may improve isolation
    - Re-culture onto solid media every 2 wks x 2 mos
  - *BACTEC*™ bd cultures may yield results in ~ 4 days

Brucellosis: Diagnosis

- Agglutination tests
  - Titers >1:160 presumptive for acute infection
    - Serum (SAT) for IgM & IgG
    - Tube (TA) for anti-O polysaccharide
  - 4-fold increase in A/C over 2 wks confirmatory (if same lab)
- ELISA & PCR are also available

Brucellosis: Acute Therapy

Acute disease

- Combination therapy a mainstay
  - Relapse 5-10% for oral combos; 30% for TMP-SMX monotherapy
- Most effective proven treatment:
  - Doxycycline 100 mg po bid for 4-6 weeks + Streptomycin*
    1 gm IM qd for first 2-3 weeks

    * Gentamicin probably a suitable alternative
- Uncomplicated outpatient disease (WHO recommended)
  - Doxy 100 mg bid + Rifampin 600 mg qd for 4-6 weeks
- Possible alternatives:
  - Ofloxacin (400 mg/d) + Rifampin (600 mg/day) for 4-6 weeks
    or
  - TMP/SMX (80 mg/400 mg) qid for 4-6 wks +/- Gentamicin
    5 mg/kg IV q first 5 days

Brucellosis: Acute Therapy

Special populations

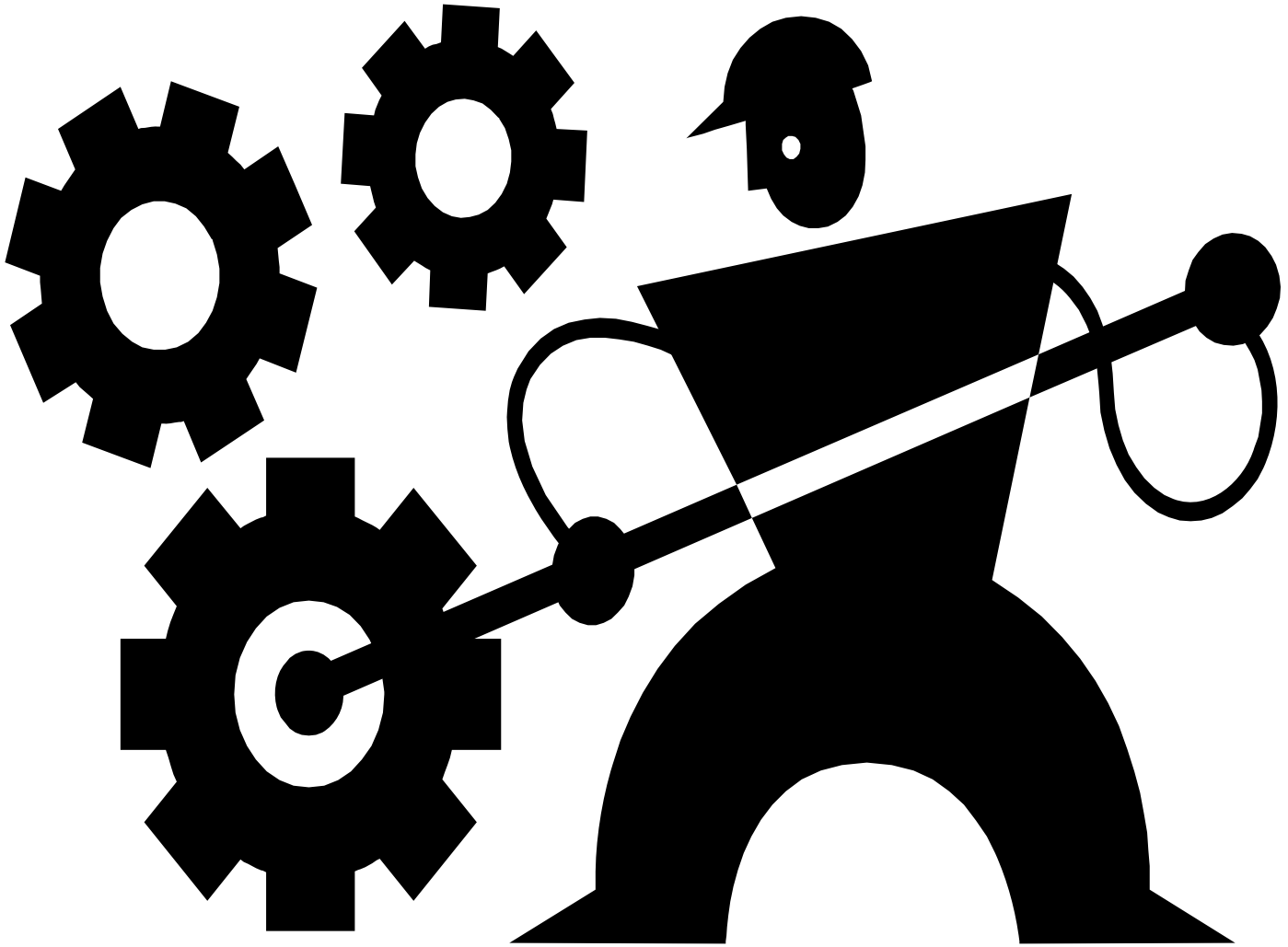
- Acute, uncomplicated disease in *children* < 8 yo age
  - TMP-SMX + Rifampin for 4-6 weeks
  - Dose by weight
- Acute, uncomplicated disease in *pregnancy*
  - Rifampin for 4-6 weeks (+ TMP-SMX after parturition)
- A quinolone-rifampin combo may be suitable alternative in both groups

Brucellosis: Therapy
Acute complicated or chronic (adult)

- May need long-term 3-drug tx for effective cure
- Doxy + Rifampin + Streptomycin *or* Gentamicin
  - At least for first 2-3 weeks, but…
  - Skeletal disease: 6-8 weeks
  - Suppurative disease (e.g., necrotizing orchitis): 6-8 weeks
  - Meningoencephalitis or endocarditis: 3-6 months
- Surgery
  - Abscess excision or drainage
  - Endocarditis (valve replacement)
  - Necrotizing orchitis, etc

Brucellosis: Prevention

- Primary prevention
  - No human vaccine
  - Live animal vaccines (B. abortus (very effective), B. melitensis (less effective) to eliminate livestock dz
  - Boil, pasteurize all dairy products (e.g., cheeses)
  - Deployed? Prohibit eating on the local economy
  - Standard precautions in clinical setting
    - *Not generally transmitted person-to-person*
    - Respiratory procedures, body fluid handling: Mask, gloves, eye protection
- Secondary prevention (Post-exposure prophylaxis)
  - Natural (animal) exposures: *Not* recommended
  - High-risk lab or aerosolized BW exposures:
    - Doxy-Rifampin x 4-6 wks



Q Fever

(*Coxiella burnetii*)

Q Fever: History

- Described, 1935 (Derrick)
  - Brisbane, Australia, abattoir workers: “Query” Fever
  - In U.S., lab-acquired infection: “Nine-Mile agent”
- Isolated, 1937 (Burnet)
- Tick Xmission shown, 1938 (Cox)

↓

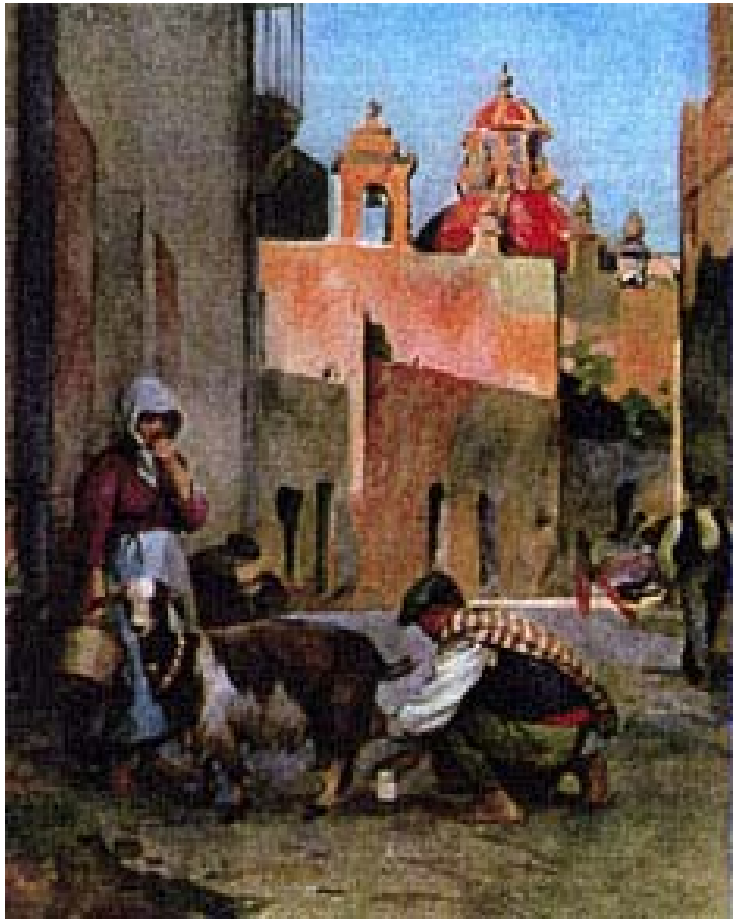
Coxiella burnetii

Q Fever: History in War

(Europe, Central Asia)

World War II:

- Serbia, 1942
  - *Balkangrippe*
  - 100s of German cases
- Italy, late 1944
  - 5 confirmed outbreaks
- Grottaglie AB, Italy, 1945
  - 1,700 cases in U.S. airmen



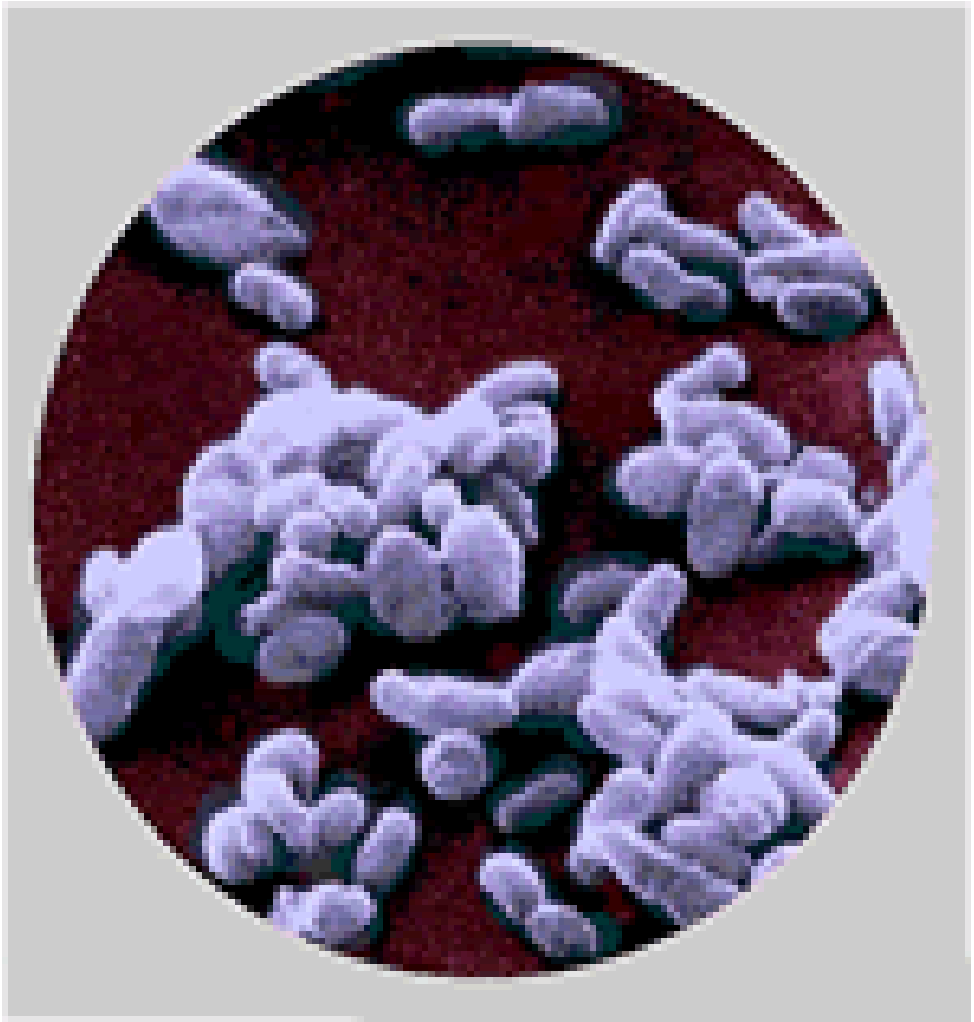
Turko-Cypriot War:

- 78 cases in British troops, 1975

- ***OIF***
  - >50 cases

Q Fever: Microbiology

- Rickettsia-like, gram-negative organism
  - Hardy, "spore-like" stage
    - Resists desiccation
    - Easily dispersed
  - Only 1 to 10 organisms necessary for infection



Q Fever as a Bioweapon

- *C. burnetii* easy to acquire & maintain
  - Hardy, spore-like stage
- Easy aerosolization
- Very low infectious dose
- Significant lab hazard
- Can cause chronic debilitating disease
- More incapacitating than lethal

Q Fever in ***Animals***

- Worldwide, except New Zealand
- Extensive wildlife reservoir – unlike Brucella
- Primary reservoirs: Sheep, cattle, goats
  - Also: Cats, rabbits, Dogs
- Ticks – reservoirs and vectors
  - Important for *animal* transmission only

Q Fever: Transmission

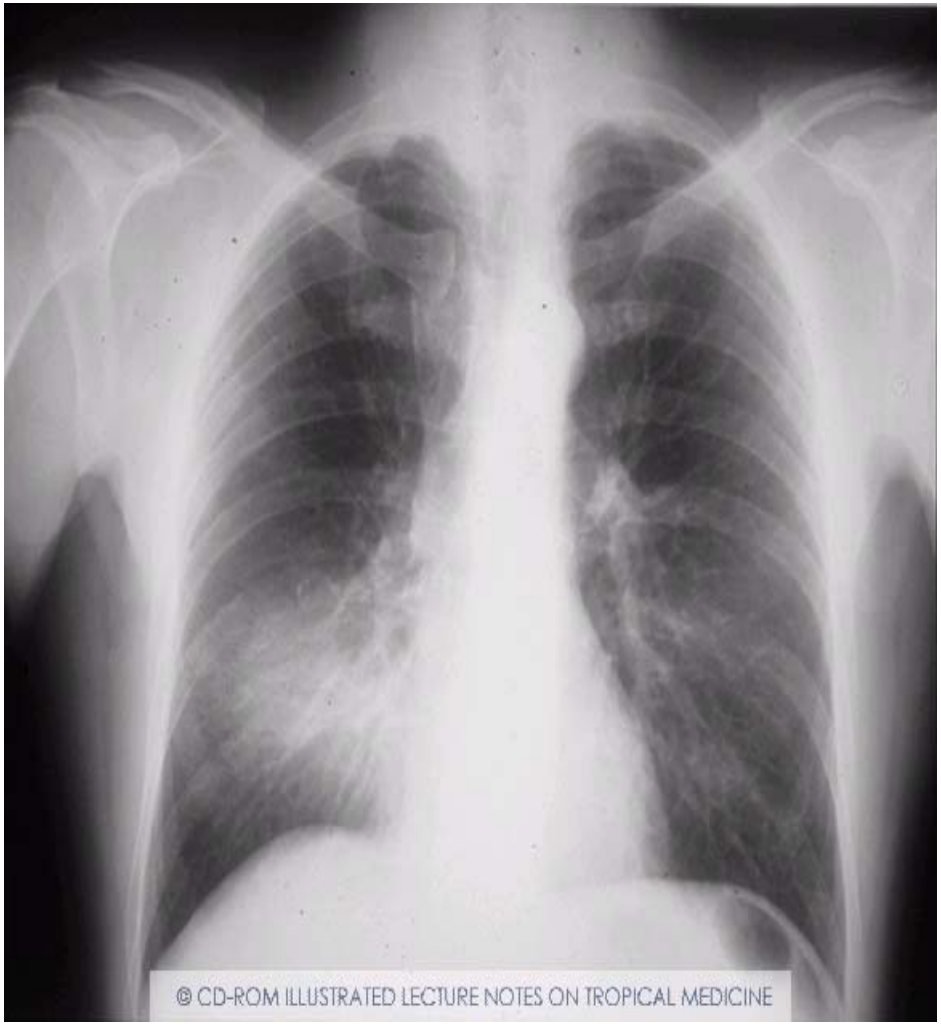
- Localizes in uterus, mammary glands
  - Excreted in milk, urine, feces of chronic carriers
  - As in *Brucella*
- Infected animals usually asymptomatic
- Multiplies in placentas, especially
  - Causing spontaneous abortion in animals
  - Shed organism massively – at parturition
    - 10^9 gp infective doses/gm tissue

Q Fever in ***Humans***

- At risk:
  - Abbatoir workers, veterinarians, farmers, those around farms/farm animals
- Aerosol exposure (most common)
  - Farm vehicles on roads
  - Animal husbandry
  - Clothing of cat owners
  - Lab workers
- Direct contact with animals (or parts)
  - Skinning infected rabbits, other animals
- Ingestion of raw milk (?)
- Rare: percutaneous (crushing ticks), bld transfusion, autopsy, vertical (mother-infant), sexual
- Outbreak related to playing poker

Q Fever: Clinical Features

- Incubation period: 7- 21 days
  - avg 2 wks
  - Dose-response
- Asymptomatic in ~ 60%
- If clinical:
  - Self-limited febrile illness
    - Atypical pneumonia (~ 20%)
    - Mild hepatitis (~ 20%)
    - Meningoencephalitis (~ 1%)
    - Pericarditis/myocarditis (~ 1%)
  - Fever will last 5-14 days
    - Or up to 2 months if untreated



Q Fever: Acute Signs & Symptoms

Nonspecific, febrile syndrome

Fever	99%
Weight Loss	82%
Headache	68%
Shortness of Breath	**64%**
Myalgias	54%
Cough	**51%**
Chest Pain	**45%**
Arthralgias	27%
Neurologic symptoms	23%

Q Fever: Clinical Features

- 1- 2% of *acute* cases → *chronic*
  - Endocarditis (Cx negative) in 2/3rds
    - Majority with pre-existing valvular disease
  - Osteomyelitis, especially in
    - Pre-existing bone disease
    - Prosthetic hardware
  - In pregnancy:
    - Fetal death
    - Prematurity
    - Low birth weight if in 1st or 2nd trimester

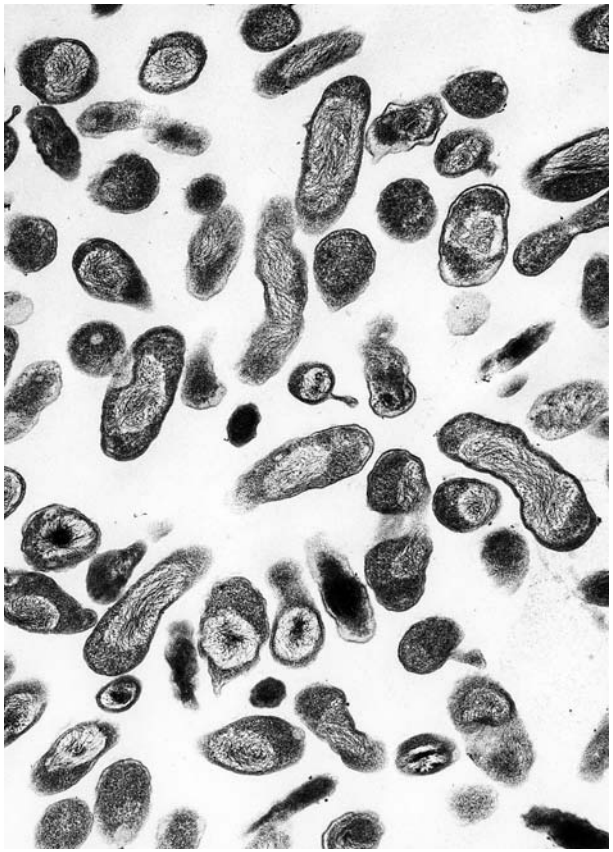
Laboratory findings

- WBC ct – usual nl (elevated in 1/3)
- Thrombocytopenia in 25%
- Slight elevation in transaminases
  - Nl bilirubin
- Classic feature on liver biopsy
  - Donut granulomas – central lipid vacuole with fibrin deposits

Q Fever: Diagnosis

- ***Best method*:** Indirect fluorescent antibody
  - IgM & IgG antibody profiles enable acute *vs* chronic forms to be distinguished
- Complement fixation
- ELISA

- Culture less sensitive than serology
  - Cell (not blood) cx is possible
  - ***A significant laboratory hazard***
    - Done in BSL-3 labs only
- PCR available in specialized labs



Q Fever: Acute Therapy

98% self-limited, but always tx if found

- Doxy 100 mg po bid for ≥ 14 days, *or*
- TCN 500 mg po qid for ≥ 14 days, *or*
- Fluoroquinolones (14-21 days), *or*
- TMP-SMX (14-21 days)

Q Fever: Acute Therapy

Special populations

- Children < 8 yo age
  - TMP-SMX *or* Macrolides
- Pregnancy:
  - ***During*:**

    TMP-SMX 160 mg/800mg po bid
  - ***After delivery (if serology positive)*:**

    Standard 2-3 wk course of doxy* or quinolone

*Contraindicated if breastfeeding

Q Fever: Chronic Therapy

Chronic endocarditis

- Doxy 100 mg BID plus …
  - Hydroxychloroquine 200 mg tid for ≥ 18 mos
    - Until IgG & IgA levels drop to ≤ 1:200

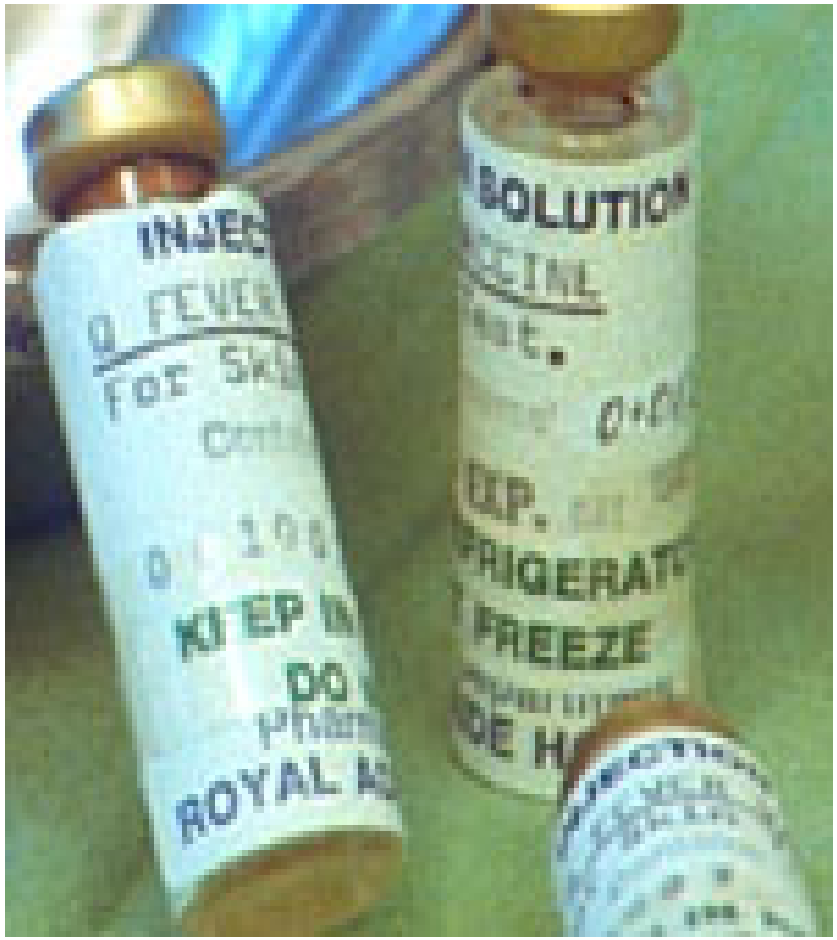
or

  - Ofloxacin 200 mg tid for ≥ 3 years

- Cipro 750 BID + rifampin 300 BID
- Possible valve replacement

Q Fever: Prevention & Control

- Formalin-inactivated whole-cell vaccine
  - Licensed in Australia (Qvax)
    - Production stops in 2007
  - Similar, IND version in the U.S.
  - One dose provides > 5 yrs protection
- Vaccine problematic in already-immune patients
  - Sterile abscesses possible
  - Contraindicated if skin testing is positive

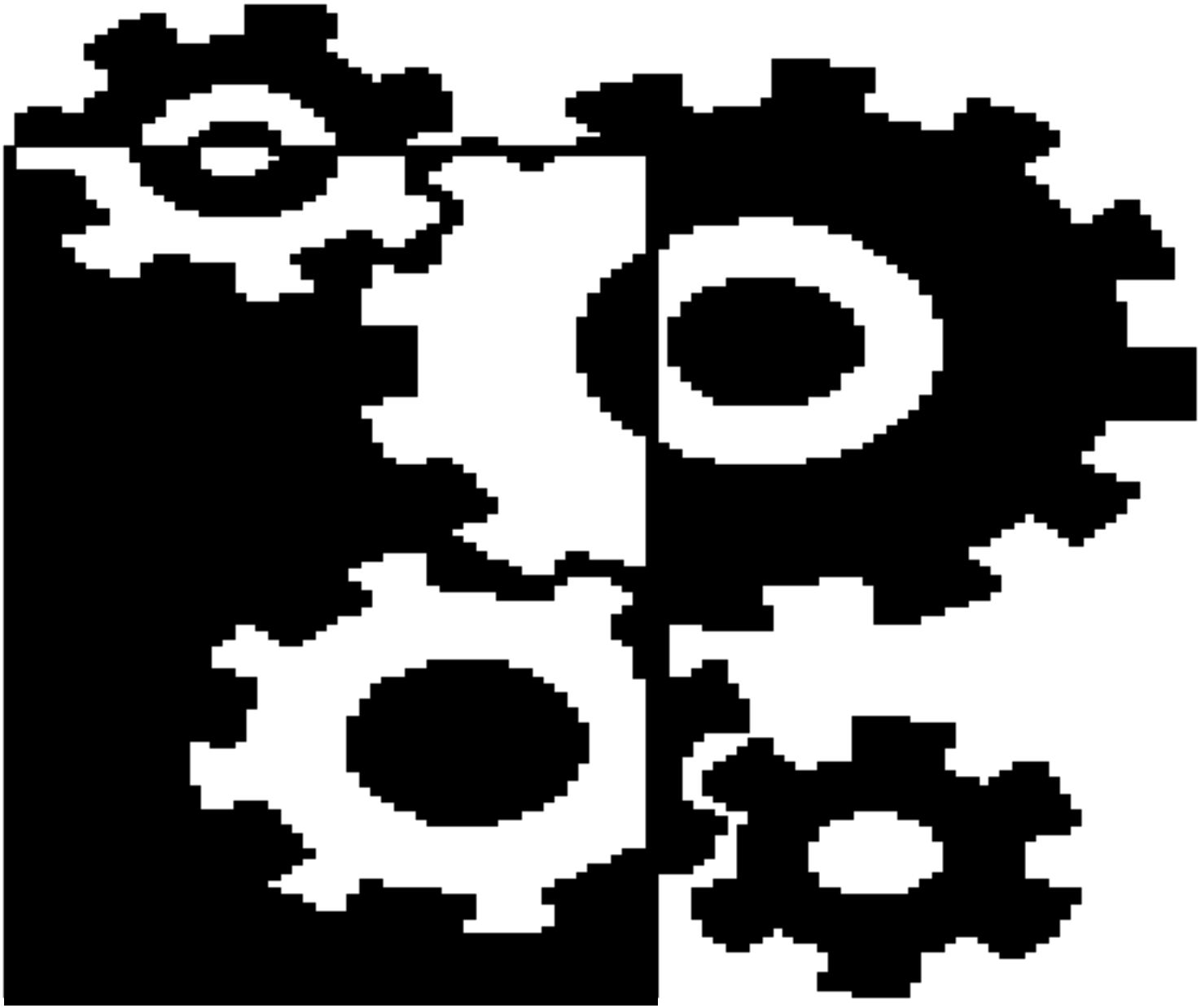


Q Fever: Post-exposure Prophylaxis

- Doxy or TCN x 5 to 7 days

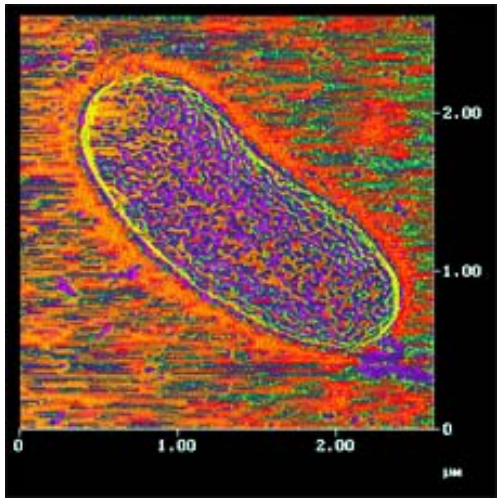
  … started 8-12 days post-exposure

  – If started on Day 1: *Disease occurs 3 weeks later*
  – If started on Days 8 to12: *Disease is prevented*

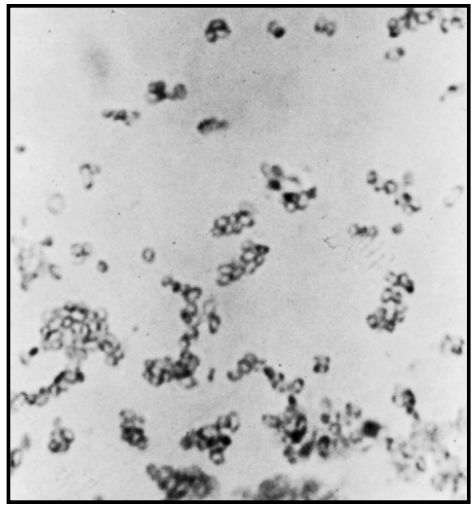


Burkholderia spp

Glanders & Melioidosis



Burkholderia spp



Two similar, but distinct, diseases:

B. mallei = **Glanders**

- Primarily: Disease of equids: horses, donkeys, mules
- Humans seldom infected despite close contact

B. pseudomallei = **Melioidosis**

- Widely distributed in soil & water
- Primarily infects humans, but occasionally animals

Glanders & Melioidosis: History

- **Glanders** [Old Fr, *glandre*, "gland"]
  - Recognized by Hippocrates
  - Occupational risk for horse handlers
  - 1st BW agent (w/anthrax) ever used (Germany, WWI)
- **Melioidosis** [Greek, *melis*, "distemper of asses"]
  - 1912: Morphine addicts in Rangoon, Burma
    - Whitmore & Krishnaswamy
  - Isolated from troops of virtually all nationalities serving in endemic tropical areas
- **1992:** Seven spp *Pseudomonas* →*Burkholderia*

Burkholderia spp
BW significance

- **Glanders**:
  - Classically: For use against animals
    - Disrupted transportation assets in WWI
    - *E.g.*, 1915 – Baltimore (Anton Dilger, MD)
    - Interest declined after advent of the auto/truck

- **Melioidosis**:
  - WW II & Vietnam:
    - Many US military personnel in Asia acquired it
  - Weaponization:
    - U.S. studied BW potential, but never weaponized
    - U.S.S.R. reportedly evaluated its BW potential

Glanders vs Melioidosis
B. mallei vs *B. pseudomallei*

SIMILARITIES:

- Both protean in clinical manifestations
- Most aspects of both dx are identical
  - Serologically indistinguishable
  - Isolation of organism required for specific dx
- Most aspects of both tx are identical
  - Antibiotic sensitivities similar
  - No vaccine available for either
- Weaponized form (inhalational)
  - Clinically indistinguishable
  - Without tx, both almost always fatal

Glanders vs Melioidosis
B. mallei vs *B. pseudomallei*

DIFFERENCES:

- Distribution:
  - *Glanders*: Zoonotic disease, once worldwide
  - *Melioidosis*: Truly (exclusively) tropical disease
- Reservoirs:
  - *Glanders*: Only found in susceptible animals
  - *Melioidosis*: Ubiquitous in soil, water, mud
- Relapse of meliodosis is common:
  - May remain dormant after tx & re-emerge decades later
- Knowledge of human disease:
  - *Melioidosis*: Moderately well known
  - *Glanders*: No human cases (but 1) in 3 generations

Glanders & Melioidosis as BW Agents

Both are considered potentials because…

- Spread by aerosol known to be efficient
  - Observed highly infectious to lab workers
  - Biosafety level 3 containment practices required
- Pulmonary disease could rapidly progress to sepsis, death
- No available vaccine & abx therapy poorly described
- Could be made (or already be) abx resistant
- Might be acquired easily (especially melioidosis)

However…

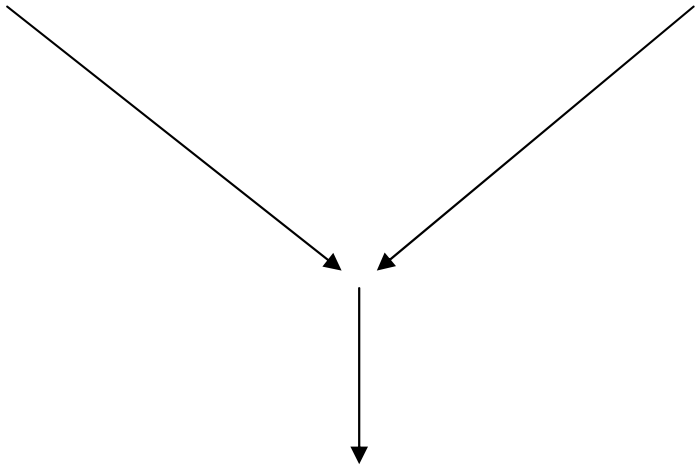
- Person-to-person spread rare or unknown (more concern with glanders)
- Non-inhalational forms probably more incapacitating than lethal

Glanders

- Natural Hx
- Clinical Forms

Melioidosis

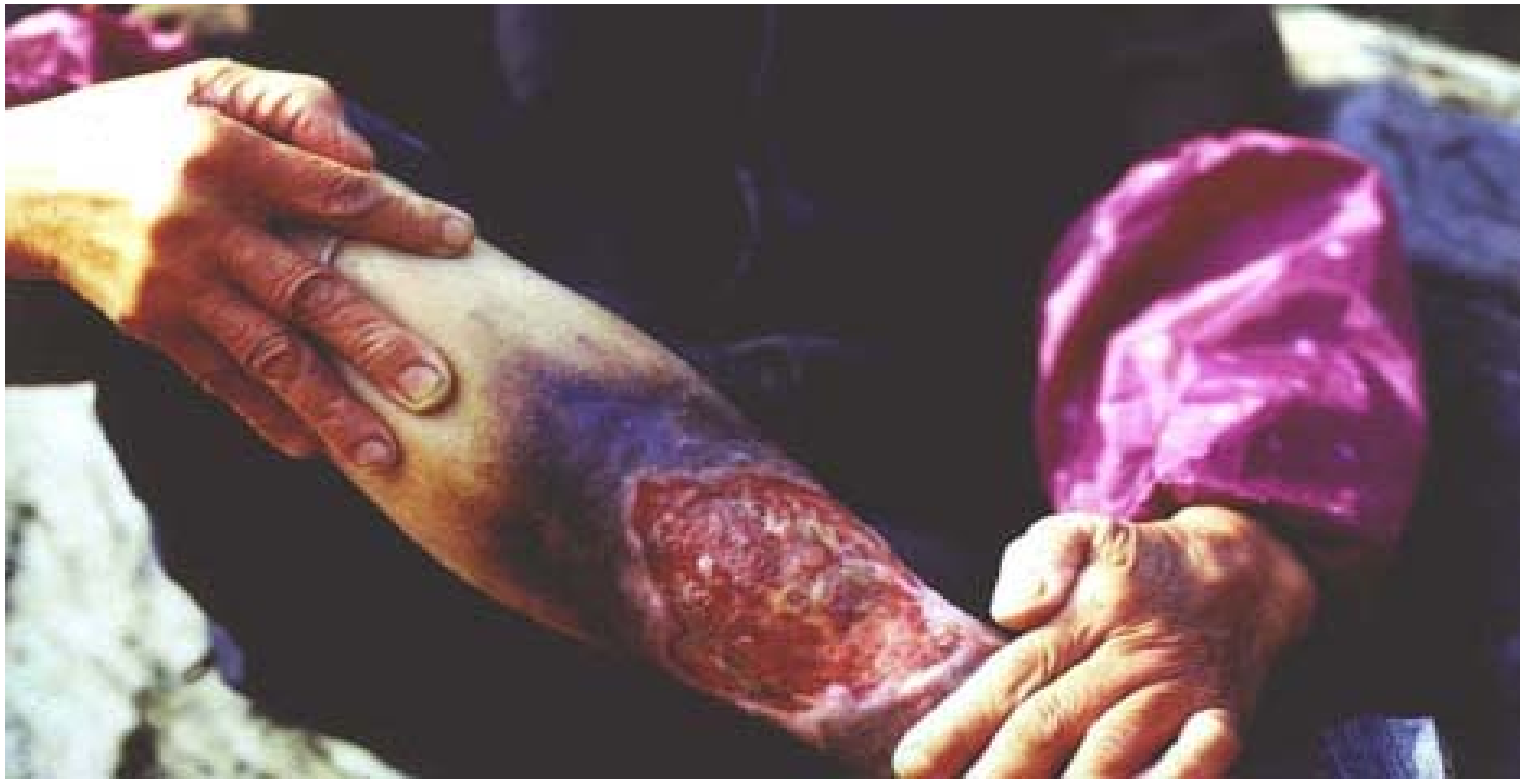
- Natural Hx
- Clinical Forms



Diagnosis

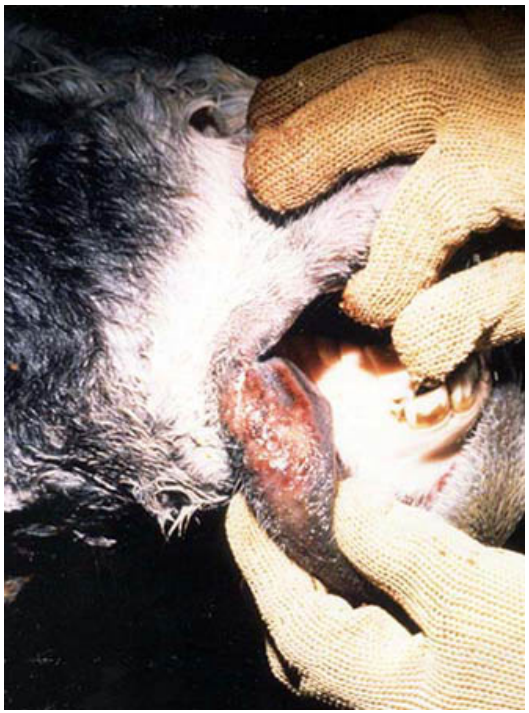
Treatment

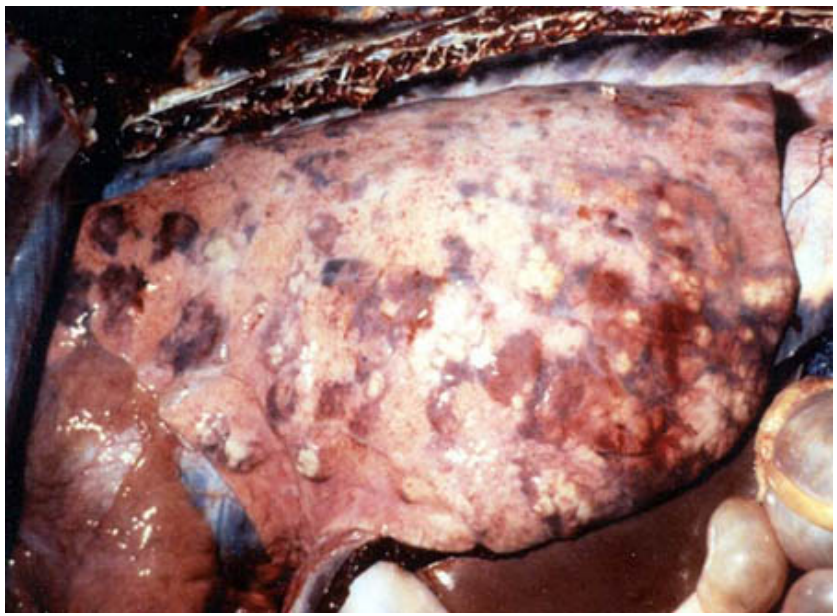
Glanders
(*Burkholderia mallei)*



Glanders in ***Animals***

- Exists in nature only in infected equid hosts
  - Eradicated in most of the world
  - Enzootic in Africa, Middle East, Mediterranean
- Portal of entry
  - Primarily: Breaks in hide, mucous membranes
  - Also: Inhalation of contaminated aerosols





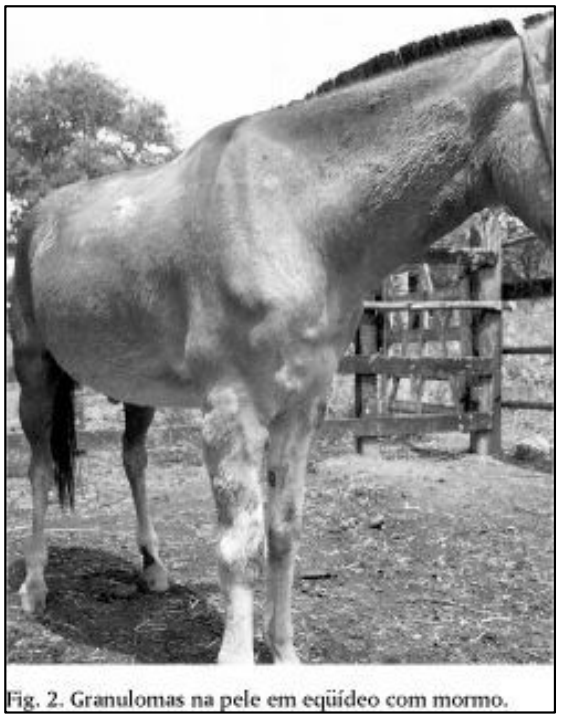


Fig. 2. Granulomas na pele em eqüídeo com mormo.

Glanders In ***Humans***

- Natural infections in humans: Rare
  - Requires contact with infected equids
  - Early 20th Century:
    - Sporadic cases in Asia, Africa, Near East, South America
  - Last naturally-acquired U.S. case: 1942
- Lab-acquired infections more common
  - USAMRIID: 1944-45 -- 6 lab infections
    1953 -- 1 " "
    2000 -- 1 " "
  - 2000 case: 1st reported human case in the English-language literature since 1949

Glanders: Clinical Forms

Correlate with route of entry:

- Cutaneous
- Mucocutaneous
- Oronasal or Ocular
- Inhalational

Any can progress:

- Disseminated infection & septicemia

Glanders: Incubation Periods

- Cutaneous 1-5 d (Range 1-21 d)
- Mucocutaneous 1-5 d (Range 1-21 d)
- Inhalational 10-14 d

According to animal models:

- High-dose, inhalational exposure

1-4 d*

*Also true for meliodosis

Glanders: Clinical Forms

- Cutaneous
  - Inflammatory nodules & subsequent lymphangitis
  - Sometimes: Sporotrichoid nodules
  - Nodules may break down & ulcerate
- Mucocutaneous
  - Acute or subacute onset of constitutional signs:
    - Fever (low-grade or recurring), rigors, sweats, headache, fatigue, & myalgias
  - Localized nodular → Erosive infection, mucopurulent discharge, & regional lymphadenopathy → Liver/spleen involvement common

Cutaneous Glanders

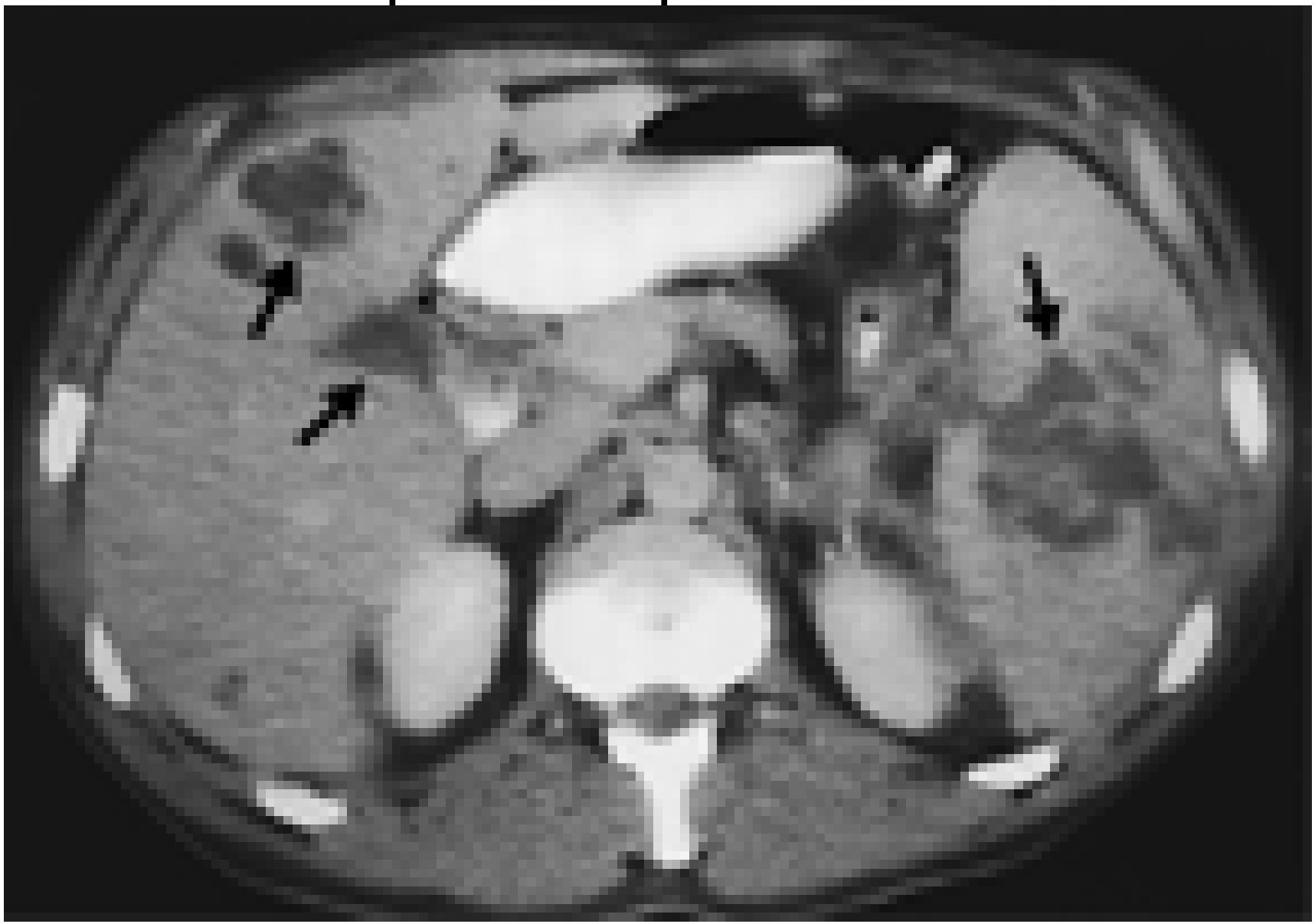
Glanders: Clinical Forms

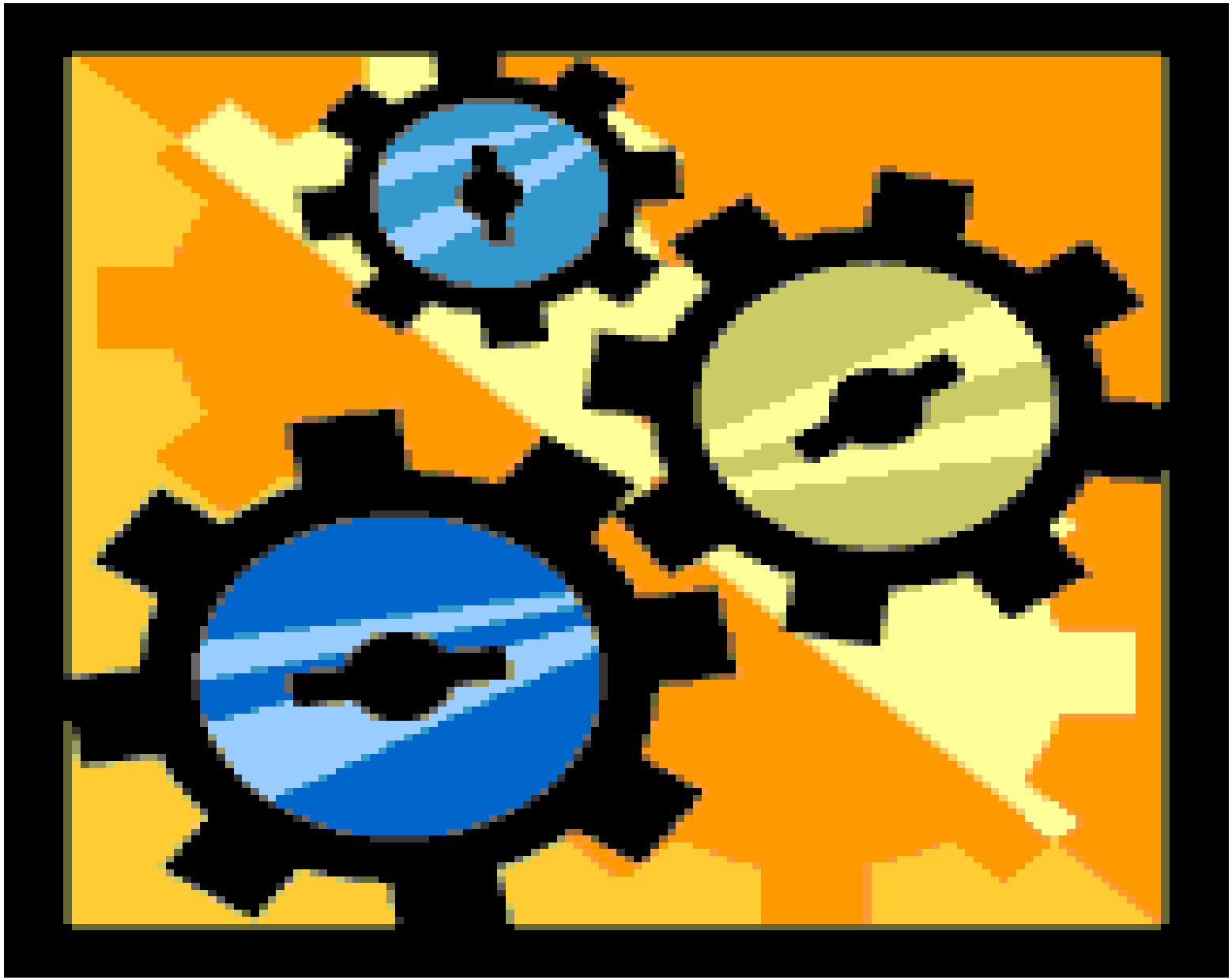
- Oronasal *or* ocular
  - Severe h/a, photophobia, lacrimation, mucopurulent nasal (ocular) exudates → Ulceration
  - Chronic infection & erosion of the nasal septum & turbinates → Severe disfigurement
- Pulmonary
  - May follow direct inhalation of organisms *or* secondarily via hematogenous spread
  - Pulmonary involvement → Pleuritic chest pain
  - Cervical adenopathy, pharyngitis, purulent rhinitis
  - Possibly other organ signs: hepatosplenomegaly, etc

Glanders: Clinical Forms
Septicemic & Disseminated

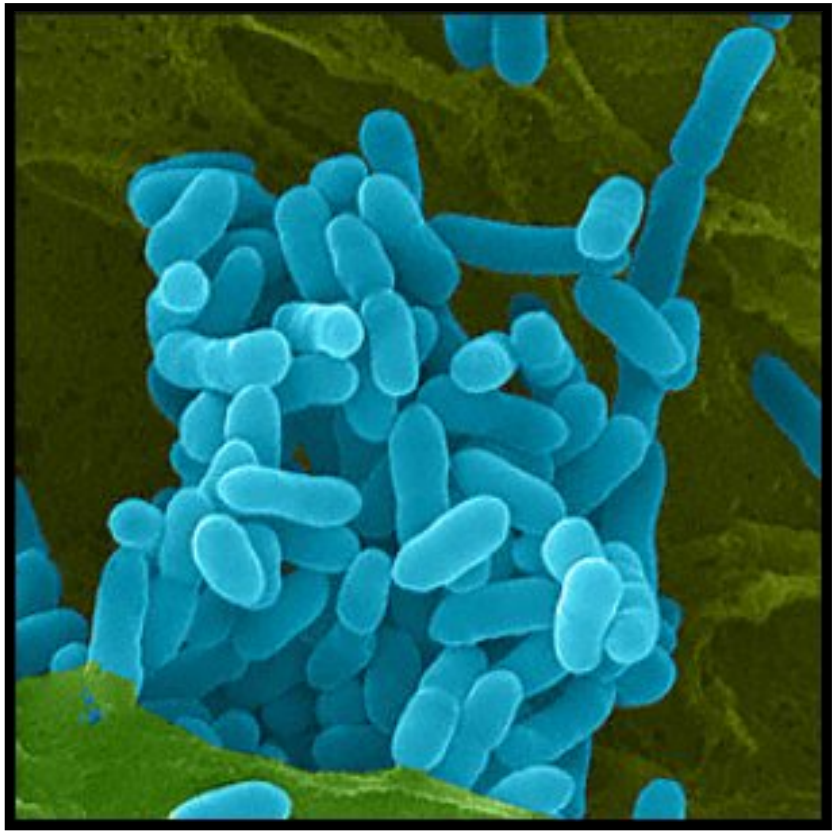
- May occur at any time during infection, regardless of portal of entry
- Rapidly progressive
- May include any of the previous signs & sx's
  - Plus: Tachycardia, jaundice, diarrhea, granulomatous & necrotizing lesions in virtually any organ (especially liver, spleen, lungs)
  - Cutaneous – can also have a diffuse papular/pustular rash that can be mistaken for smallpox

Case at Fort Detrick from 2000: Hepatic & Splenic Glanders



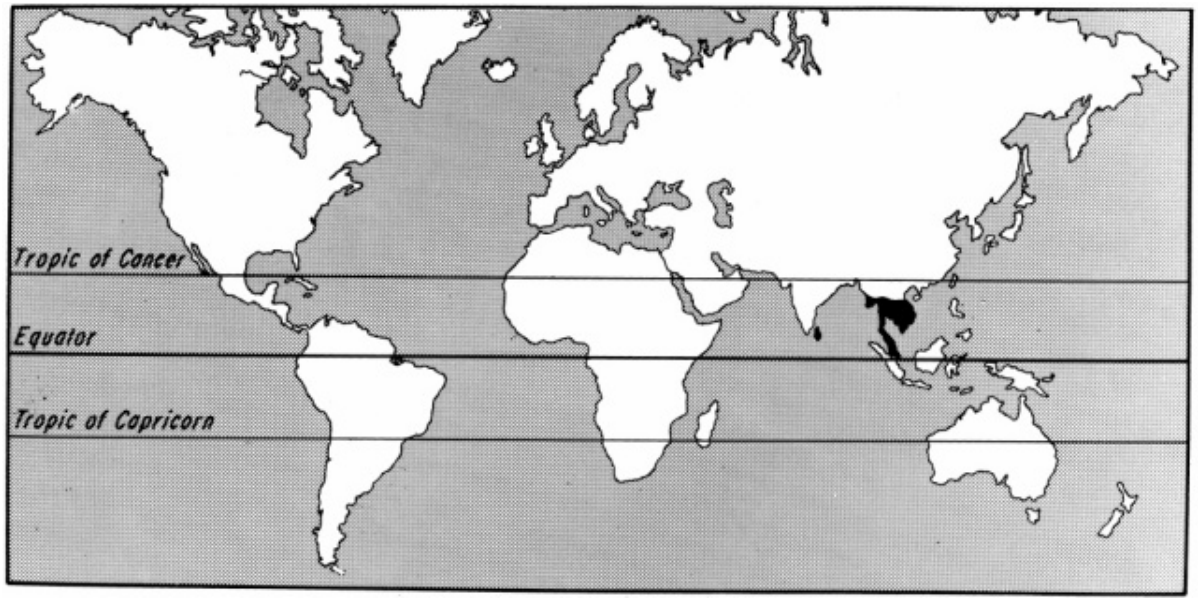


Melioidosis
(*Burkholderia pseudomallei*)



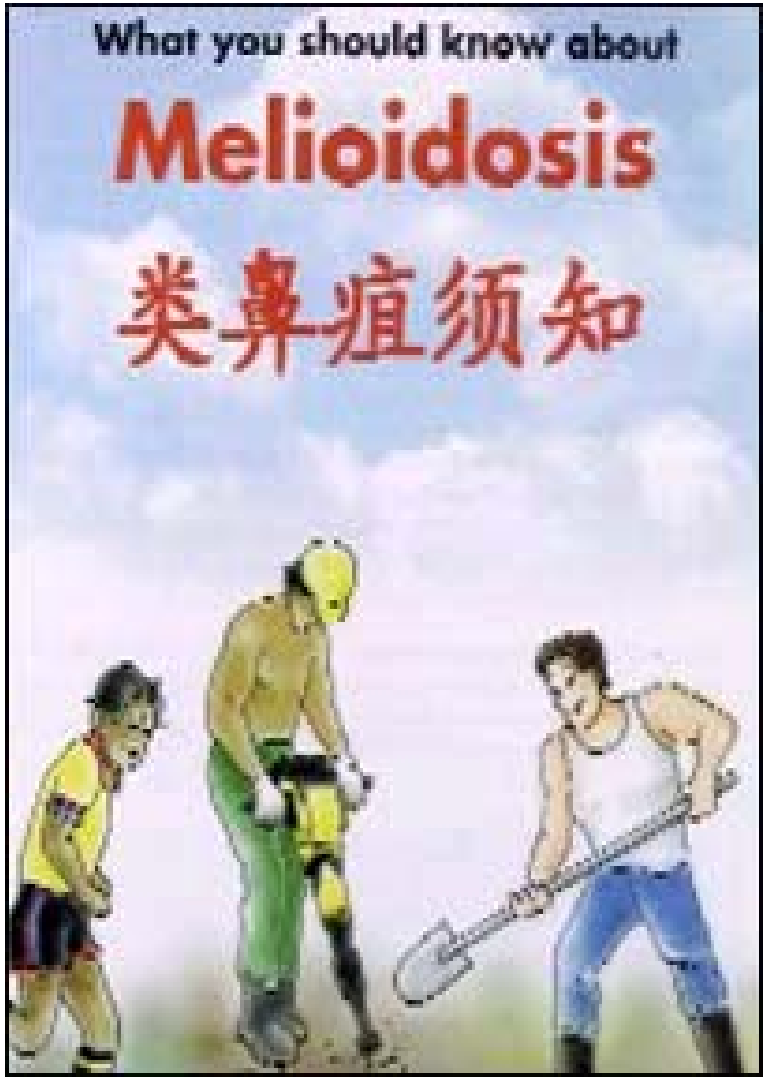
Melioidosis *au natural*

- Inhabits soil, stagnant ponds & rice paddies
  - Not necessarily associated with animals
- Distribution thru out tropics/subtropics
- Especially endemic in Thailand, Malaysia, Singapore



Melioidosis *au natural*

- Opportunistic behavior
  - 50-70% of clinical cases had predisposing medical conditions
  - Diabetes, alcoholism, cirrhosis, CF, renal disease, thallassemia, corticosteroid use, heroin abuse
- Bimodal age distribution
  - Children
  - Middle-aged adults (40-60 years)
- Many asymptomatics
- No person-to-person spread



Melioidosis *au naturale*

- In NE Thailand:
  - Most common cause of community acquired pneumonia & sepsis
  - 40% of sepsis deaths
- Can reactivate after many years!
  - "Vietnamese Time Bomb" in U.S. vets

Melioidosis: Clinical Forms

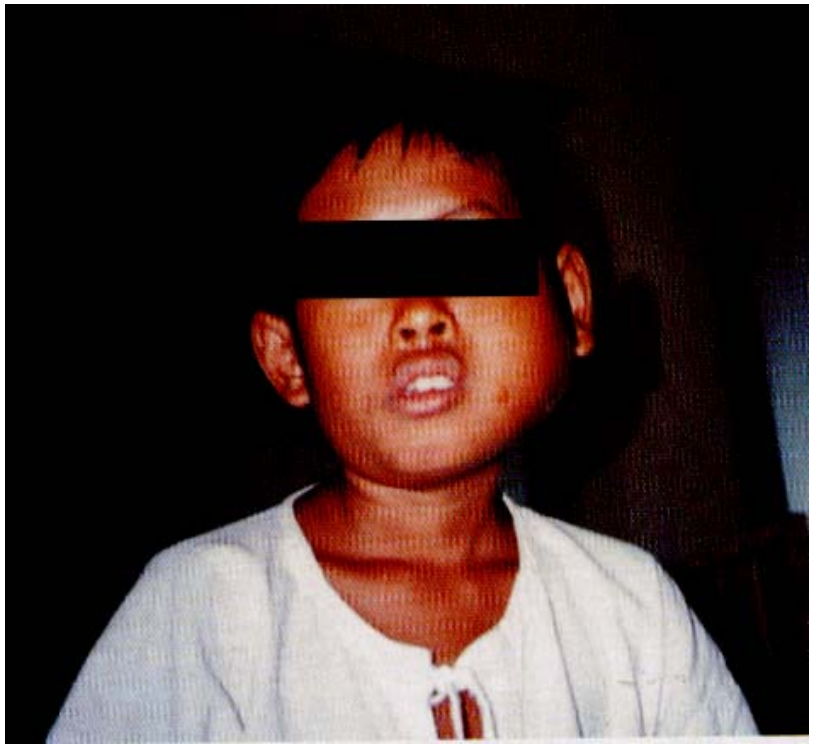
- Portal of entry: Cutaneous, mucocutaneous
  - Incubation period:
    - 1 day to 62 years!!
    - Usual: 1 to 21 days
  - Nodule & abscess formation & regional lymphadenitis
  - Rarely, presents as a distal, focal abscess without obvious portal
  - Often presents as pneumonia (hematogenous spread?), as sepsis, or as both

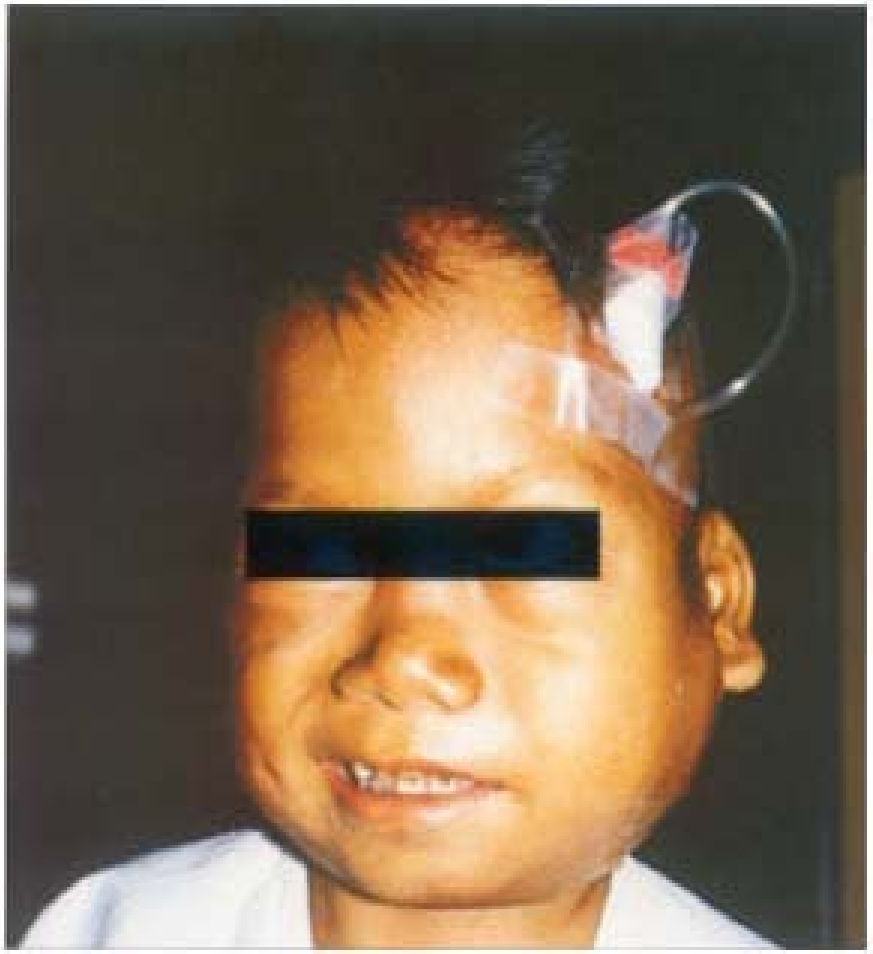
Melioidosis: Clinical Forms

- Pneumonia
  - Present in 50-80% of melioidosis patients
  - Sputum is often purulent; Hemoptysis may be present
  - Several possible forms:
    - Lobar or segmental consolidation (especially upper lobes)
    - Multiple, widespread 0.5-1.0 cm nodules
    - Cavitation is common
- Acute Parotitis
  - Primary purulent infxn in children (seen in Thailand)
- Prostatic
  - Primary abscess (seen in N Australia, 2-15% of cases)
- Septicemia

Melioidosis: Clinical Features

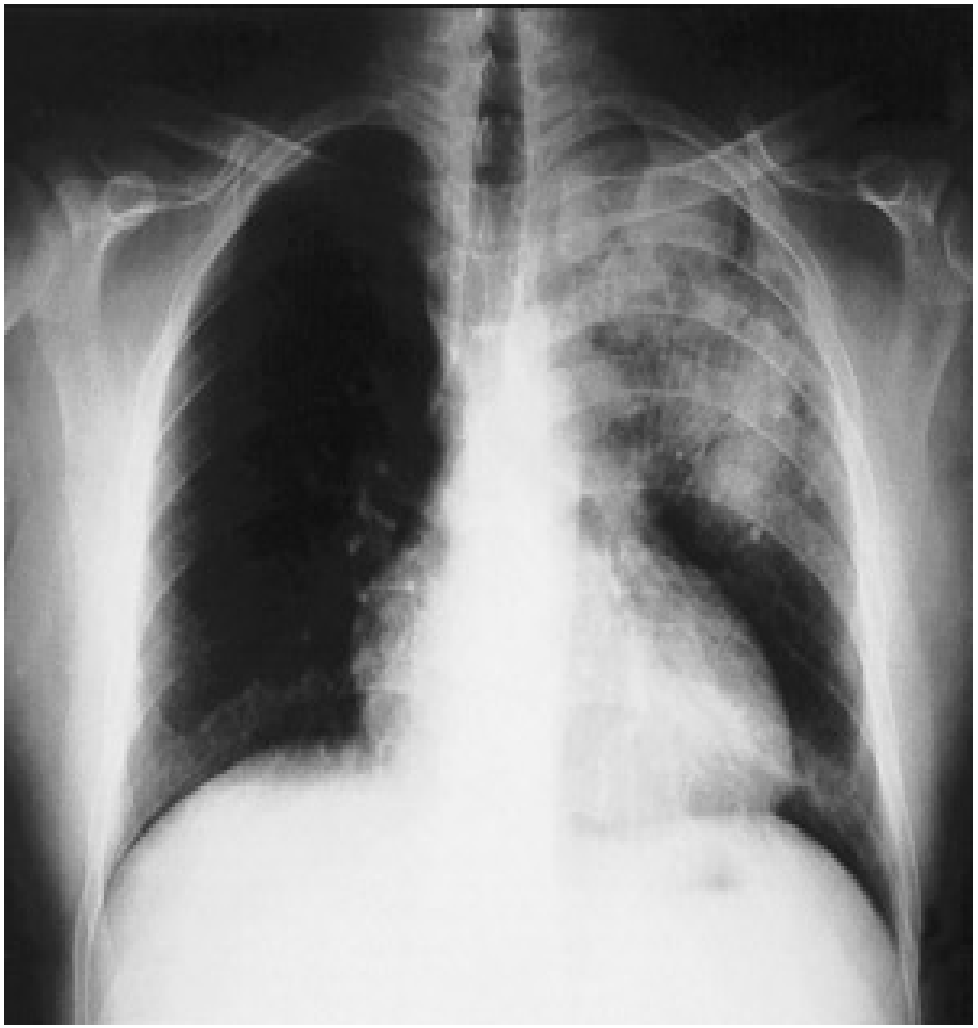
Acute Parotitis

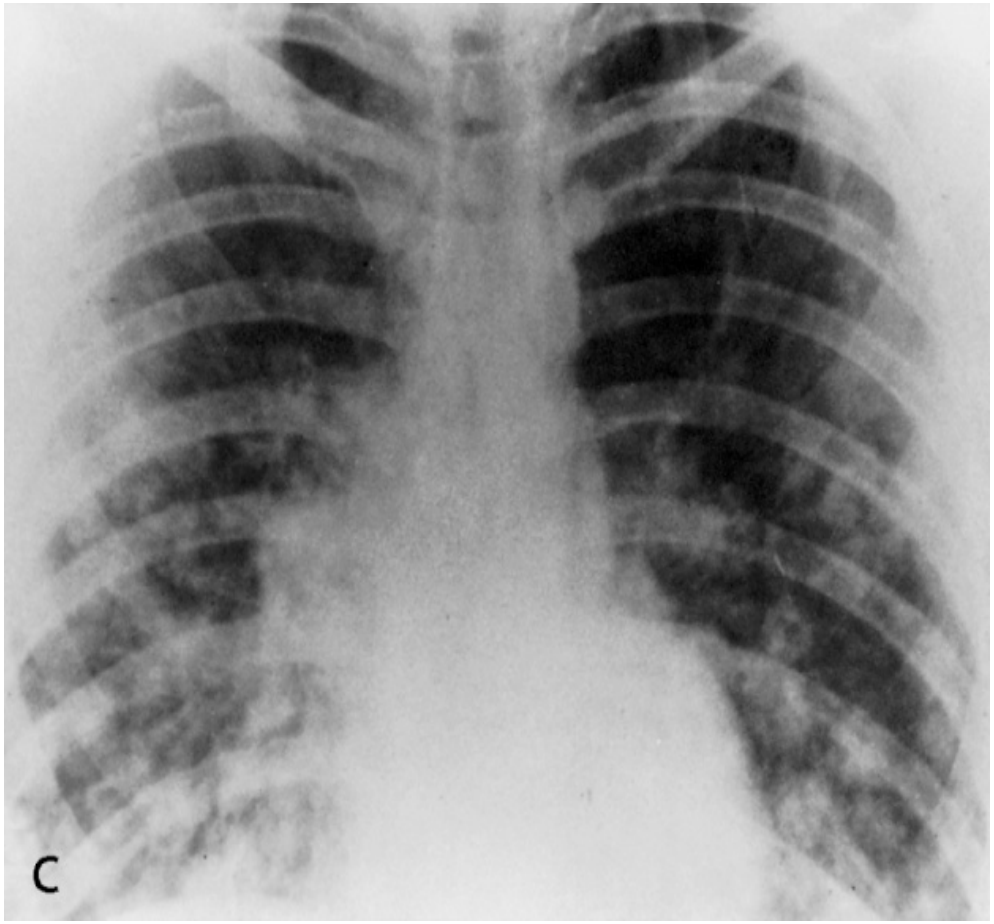




Melioidosis: Clinical Features

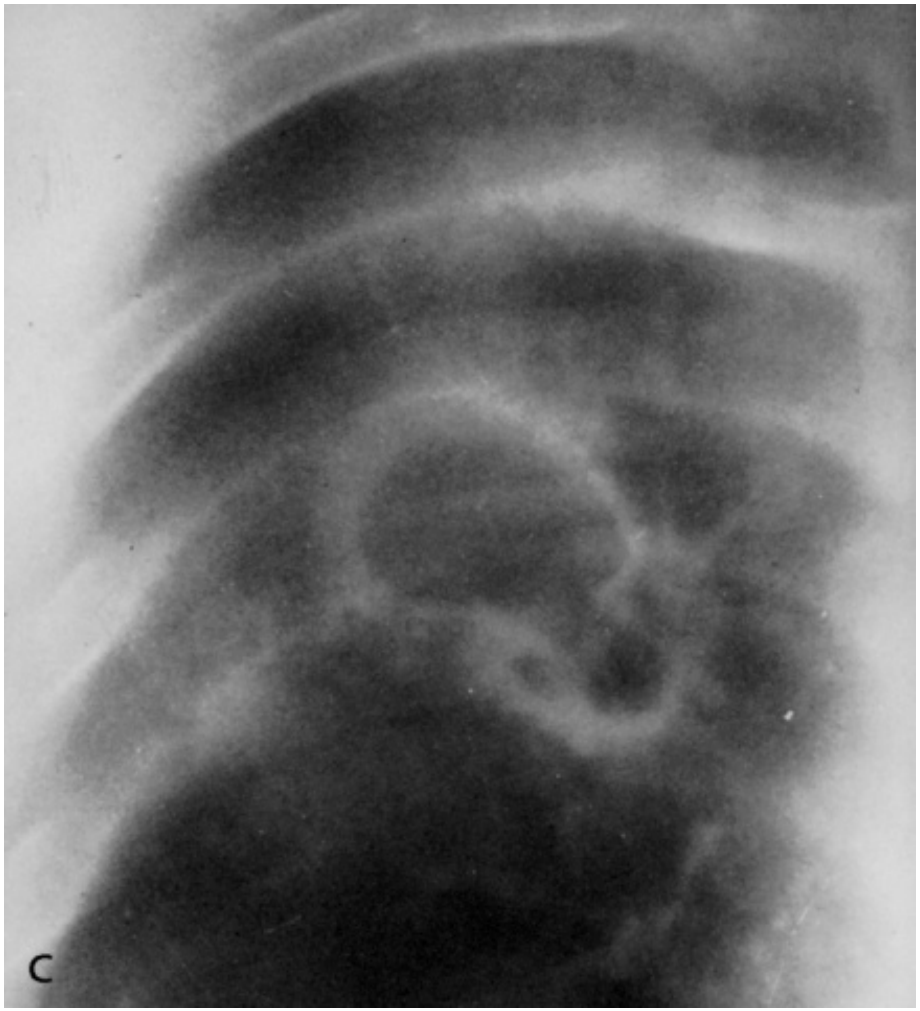
Pneumonia

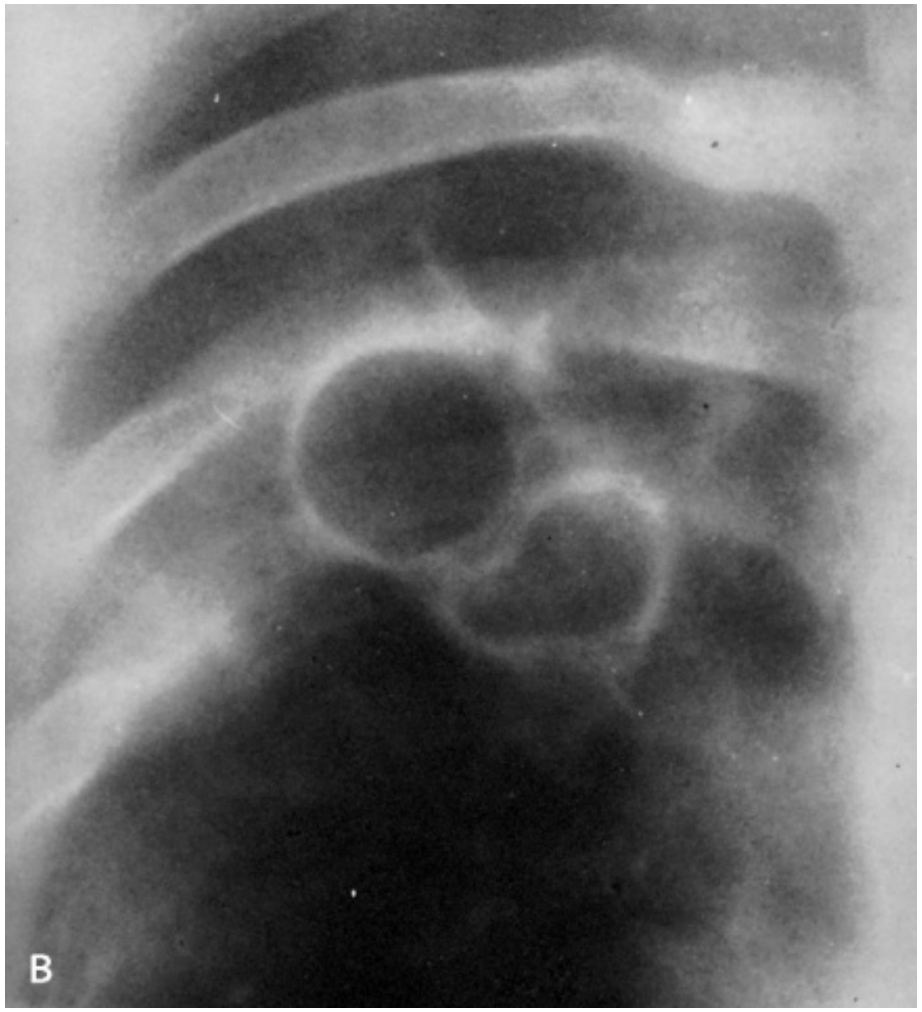




Melioidosis: Clinical Features

Melioid Cavitation



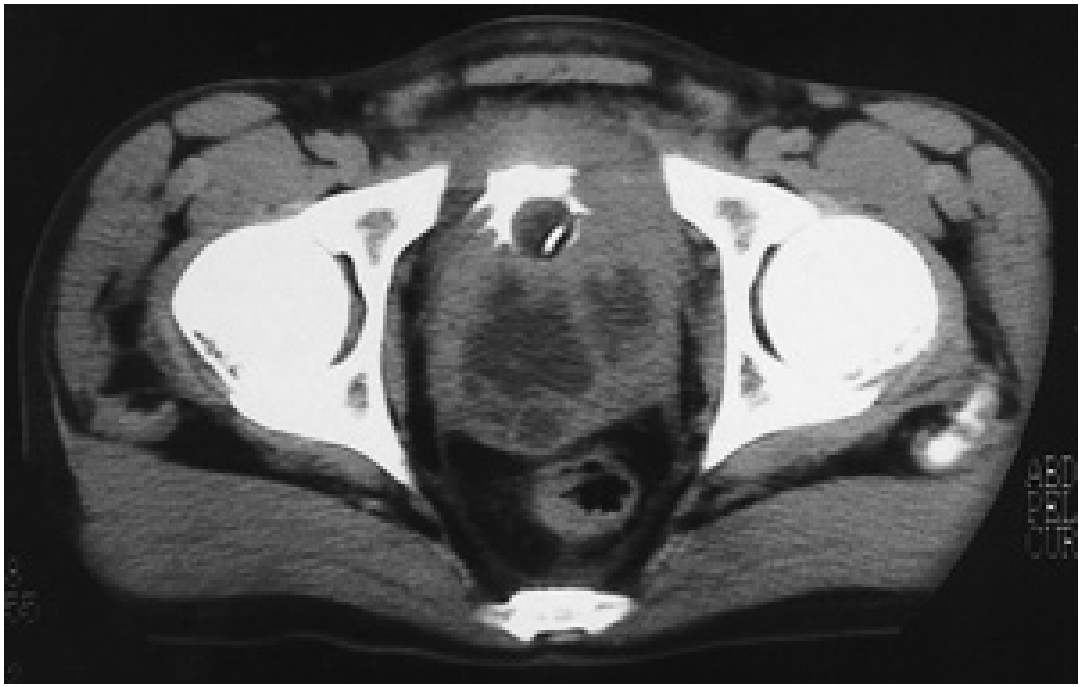


Melioidosis: Clinical Features

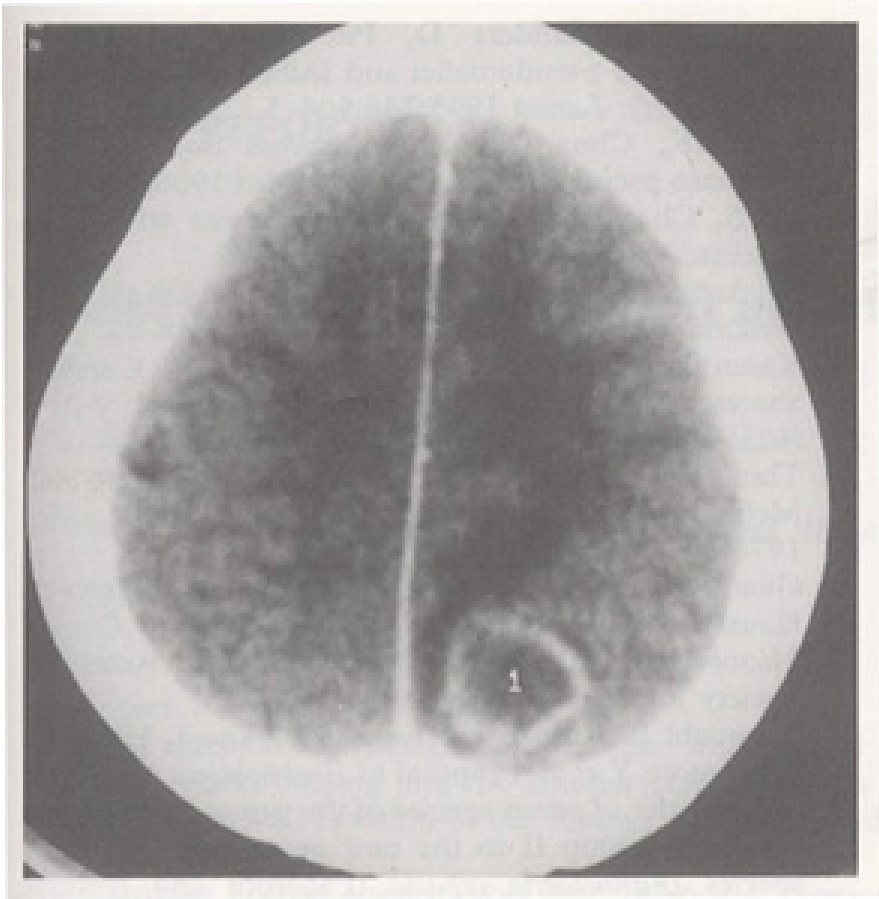
Melioid Abscess Formation

Melioidosis: Clinical Features

Abscesses



Prostatic

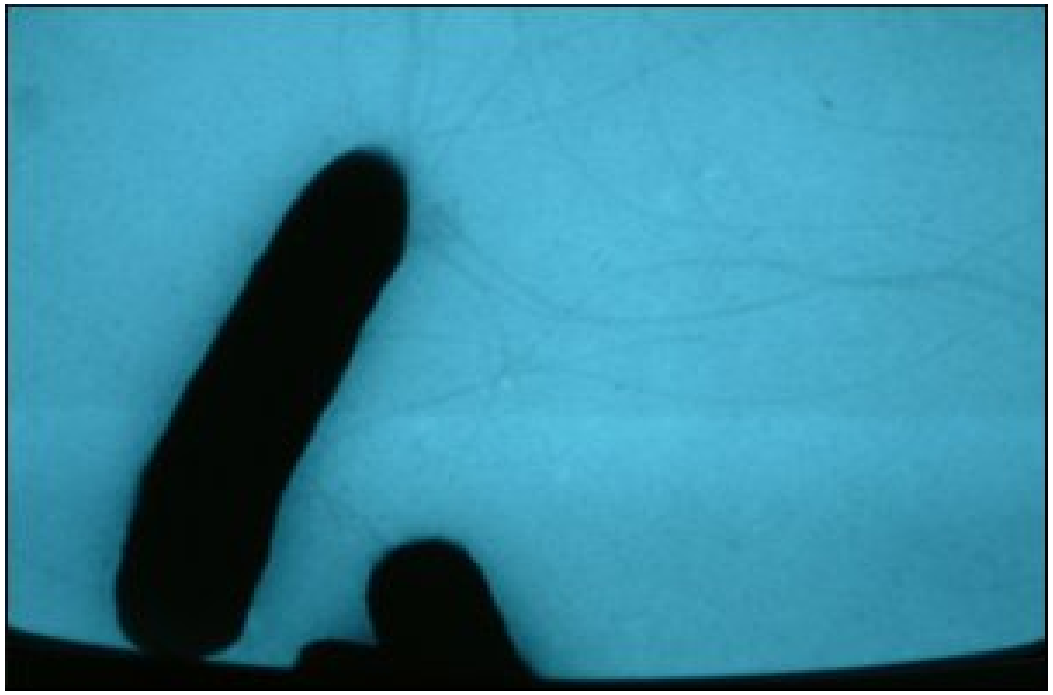


CNS

Glanders & Melioidosis!

Diagnosis

- Gram stain:
  - Small, irregularly staining, gram-negative bacilli
- Methylene blue or Wright's stain:
  - Bipolar "safety pin" staining (a la *Yersinia* spp)



Classic Wrinkled Colonies of *Burkholderia mallei*
on Ashdown's Medium

Glanders & Melioidosis: Diagnosis

- Serology diagnostic for "*Burkholderia*"
  - *B. mallei* vs *B. pseudomallei* indistinguishable
    - Agglutination titers not positive until 7-10 days into disease
    - IgM > 1:160 is diagnostic
    - IgG:
      - A single value hard to interpret in high seroprevalence regions
      - A 4-fold ↑, acute → convalescent is diagnostic
- Thus, **blood cultures** are best:
  - Grow within 48-72 hr at 37.5 °C in agar; Faster in automated systems
  - Sputum, pharyngeal cx's may require special media (Ashdown's medium)
- PCR is sensitive & specific, but not widely available

Glanders *or* Melioidosis: Treatment

Localized disease w/o toxicity

- No true consensus: Very little clinical experience
- TMP-SMX (2 mg/kg bid),

  or Doxy (100 mg bid),

  or Augmentin (20 mg/kg tid) – higher relapse in eradication phase

  … for 60-150 days
- Acceptable alternatives (?):

  Azithromycin *or* clarithromycin

Glanders *or* Melioidosis: Treatment

Severe Disease

- Ceftazidime (120 mg/kg/d IV in 3 divided doses),
  Or
- Imipenem (60 mg/kg/d IV in 4 divided doses, max 4 g/day),
  Or
- Meropenem (75 mg/kg/d IV in 3 divided doses, max 6 g/day)

Plus, some add…

- TMP-SMX (TMP 8 mg/kg/d IV in 4 divided doses)
  …*especially is septicemic*
  - Oral TMP-SMX *OK* if IV formulation is not available

Glanders *or* Melioidosis: Treatment
Severe Disease (Cont'd)

- Ceftazidime or a carbapenem not available?
  - Ampicillin/sulbactam (*Unasyn*®)
    - other IV beta-lactam/beta-lactamase inhibitor combinations

    … *MAY* be adequate alternatives
- Initial intensive tx:
  - IV abx until clinical improvement, but for ≥ 14 days
- Eradication tx:
  - Oral abx for 4-6 months
  - For melioidosis: Lifelong follow-up indicated to identify relapses

Melioidosis: Treatment
Septic Shock

- Australian Research:
  - Granulocyte colony-stimulating factor (G-CSF) 300 µg IV per day for 10 days (or longer if clinic shock persists)
    - Mortality in study pts dropped from a historic value of 95% → 10%
    - But: IV abx, plus limitations in the study preclude attributing success entirely to G-CSF

Glanders & Melioidosis: Prognosis in Severe Forms

- ***Glanders: ?***
- ***Melioidosis:***
  - Overall mortality for severe, treated melioidosis:
    - ~ 50% in Thailand
    - ~ 19% in Australia
  - Without proper tx most septicemic patients die in 2-3 days
  - Poor prognostic indicators
    - Positive bd cx in < 24 hours of incubation
    - Neutropenia
  - Even after prolonged abx tx, relapse is common

Glanders *or* Melioidosis : Post-exposure Prophylaxis

- No consensus
- Based upon animal studies:
  - TMP-SMX *or* doxy might work
  - Fluoroquinolones *may* be an alternative
    - Associated with higher relapse rates in animals
  - Duration: Unknown, but ≥ 10 days probably prudent

Questions?

Tularemia: An Occupational Hazard for “Weekend Warriors”, a BW Threat for the Soldier

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USAMRIID, Fort Detrick, MD

Lesson Objectives

- Describe the natural epidemiology of tularemia
- Identify the organism that causes tularemia and its basic microbiology and pathophysiology
- Describe the two clinical forms of tularemia
- Summarize the clinical management of tularemia
- Describe mechanisms to prevent disease and/or transmission of tularemia

History

- Bacterial zoonosis caused by *Francisella tularensis*
- Recognized as a human disease since early 1800's
  - Organism first identified during 1911 investigation of enzootic plague-like illnesses in ground squirrels in Tulare county, CA
  - First confirmed human case: 1914
- U.S. early 20th C.: Large outbreaks (waterborne)
  - 1939 - 2,291 cases
  - 32,749 cases 1927- 1967
  - Frequency declined due to improved hygiene and sanitation
  - Removed from national notifiable disease list 1994-99, returned 2000 due to BT threat

Military History & Relevance

- WWII
  - Suspected use as BW agent:
    - Studied at Japanese Germ Warfare research units
    - Outbreak in Russian troops and civilians
      - >100,000 affected
        - » BW allegations, probably related to poor sanitation (German troops also affected)
- Post-WWII
  - US and Soviets developed means to weaponize
- Very high infectivity with aerosolization
- Can/has been weaponized in wet and dry forms for delivery

Tularemia as a BW Threat

- 50 kilograms of aerosolized *F. tularensis* dispensed 2 Km upwind of a population center of 500,000 under ideal weather conditions would kill up to 155,000 people (WHO, 1970)
- Local disease cycles could occur involving other mammals, ticks and biting flies
- Contamination of water supplies also a concern

Tularemia
Do Not Underestimate!

- 10% overall mortality reported
- In pre-antibiotic era, mortality rate of pneumonic tularemia was 40-60%

Infectious dose

- 10-50 organisms by inhalation/intradermal
- 10^8 organisms orally

Synonyms

- U.S.:
  - Rabbit fever
  - Deer fly fever
  - Market men's disease
- Japan
  - Wild hare disease (yato-byo)
  - Ohara's disease
- Russia
  - Water-rat trapper's disease

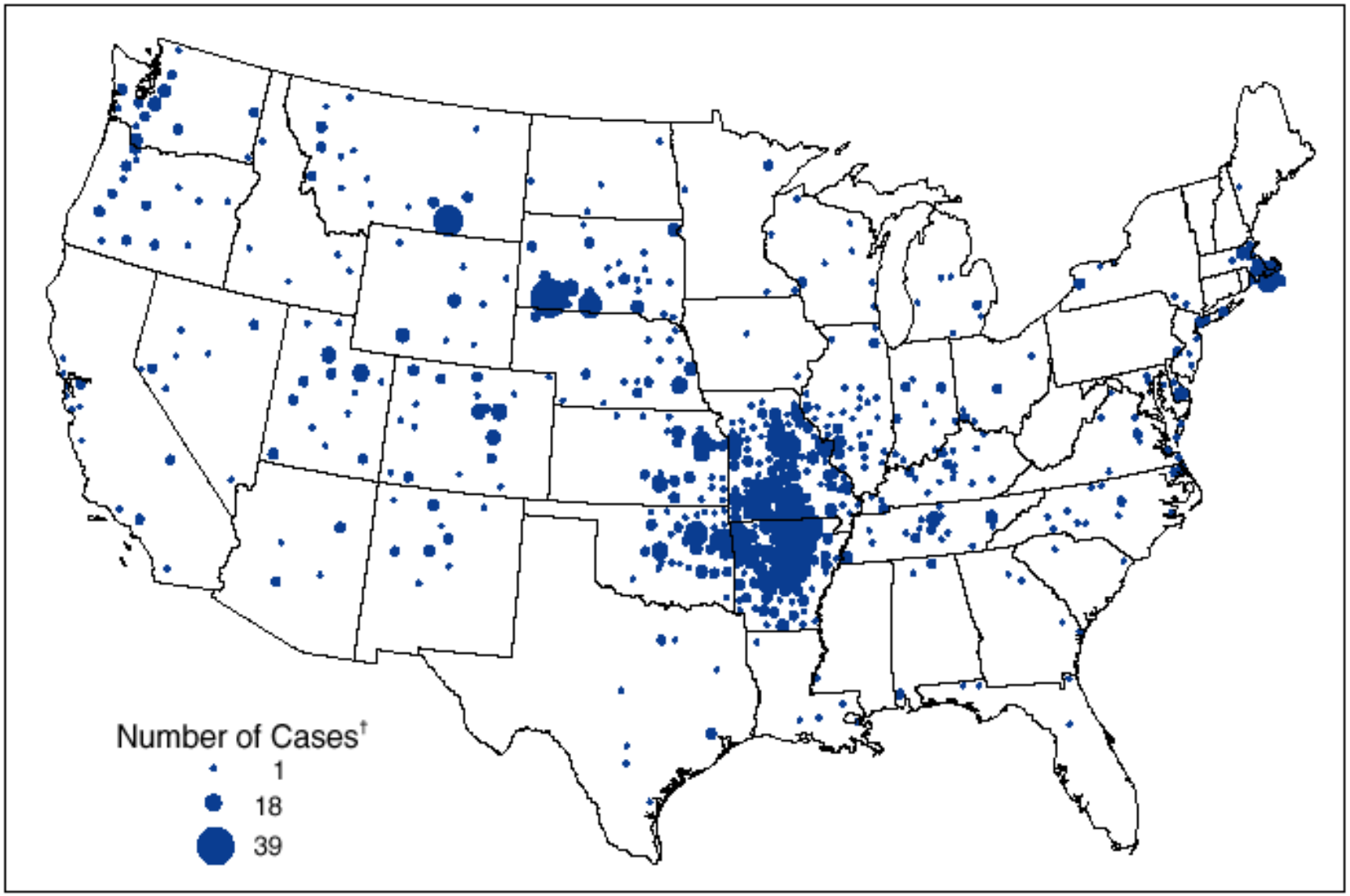
Tularemia Distribution

- Northern Hemisphere disease (natural)
  - Between 30 ° & 70 ° N latitude predominantly
- Two peaks in incidence (U.S.)
  - Summer: vectorborne, mowing season
  - Fall: varmint hunting season

World Geographic Distribution

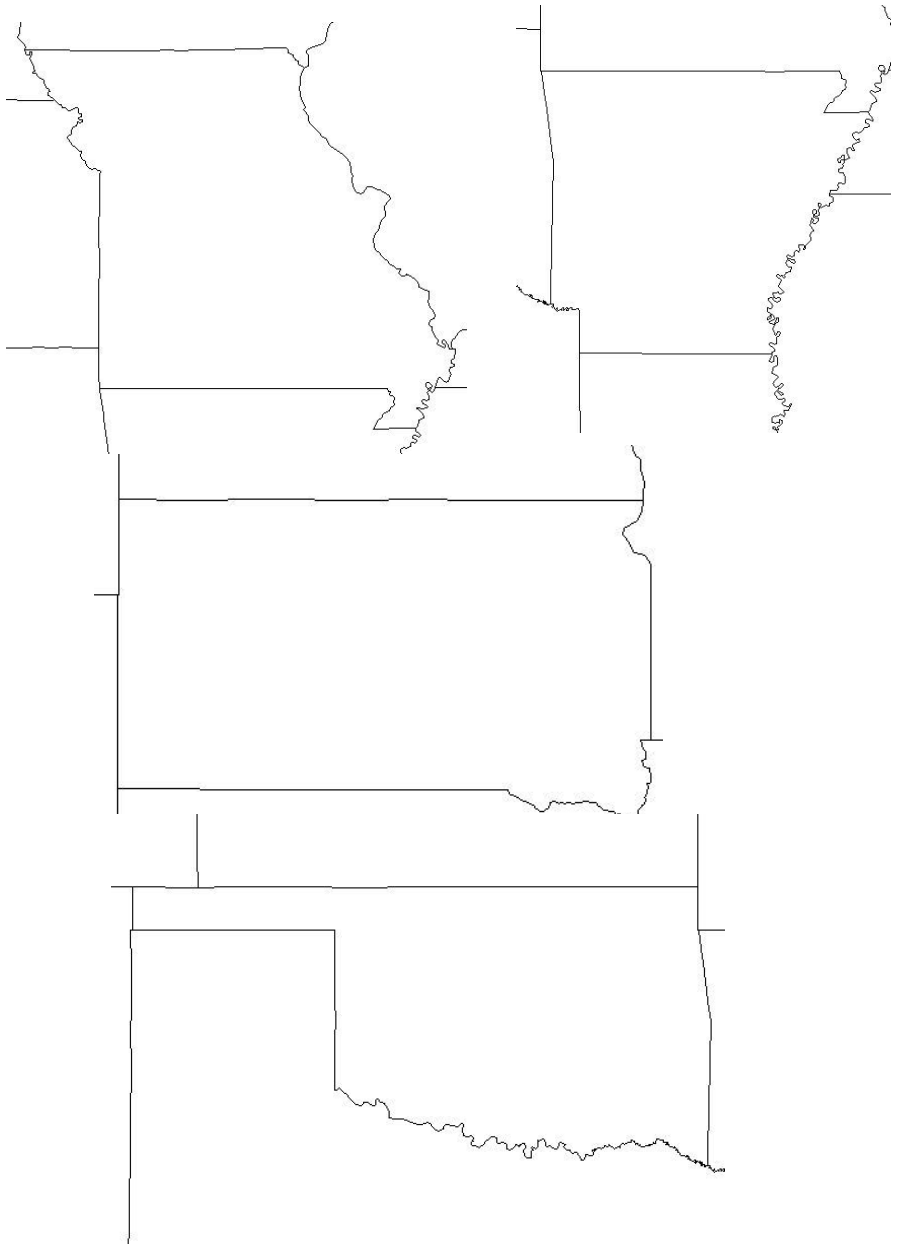
U.S. Geographic Distribution

FIGURE 2. Reported cases* of tularemia — United States, 1990–2000

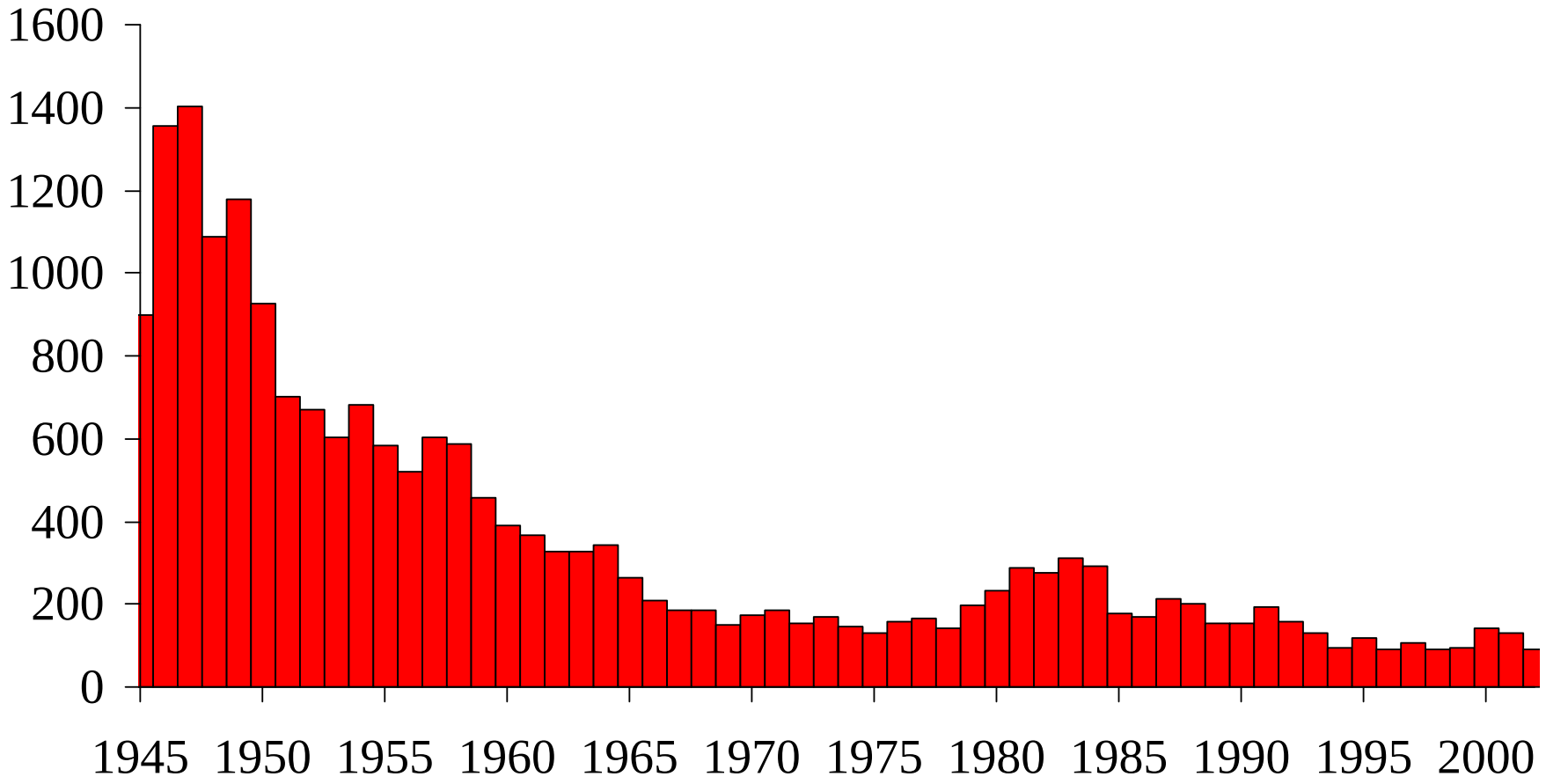


Tularemia in the U.S.A.

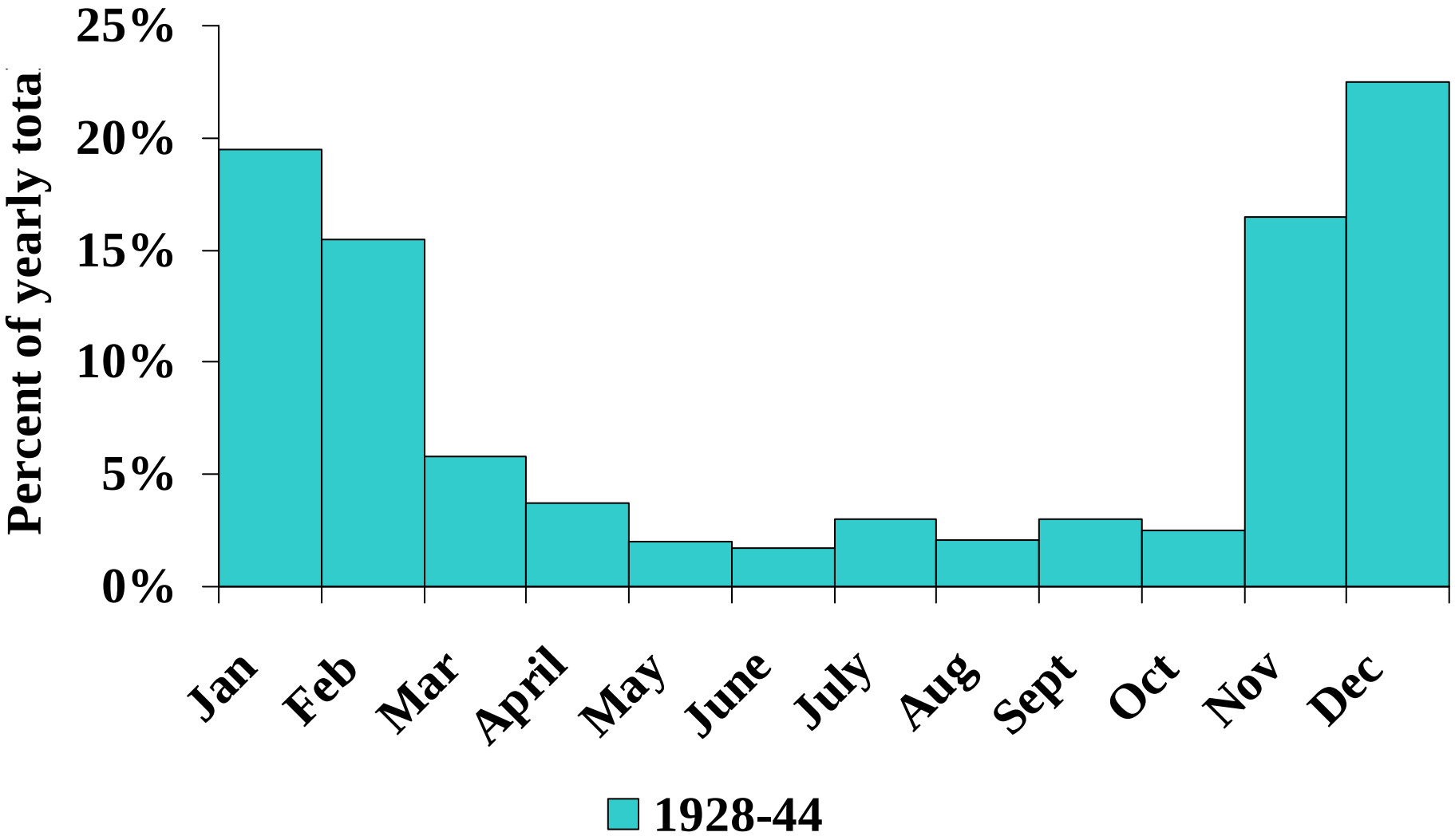
- 32,749 cases 1927- 1967
  - 2,291 cases in 1939
- 1,368 cases 1990-2000
  - Avg. 124/yr (Range: 86-193)
- 56% from four states
  - Missouri (265 cases, 19%)
  - Arkansas (315 cases, 23%)
  - South Dakota (96 cases, 7%)
  - Oklahoma (90 cases, 7%)



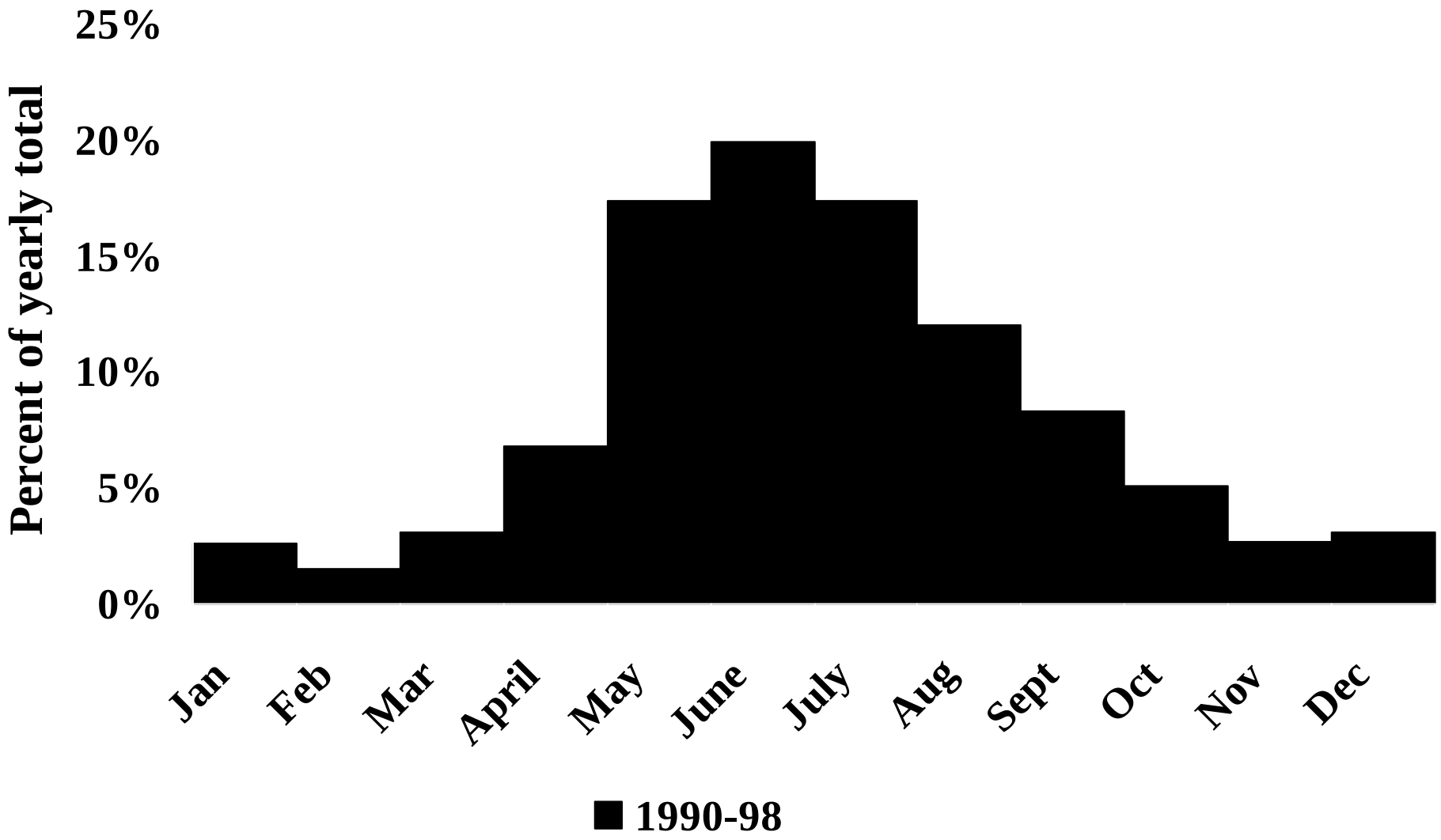
Reported Tularemia Cases, U.S., 1945-2002



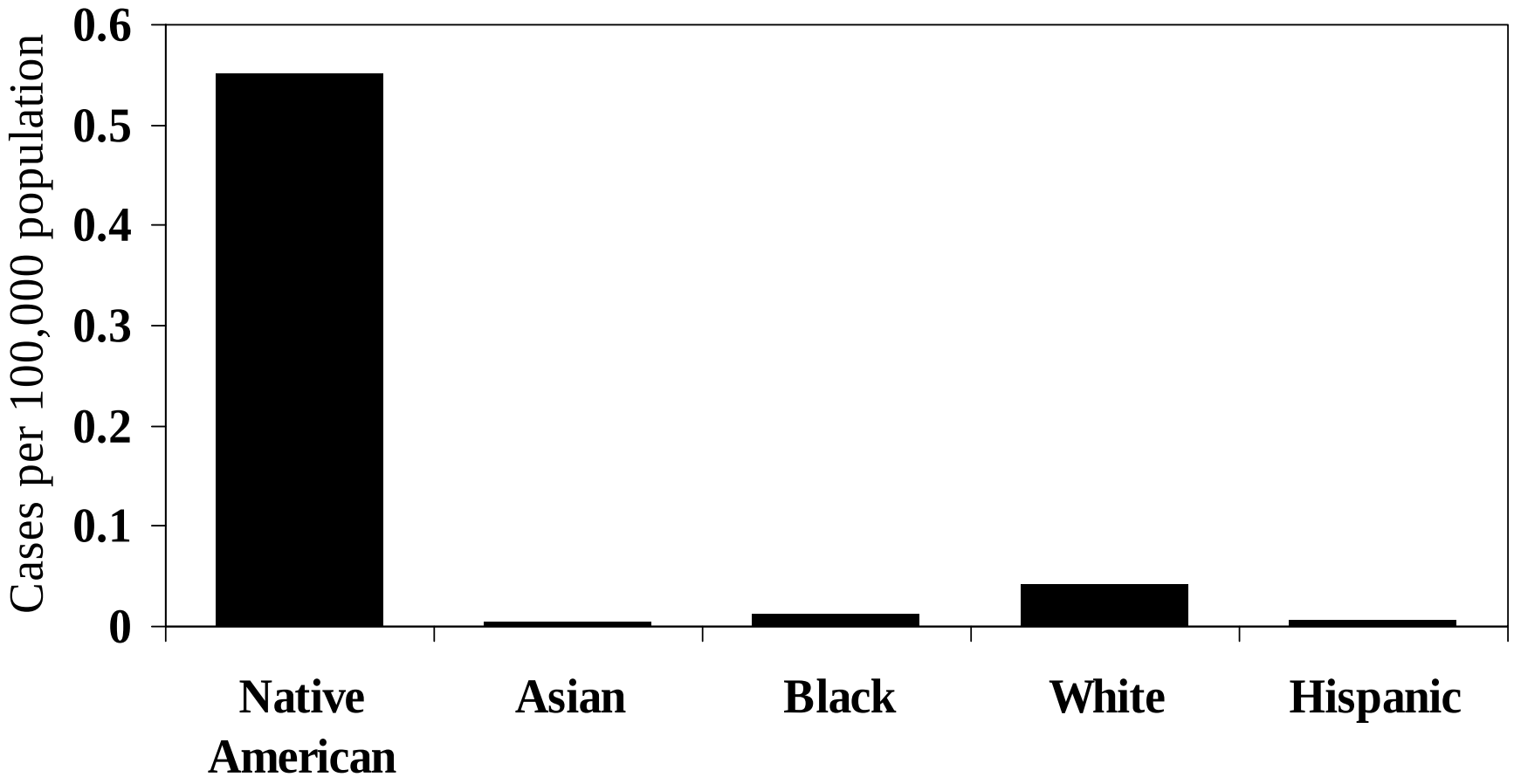
Seasonal Distribution of Tularemia Cases 1928-44



Seasonal Distribution of Tularemia Cases 1990-98

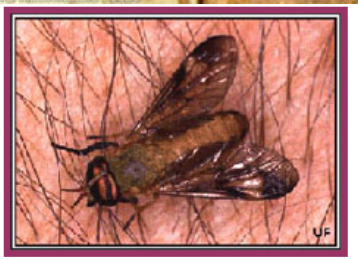


Tularemia Incidence among Ethnic Groups (1990-1998)



Reservoirs & Vectors

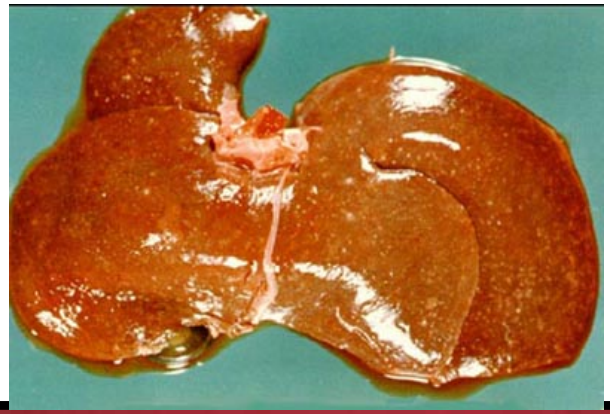
- Reservoir
  - North America: Tick, occasionally dog
  - Other regions: Water rat, other aquatic animals
- Vectors
  - Tick (hard shell), deerfly, mosquito
  - Small mammals
  - Contaminated food, water
  - Aerosol (contaminated dusts)
- Disease
  - Many small mammals, wild and domestic
  - Humans

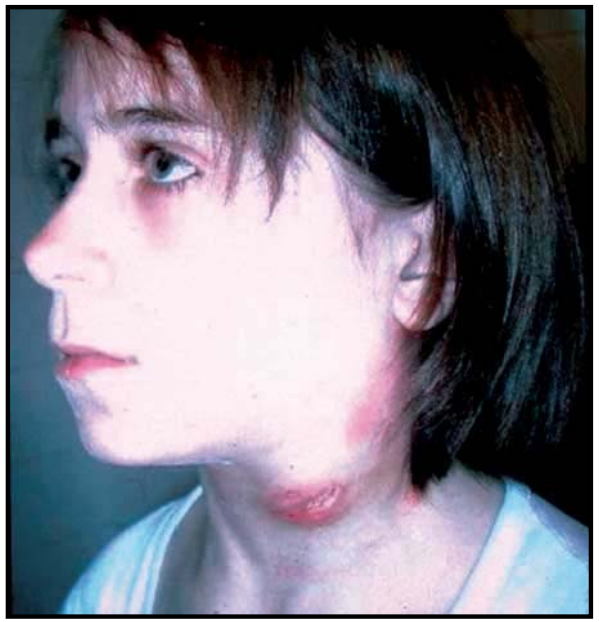


Multiple Modes of Infection

- Cutaneous
  - Injection: Bloodfeeding arthropods and flies
  - Abrasions: Skinning and dressing animals
- Ingestion
  - Contaminated water or grains, undercooked meat
- Inhalation of aerosols
  - Water, contaminated dust or hay
  - Lawn mowing
  - Laboratory workers
- Mucous membrane contact
  - Aerosol or liquid
- No person-to-person transmission

Modes of Transmission to Humans

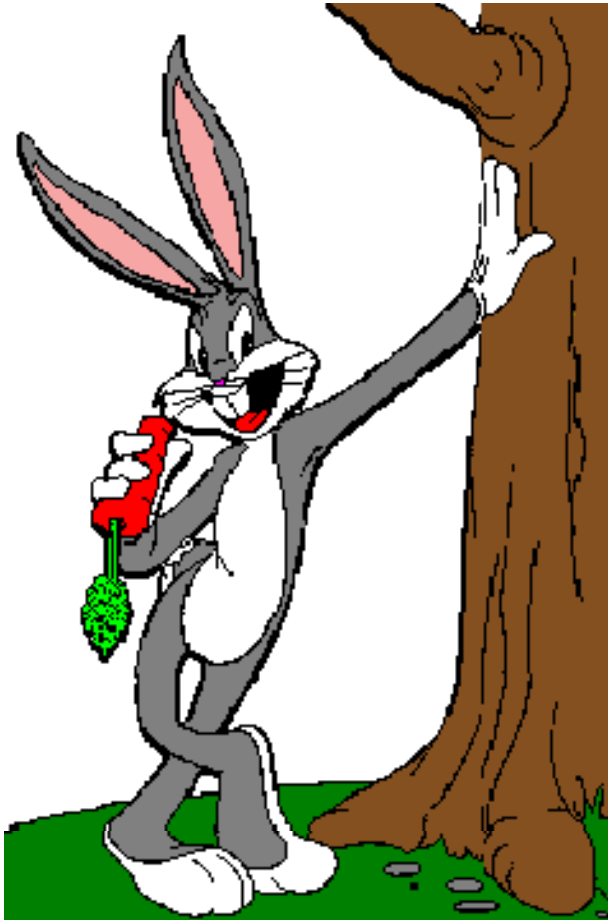




Tularemia
Epidemiology

Think:

Bugs	Bunny
Ticks	Cottontails
Deerflies	Jack Rabbits



Tularemia

Clinical signs and severity of illness depend on route of transmission and strain

Type A

- Biovar[1] *tularensis*
- Highly virulent
- 10% fatality (non-tx)
- Rabbits, ticks, and deer flies
- North America

Type B

- Biovar *palaearctica*
- Less virulent
- 1% fatality (non-tx)
- Voles, muskrats, water rats, and mosquitoes
- Europe, Asia and North America

[1]subspecies

Tularemia
Epidemiology

What if no Bugs Bunny?

Tularemia
Outbreaks

- 1942 - > 100,000 Russian cases, unknown # German cases, Battle of Stalingrad
- 1946 - 50 soldiers bivouacked in TN, tick-related
- 1966-67 – 676 cases, most typhoidal, northern Sweden, farming associated
- 1982 – 49 people drinking from infected water system in Tuscany, Italy
- 1982 – 123 cases (53 typhoidal), northern Finland, farming associated
- 1978 – 7 cases (pneumonic), Martha's Vineyard, all from same cottage
- 2000 – 15 cases (11 pneumonic), Martha's Vineyard, assoc. w/ lawn mowing and brush cutting

Tularemia in Sweden

- 1700 cases through July 1966
- 85% cases occurred in endemic area (Central Sweden)
- Most cases, July – September
  - Insect transmission
- Primarily ulceroglandular (~90%)
- 55% F / 45% male

Tularemia Outbreak!

Natural or Intentional?

- Northern Sweden - 676 cases
  - 444 in Jamtland county (4 prior cases)
  - Other cases from Vasternorrland, Vasterbotten, Norrbotten counties
- Autumn and winter of 1966-67, peak in December
- Primarily typhoidal presentation
  - 10% confirmed pts w/pneumonia
- 63% male

Typhoidal Tularemia Outbreak Northern Sweden

- Marked increase in vole population
  - Large vole die-off in December
- 83% patients – contact with contaminated hay
- December – transportation of hay from field barns
  - Voles had destroyed 50-60% of harvest
  - Farmers had to sort hay by shaking
  - Large numbers of dead voles and vole feces discovered in barns
- Conclusion: Natural aerosol transmission

2000 MV Outbreak Timeline

Date	Event
June 5	57 yo visits family doc in CT – fever, fatigue, anorexia, rhinorrhea, chest congestion for 7 days. Illness onset included eye irritation, anorexia, and diarrhea. Pt has lost 20 lbs, has temp of 102.8 C.
1st week in July	Hospital in Martha's Vineyard reports 5 cases of pneumonic illness to state health

2000 MV Outbreak Timeline

Date	Event
Mid-July	Case onset dates from May 30 – June 22. MA DPH initiates active disease surveillance, suspect tularemia
Late July	*F. tularensis* confirmed in clinical samples
July – August	15 confirmed cases from samples and blood titers

2000 MV Outbreak Timeline

Date	Event
July – August	3/15 confirmed cases from out-of–state residents
Late August	CDC is called for help!
Mid - August	Confirmed case definition developed: visitor or resident of Martha's Vineyard, sx of 1^{o} pneumonic tuli, >body titer of ≥1:128, illness, summer illness

2000 MV Outbreak Timeline

Date	Event
Mid - August	11/15 cases determined to have pneumonic form of disease, 2 ulceroglandular, 2 fever w/malaise
Pt demographics	14/15 male, mdn age = 43 yo, range 13-59, a 43 yo died

MV C-C study

Case control study	
Risk factors for tularemia	▪Male ▪Worked as landscaper ▪Used lawn mower or brush cutter ▪Cut brush or mowed over rabbit ▪Worked with bark chips ▪Worked with weed wacker ▪Worked with lumber ▪Owned a dog ▪Smoked ▪Saw dead rabbit ▪Found ticks

MV C-C study

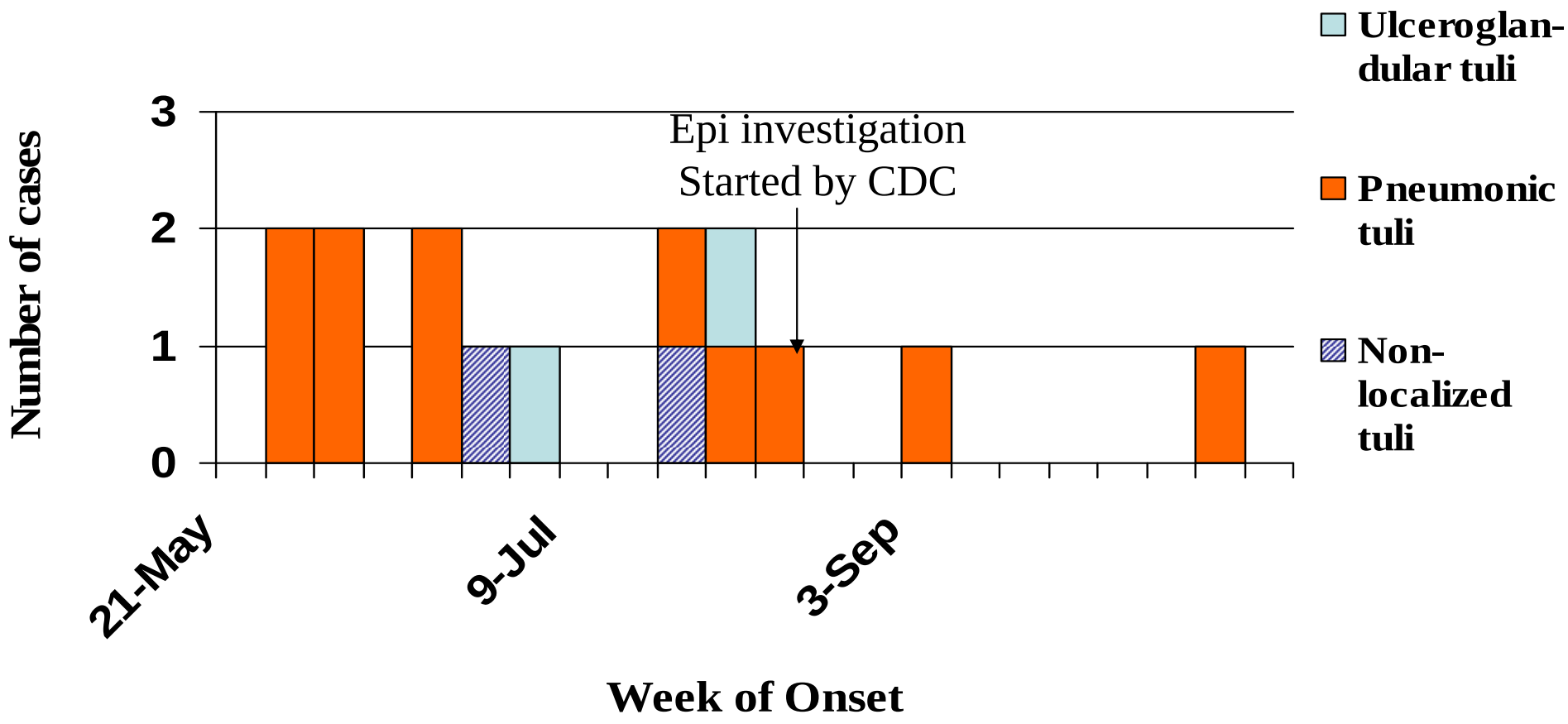
Risk factors for primary pneumonic tularemia (11 cases)	
Potential risk factors for tularemia	▪Lawn mower or brush cutter use[1] ▪Worked with bark chips[1] ▪# hrs/day spent outside ▪Smoked ▪Owned a dog at MV

[1]Significant

MV Env Investigation

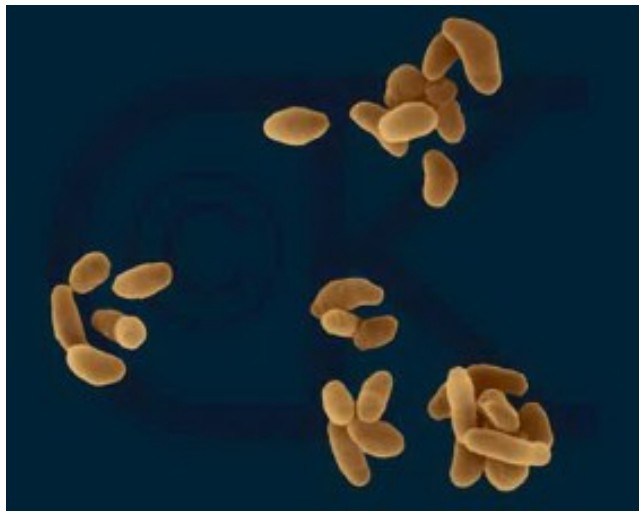
Environmental investigation	
Negative	•Lawn mower filters •Cut grass samples •Air samples •Raw water samples •Soil and mulch samples
Positive for *F. tularensis*	•1 striped skunk •1 Norway rat (*R. norvegicus*)

Cases of Primary Pneumonic Tularemia



Tularemia
Francisella tularensis

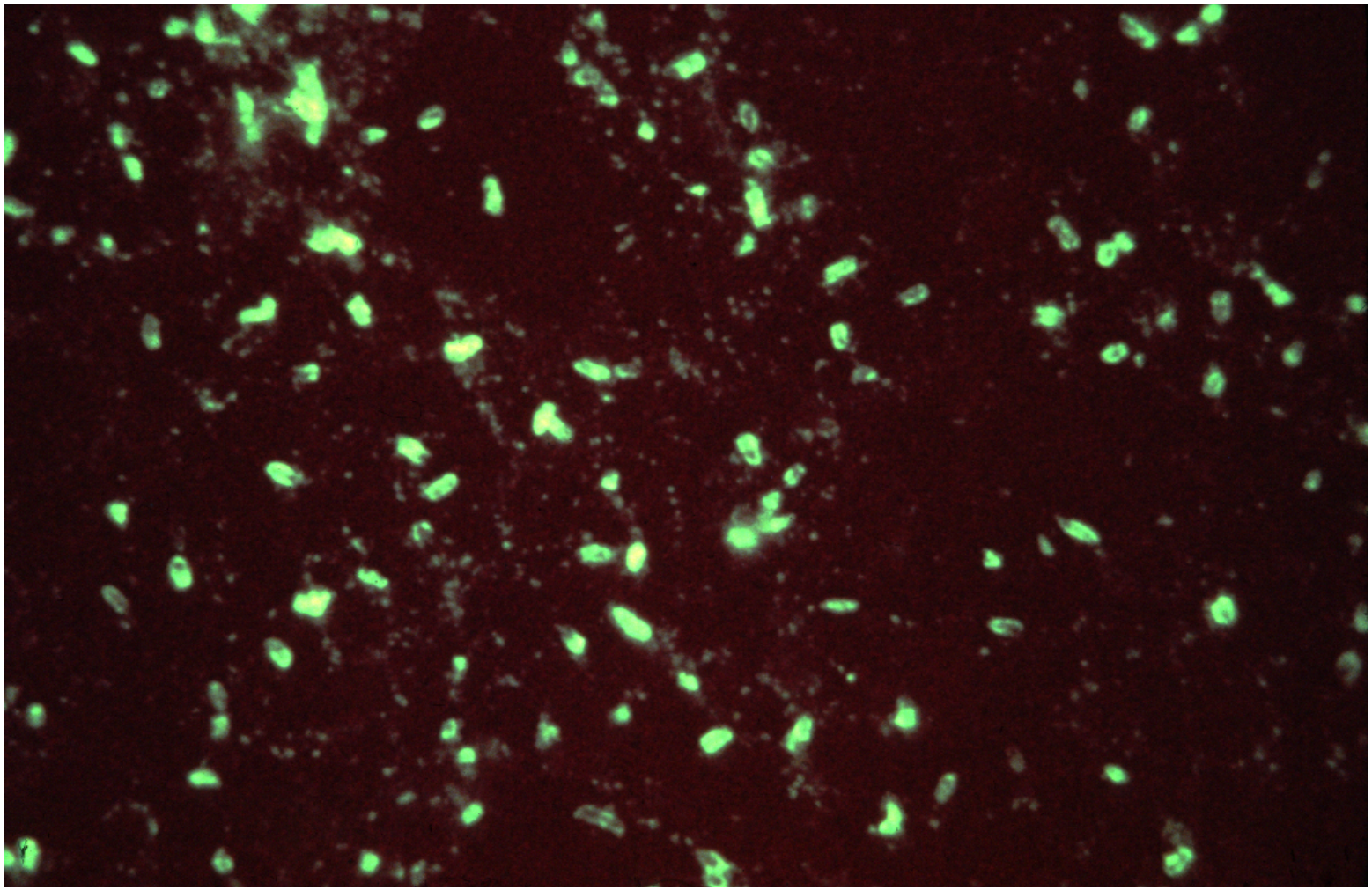
- Small (0.2 by 0.2-0.7 um)
- Gram-negative
- Nonmotile
- Coccobacillus
- Facultative intracellular pathogen
- Hardy organism, survives weeks in environment
- Types differentiated
  - Epidemiologically
  - Biochemically



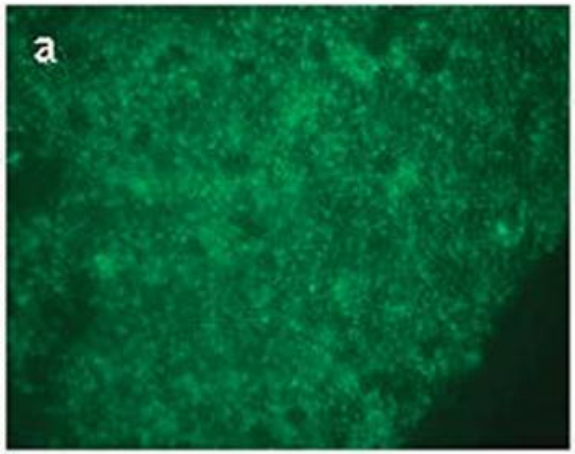
Tularemia
Laboratory Diagnosis

- Confirmatory
  - 4x rise in specific serum antibody titer (ELISA)
- Presumptive
  - Elevated serum antibody titer ≥ 1:160 (tube agglutination) or 1:128 (microagglutination)
  - Detection of organism by fluorescence
  - PCR
  - Fatty acid profile consistent
- Culture: Laboratory hazard!
  - *Warn the lab if F. tularensis is suspected*

DFA for *F. tularensis*



Magnification x 400



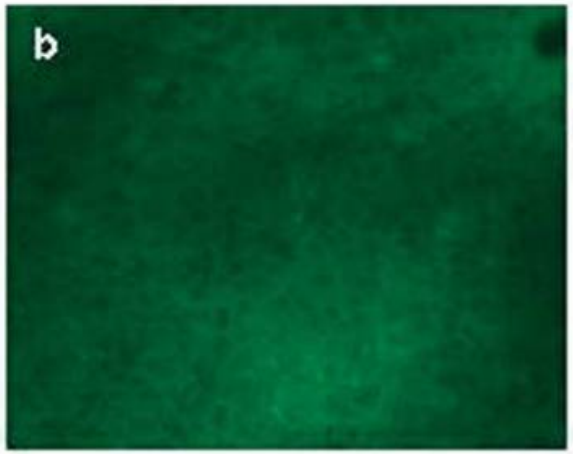
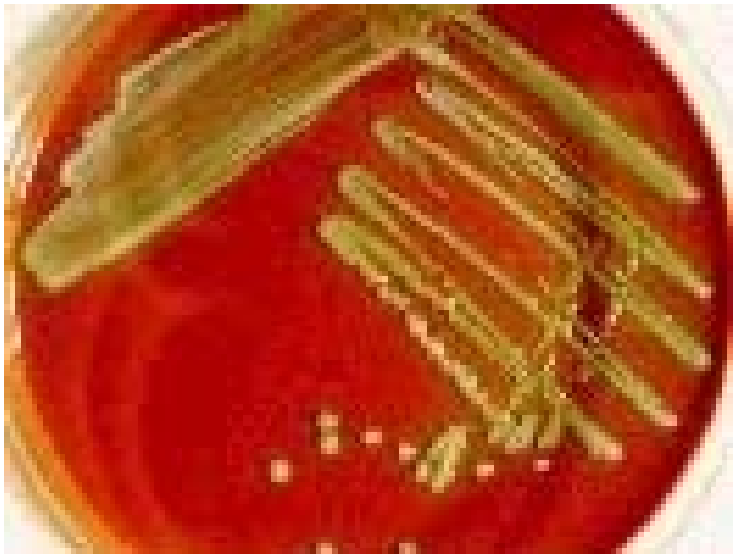


Figure 2. Direct fluorescent assay (DFA) results on spleen tissues from a seronegative (panel a) and seropositive (panel b) prairie dog.



Laboratory Diagnosis

- **Suspect:**
  - Poorly-stained, tiny gram-negative rods from patient with compatible exposure and clinical symptoms
  - Organism is slow growing (up to 72 hours) and difficult to recover in automated culture systems

Laboratory Diagnosis

"*Despite having a laboratory bioterrorism procedure in place and adhering to established laboratory protocol, 12 microbiology laboratory employees were exposed to F. tularensis and the identification of the organism was delayed due to lack of notification of the laboratory of the clinical suspicion of tularemia*."

DS Shapiro & DR Schwartz. J Clin Microbiol, 40:2278, 2002

Tularemia
Pathogenesis

- Penetration occurs through skin disruption
- Organism multiplies locally
- Skin produces papules that ulcerate, become encrusted, and form an eschar
- Microorganisms reach the lymph nodes to replicate and disseminate to the blood
- Organisms engulfed by reticuloendothelial cells and they survive intracellularly
- Microorganisms can be inhaled and pneumonic form occurs
- Ingestion of organisms can cause pharyngitis, cervical and mesenteric lymphadenopathy
- Focal necrosis of organs within the RES

Tularemia
Clinical Features of Laboratory-Acquired Infections

Flu-like symptoms
***Dry to slightly productive cough**
Minimal nasal stuffiness
Sore throat
***Vague substernal pain or tightness**

Overholt et al, Am J Med, 30:785, 1961

Lab-Acquired Tularemia
CXR Manifestations

20/43 with laboratory acquired infection

Pneumonic infiltrates - 17

- **Oval, bronchopneumonic lesions – 15**
- **Diffuse bronchopneumonia – 1**
- **Lobar pneumonia – 1**

Hilar adenopathy – 9

Pleural effusion – 5

- **Isolated finding – 2**

Perihilar linear streaking

Overholt & Tigertt, Radiology, 74:758, 1960

Clinical Forms

- Six (or more) forms previously described
  - Ulceroglandular
  - Glandular
  - Oculoglandular
  - Pharyngeal
  - Typhoidal
  - Pneumonic
- Artificial categories with frequent overlap in patients
- Lumped for simplification into two forms
  - Ulceroglandular and Typhoidal
  - Based on
    - Predominant clinical signs
    - Mode of transmission/Portal of entry
    - Prognosis

Evans et al, Medicine, 64:251, 1985

Clinical Syndromes of Tularemia

Ulceroglandular	• Most common form • Papule, ulcer at portal of entry, lymphadenopathy
Glandular	• Regional lymphadenopathy • No sign of cutaneous lesion
Oculoglandular	• Eyelids and conjuctivae inflamed, lymphadenopathy • Nodules and ulcers on palpebral conjuctivae
Oropharyngeal	• Sore throat out of proportion to physical signs • Acute (exudative) tonsillitis with cervical adenitis
Typhoidal	• Acute septicemia with no localizing signs • Secondary pleuropulmonary involvement
Pneumonic	• Most severe and lethal form • May present as unresponsive community acquired pneumonia

Tularemia

Clinical Presentations

Lumper!!

- Ulceroglandular – 75%
  - Lesions on skin or mucous membranes (including conjunctiva)
  - Lymph nodes > 1 cm in diameter
- Typhoidal – 25%
  - Systemic symptoms (80% pneumonia)
  - W/o skin or mucous membrane lesions
  - Lymph nodes < 1 cm in diameter

Evans et al, Medicine, 64:251, 1985

Basic Clinical Laboratory Findings

- WBC counts: normal to high (5K – 22K per mcL)
- Differential: usually normal; occasional late lymphocytosis
- Hgb/HCT/PLT – usually normal
- LFTs: commonly mild elevations in LDH, ALT, AST, AlkPhos (hepatosplenomegaly sometimes present)
- CSF: usually normal; mild abnormalities of glucose, protein, RBC, and WBC have been reported

Tularemia
Clinical Features

Fever
Chills
Headache
Sweating
Malaise
Myalgia
Backache
Anorexia

McCrumb, Bacteriol Rev, 25:262, 1961

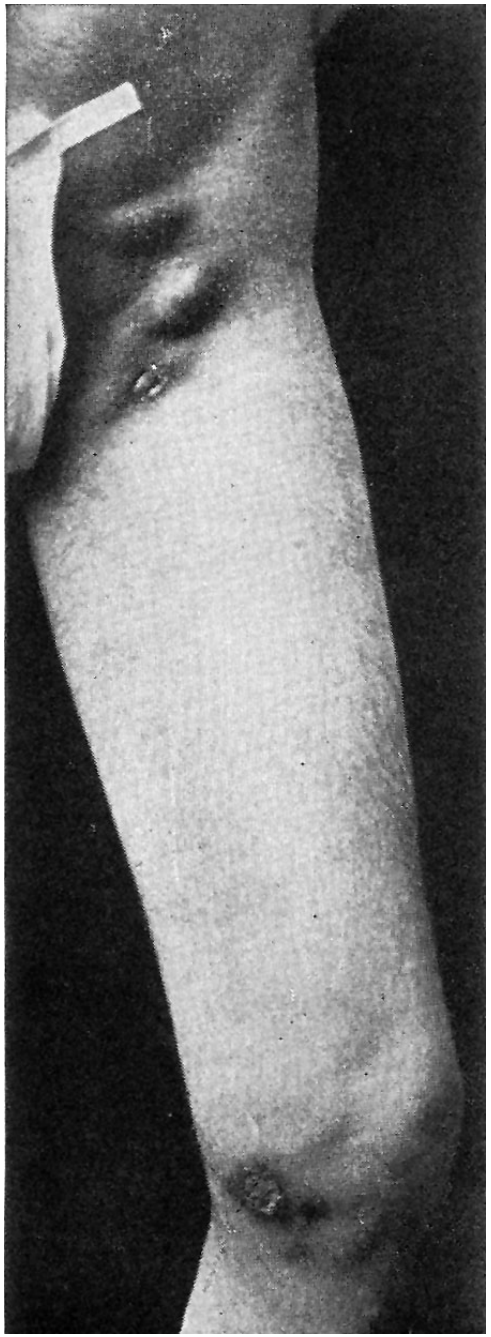
Ulceroglandular Tularemia

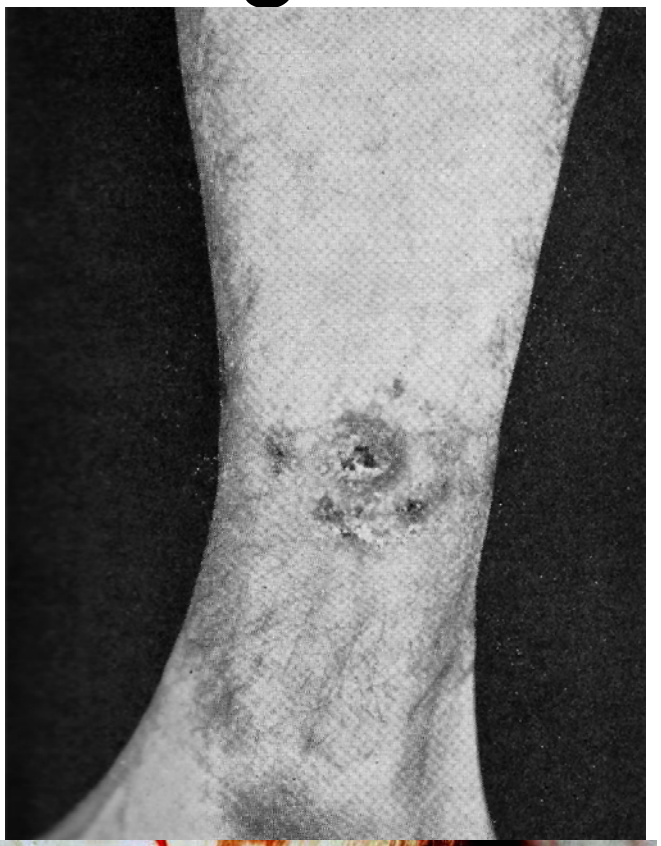
- 75-85% of naturally acquired cases
- Distinguishing characteristics
  - Lesions on skin or mucous membranes (including conjunctiva, oropharynx) *and/or*
  - Lymph nodes > 1 cm in diameter
- Mode of Transmission
  - Inoculation of skin or mucous membranes
    - Biting arthropods and insect vectors
    - Blood or tissue fluids of infected animals

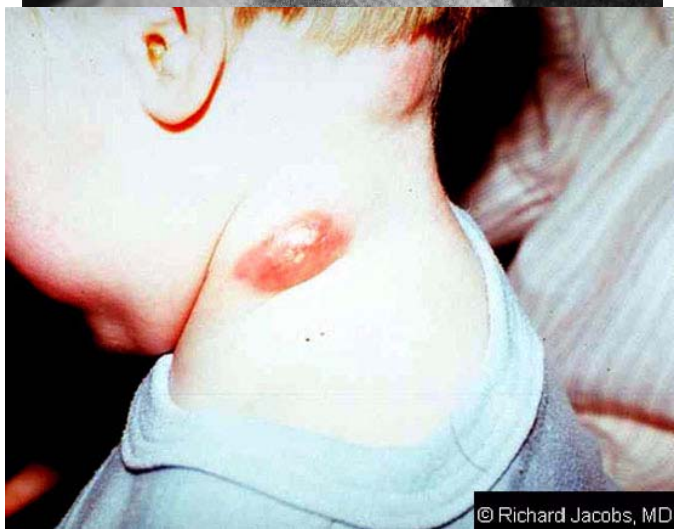
Ulceroglandular Tularemia
Signs & Symptoms

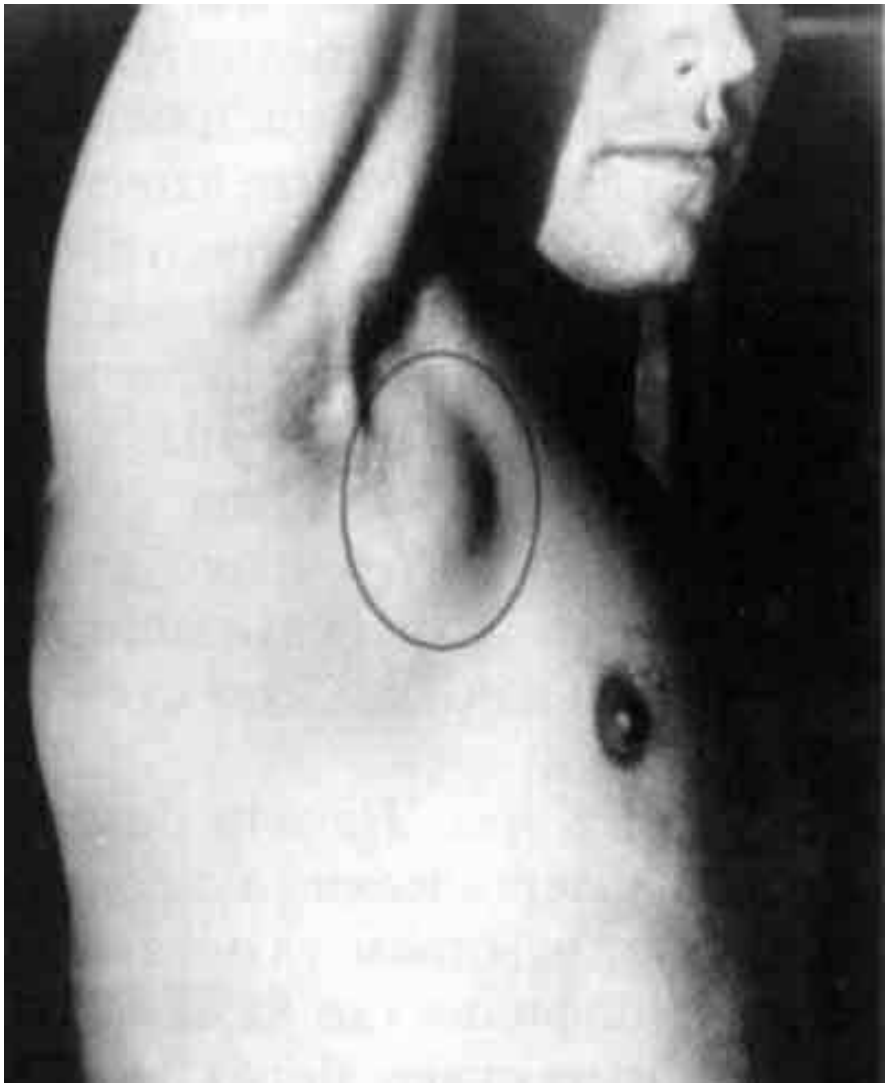
- Sudden onset of fever, chills, headache, cough, and myalgias, *concurrent with*
- Painful papule at site of inoculation
- Papule progresses rapidly
  - → Pustule → Painful ulcer
  - Development of regional lymphadenopathy
- Enlarged nodes
  - Can become fluctuant, suppurative despite treatment
  - Can persist for months or years if untreated

Ulceroglandular Tularemia

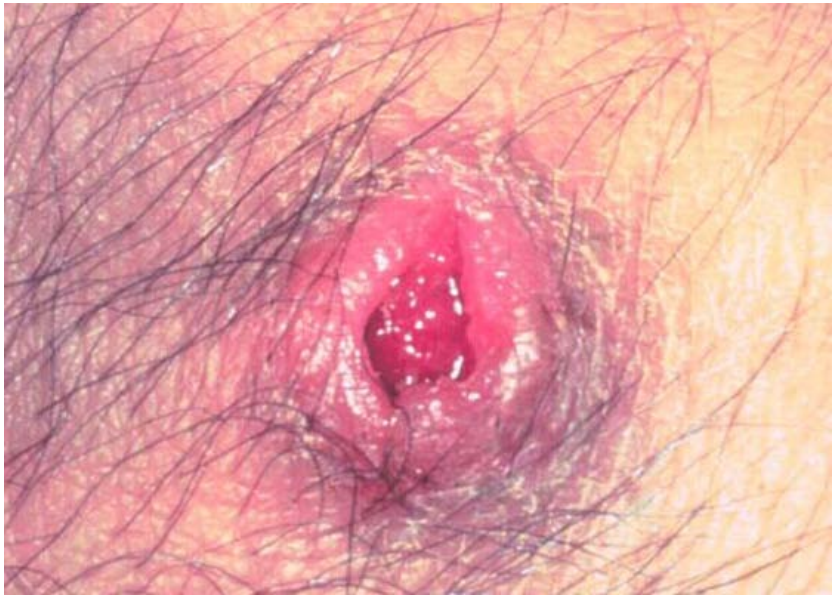


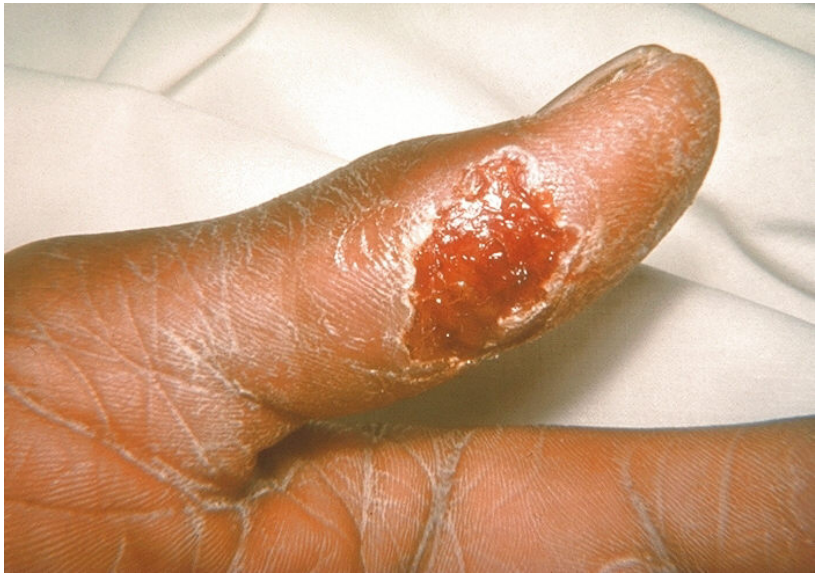


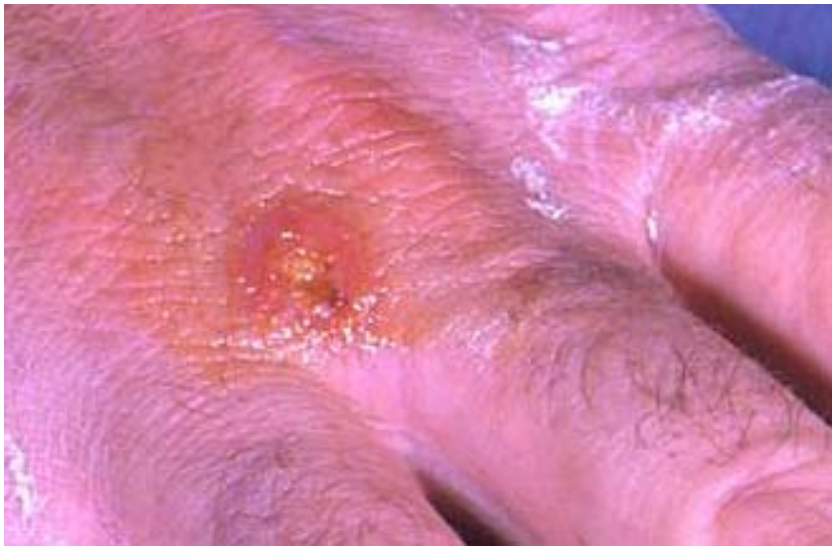


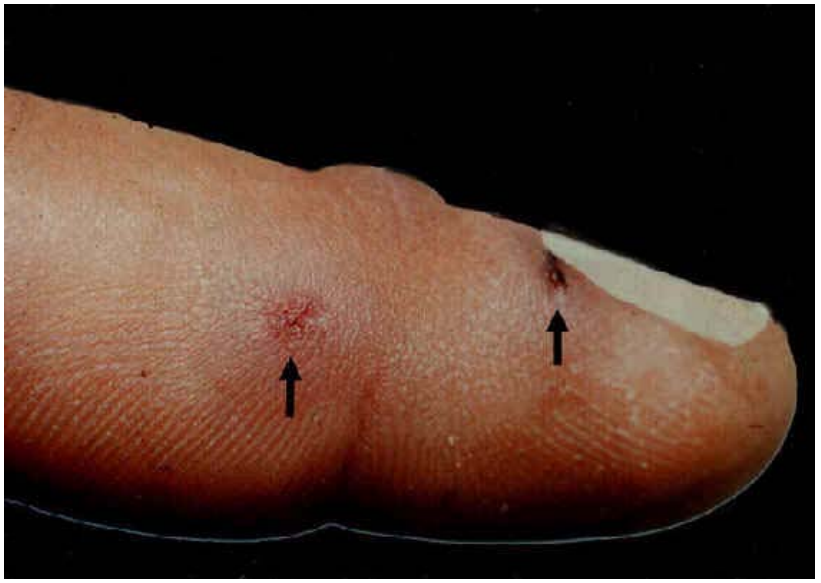


Tularemia - Cutaneous Ulcer





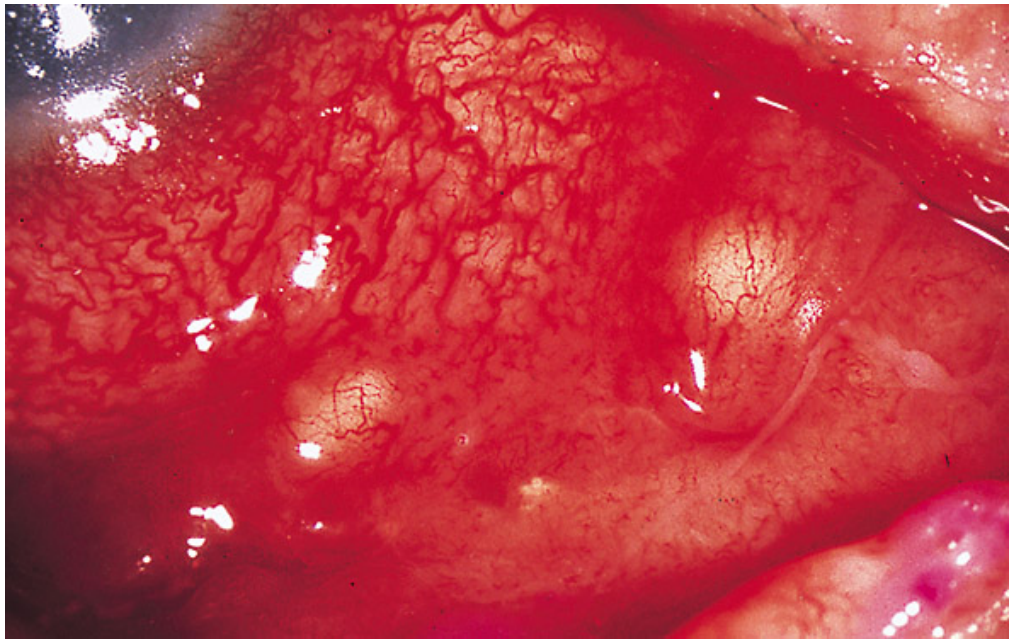




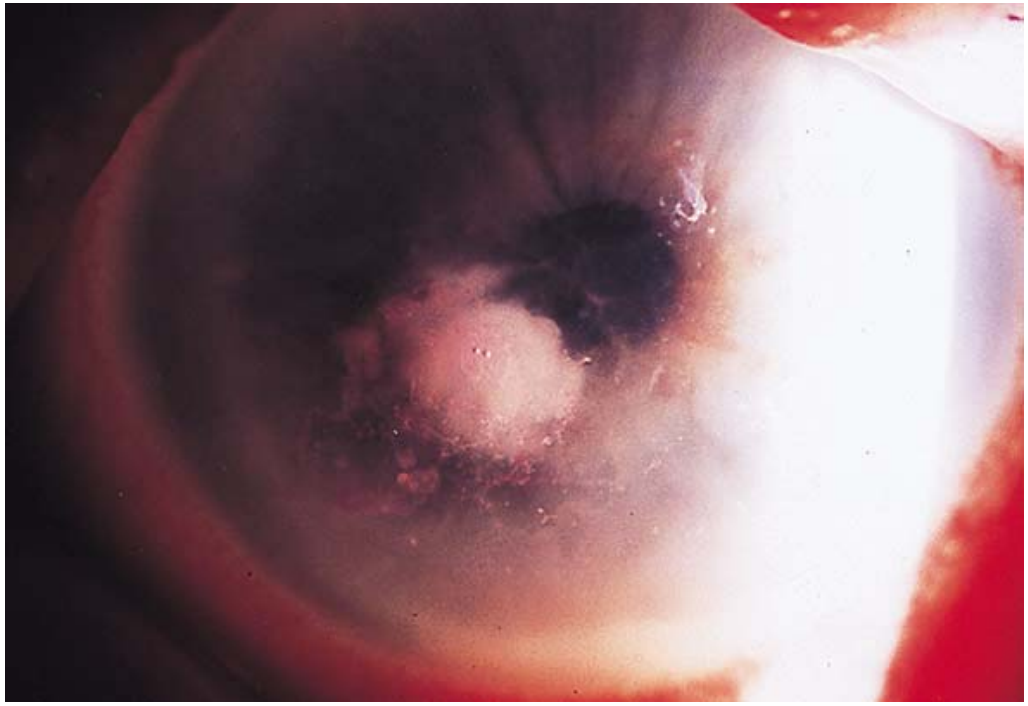
Ulceroglandular Tularemia Signs & Symptoms

- Ocular inoculation (1-2% of cases)
  - Modes: contaminated hands; splash injury; infectious aerosol
  - Painful, purulent conjunctivitis of involved eye(s)
  - Preauricular and/or cervical lymphadenopathy
  - Some patients
    - Chemosis
    - Periorbital edema
    - Nodular conjunctival granulomas
    - Conjunctival ulcers

Oculoglandular Tularemia



(Arch Ophthalmol. 1999;117:132-133)



(Center for Biologic Counterterrorism and Emerging Diseases)

Typhoidal Tularemia

- 5-15% of naturally acquired cases
- Distinguishing characteristics
  - Lymph nodes < 1 cm in diameter, *and*
  - No skin or mucous membrane lesions
- Modes of Transmission
  - Mainly after inhalation of infectious aerosols
  - Possible after intradermal or gastrointestinal challenge

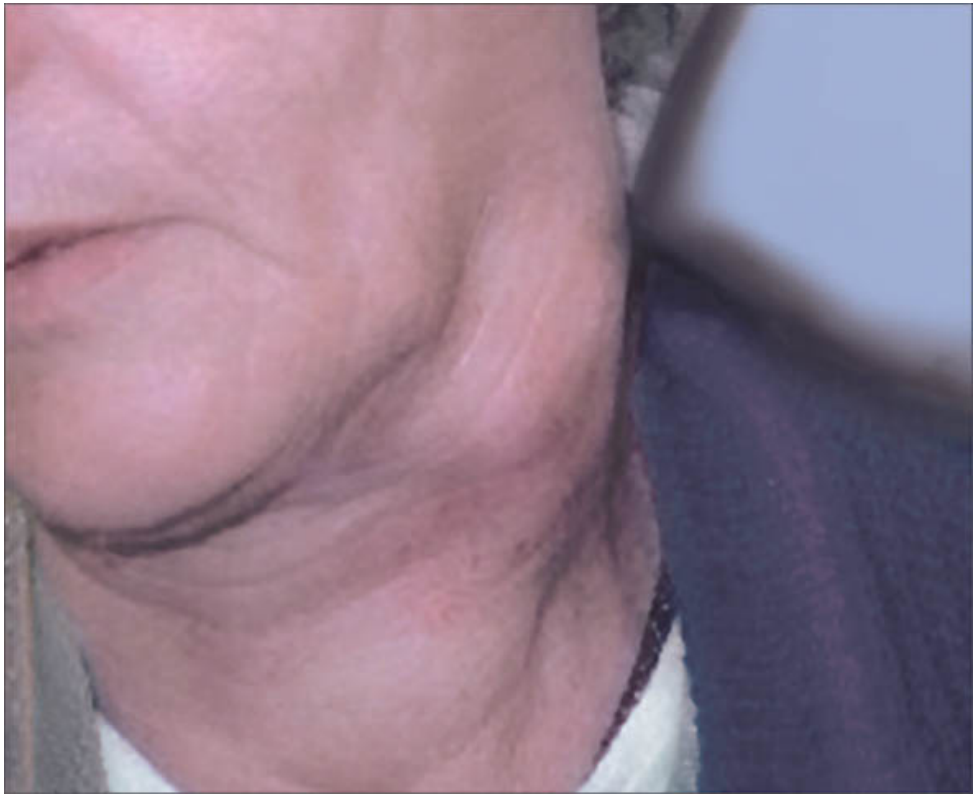
Typhoidal Tularemia
Signs & Symptoms

- Nonspecific syndrome
- Abrupt onset of fever (38-40ºC), headache, malaise, myalgias, prostration
- No obvious portal of entry
- Occasional
  - Nausea, vomiting, diarrhea, or abdominal pain
- Case fatality rate
  - Untreated: up to 35%
  - Treated: 1-3% (may be higher after BT/BW)

Tularemia Pharyngitis

- Reported in both clinical forms
  - 25% of all cases
- Acute exudative pharyngitis or tonsillitis
  - +/- mucosal ulceration
  - +/- cervical lymphadenopathy
- May be confused as
  - Strep pharyngitis → but unresponsive to penicillin, rapid strep negative
  - Mononucleosis → but Monospot negative

Glandular Tularemia



(JAMA. 2001;285:2763-2773)

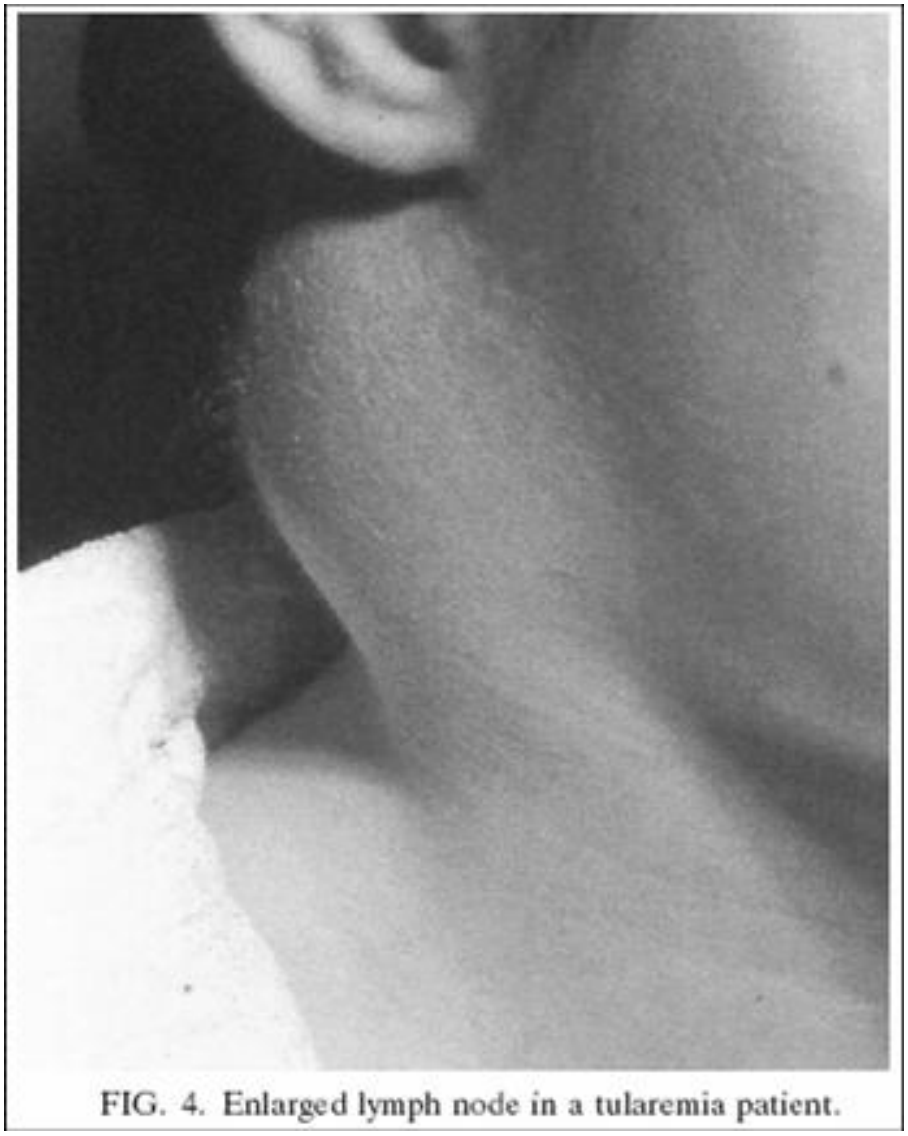
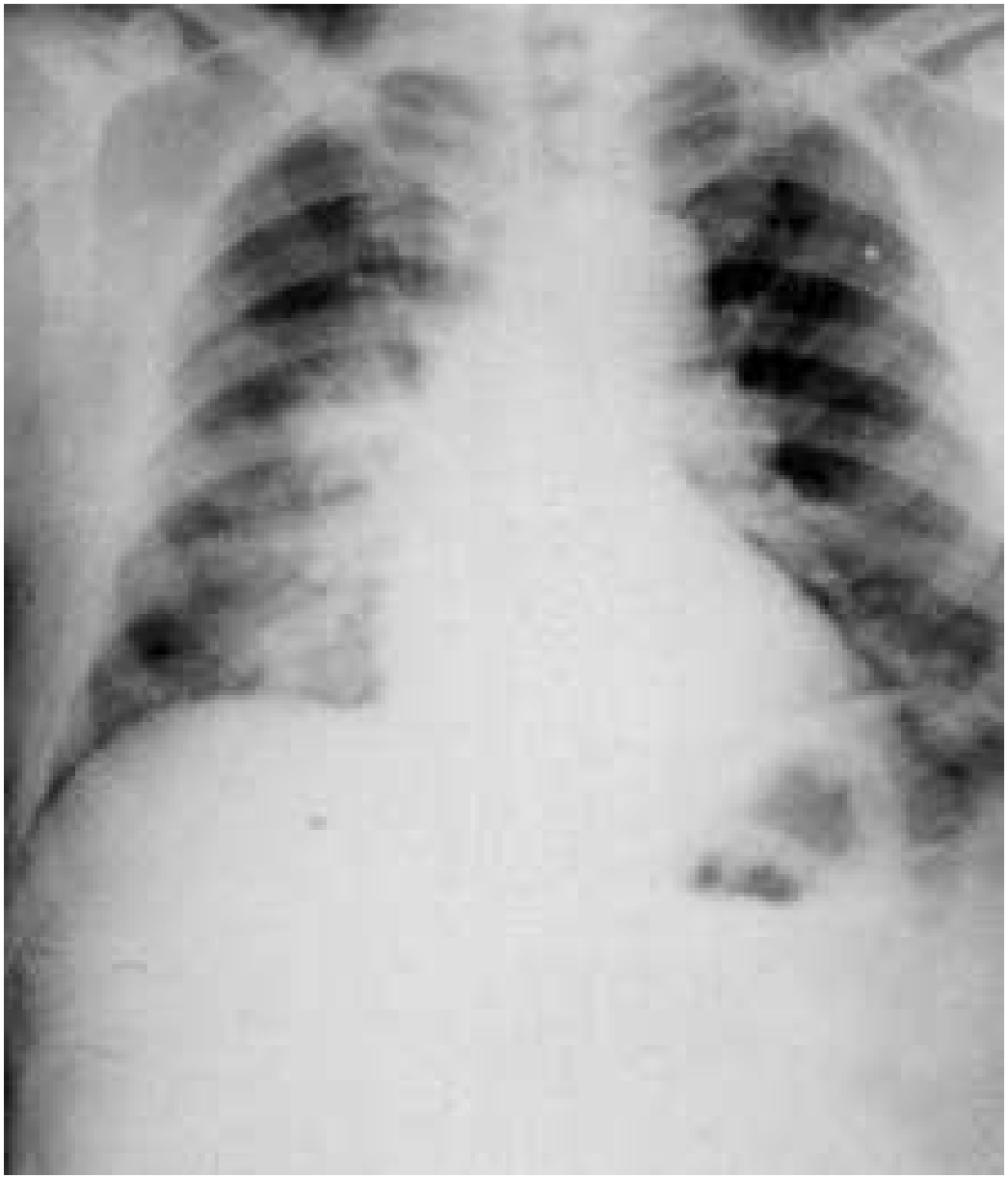


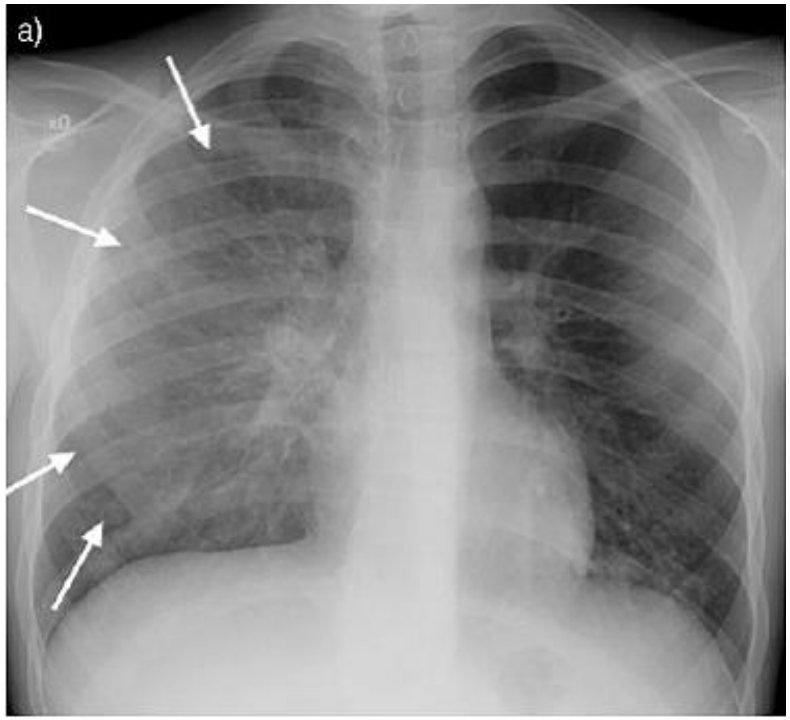
FIG. 4. Enlarged lymph node in a tularemia patient.

Tularemia Pneumonia

- Pulmonary involvement
  - Present in 47-94% of all tularemia cases
  - Variable severity: asymptomatic to fulminant, severe
  - Often under-appreciated on clinical exam
- Common
  - Atypical pneumonia or interstitial pneumonitis
  - More common and severe in typhoidal
    - ~80% of typhoidal cases vs. ~30% of U-G)
  - Hilar adenopathy
  - Pleural effusions (up to 15% of patients)
- Also reported
  - Fulminant lobar pneumonia
  - Bronchiolitis
  - Cavitary lesions
  - Bronchopleural fistula
  - Chronic granulomatous disease

Tularemia Pneumonia CXR





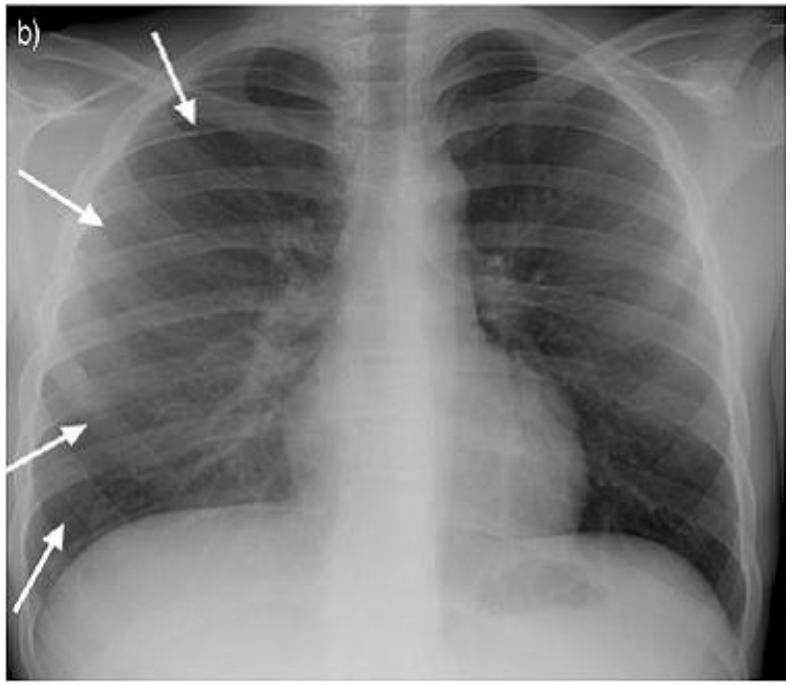
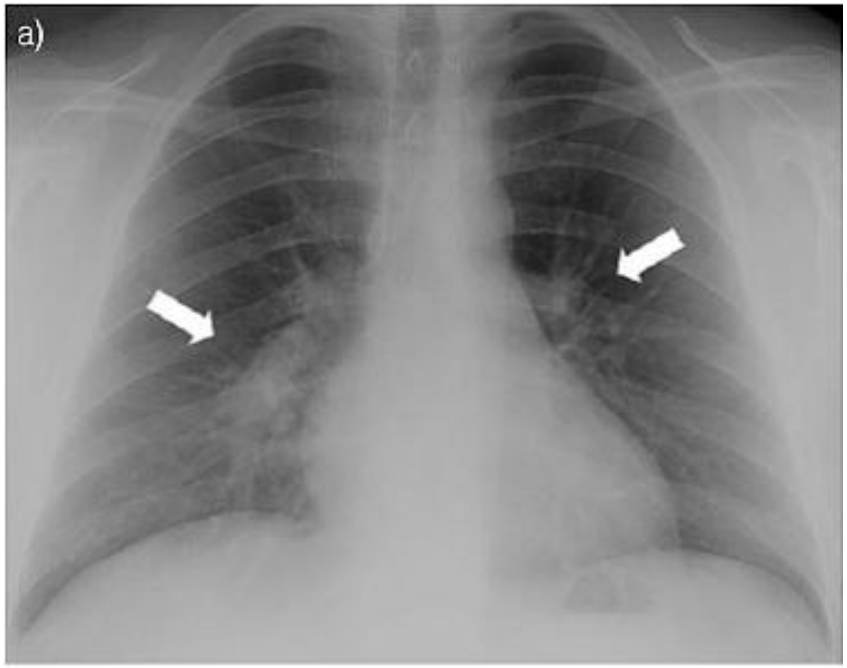


Fig. 2.–Extended right-sided consolidation (arrows) in a 16-yr-old male (case number 4) with fever and productive cough. Radiography was performed a) 11 days and b) 2 months after onset of disease.



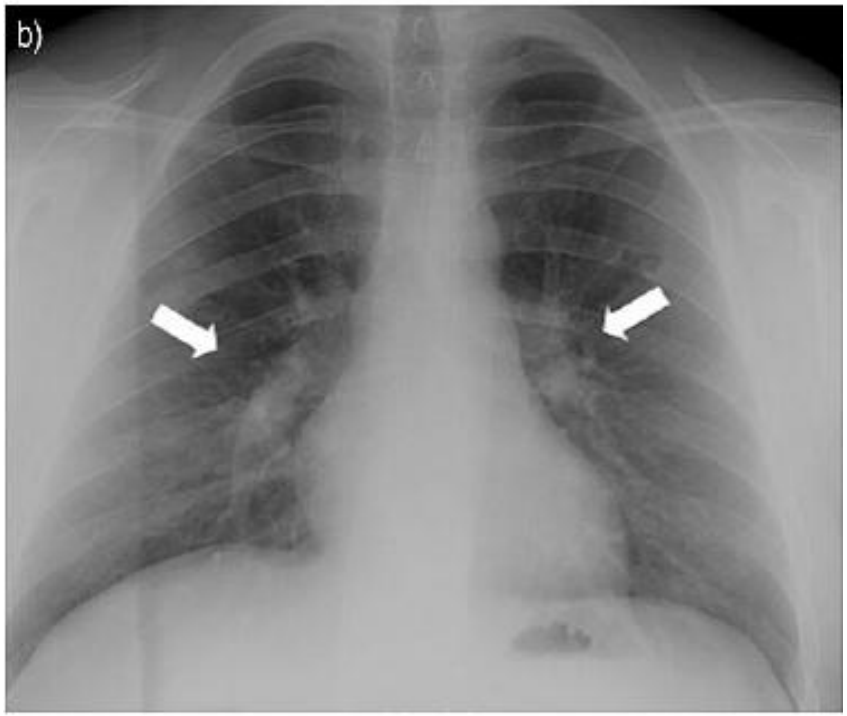
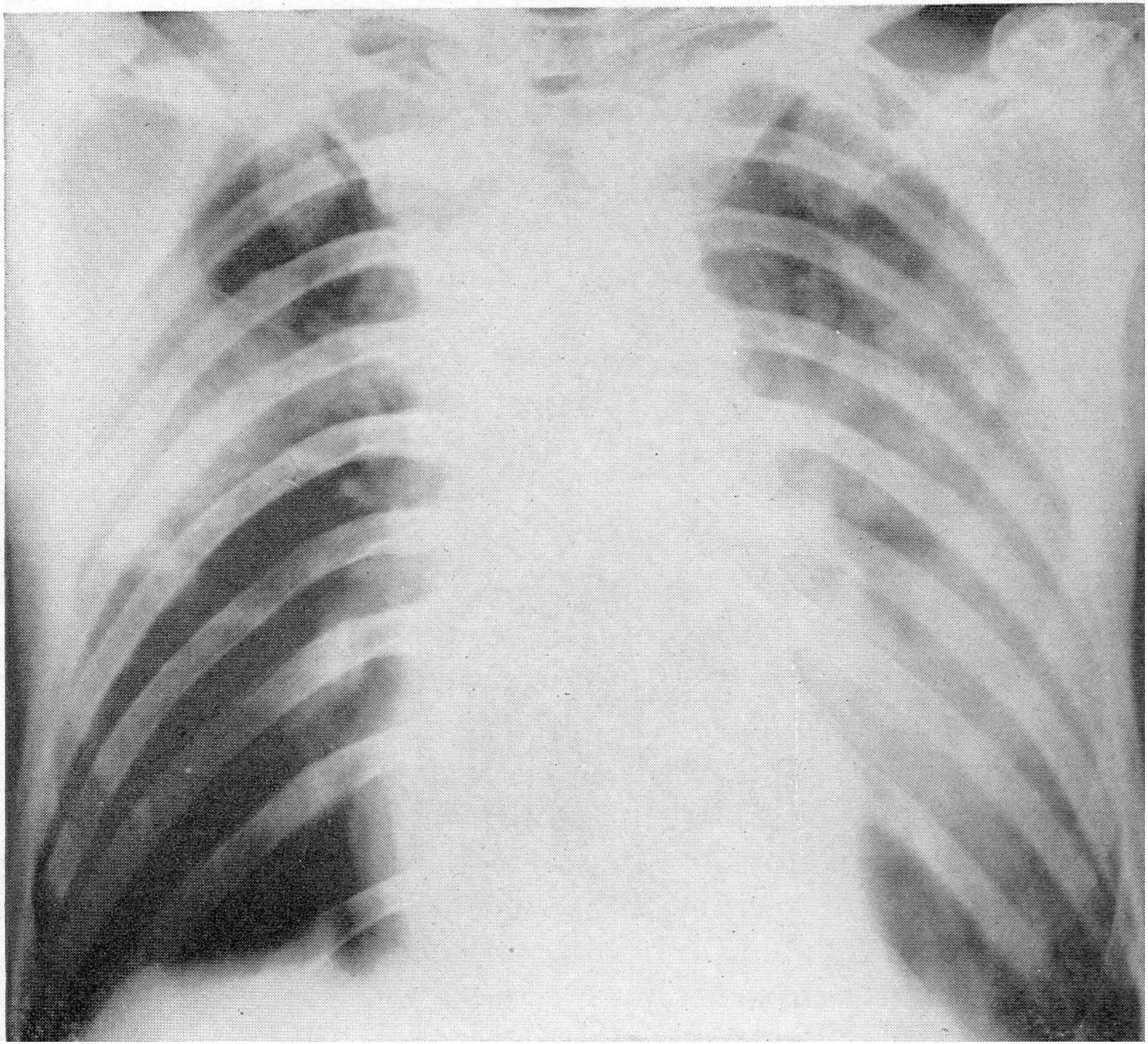
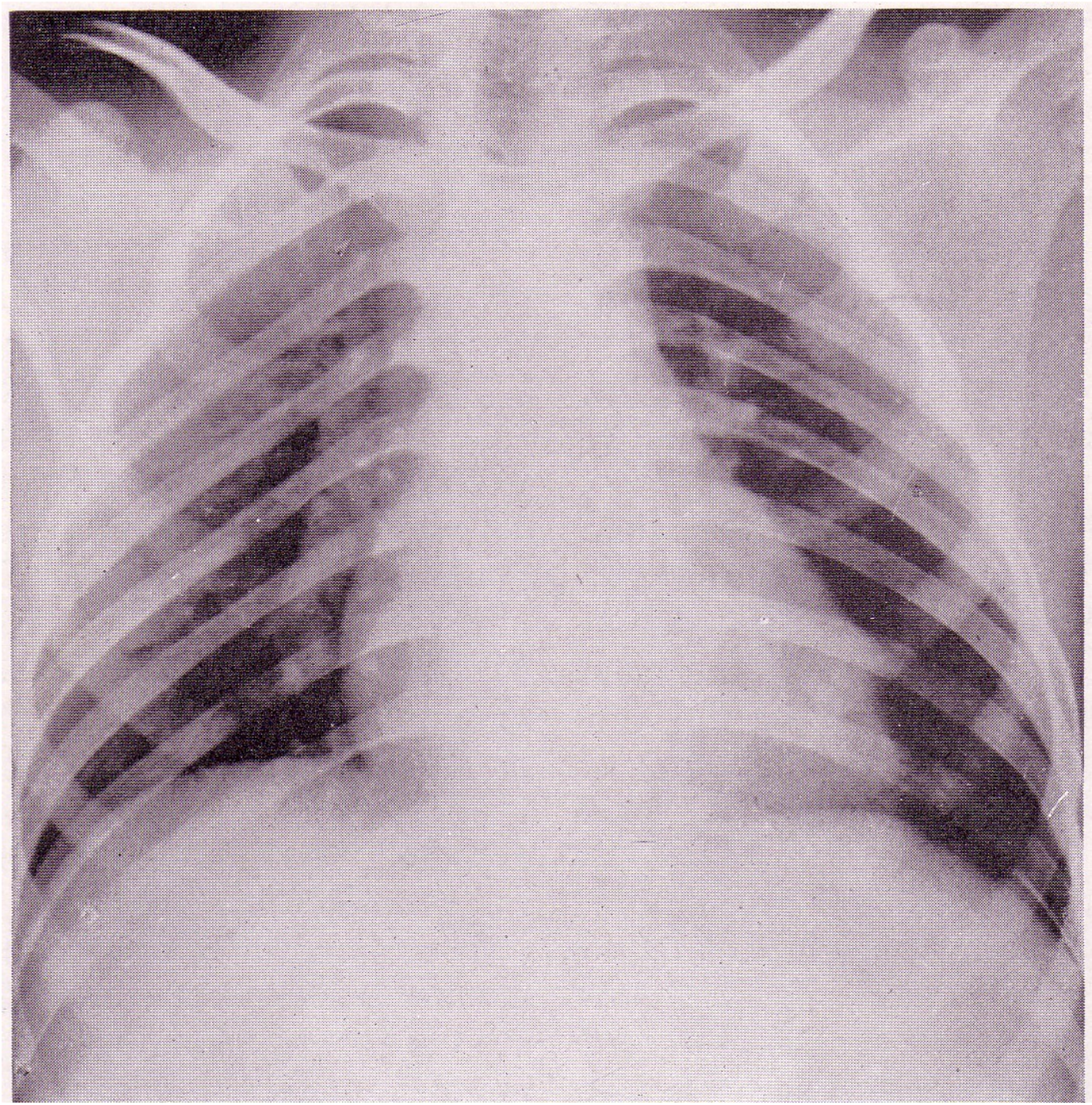


Fig. 1.–Hilar enlargement (arrows) in a 24-yr old farmer (case number 1) with fever but with no lower respiratory tract symptoms. Radiography was performed a) 13 days and b) 10 weeks after onset of disease.





Tularemia Differential Diagnosis

- Viral pneumonia
- Lymphogranuloma venereum
- Cat scratch disease
- Pharyngitis
- Mononucleosis
- Legionairre's disease
- Plague

Tularemia Therapy

- Aminoglycosides are bactericidal
  - Drug of Choice - Streptomycin 1 g IM bid x 10-14 d, or
  - Alternative - Gentamicin 3-5 mg/kg IV/d x 10-14 d, or
- Alternatives
  - Ciprofloxacin 400mg IV q 12hr, or
  - Doxycycline 100mg IV q 12hr
- Tetracycline and chloramphenicol are bacteriostatic
  - Chloramphenicol 15mg/kg IV qid
- Systemic signs classically resolve quickly with appropriate antibiotics
  - Dramatic improvement in 24-48 hours

    - **NOTE: Relapse common if Rx duration < 14 days**

Clinical Diagnosis

- Nonspecific nature makes diagnosis challenging
- Suspect if:
  - Pneumonia with negative blood cultures, throat cultures, serologies for other common organisms
  - No response to beta-lactam antibiotics (PCNs)
- Consider if:
  - Clustering of acute, severe respiratory illness progressing to life-threatening pleuropneumonitis
  - Respiratory outbreak with occasional ulceroglandular disease intermixed
- Suspect foul play if:
  - Tularemia outbreak in urban setting
  - No difference in susceptibility by age or sex

Pre-exposure Prophylaxis

- Tularemia LVS Vaccine
  - LVS = Live Vaccine Strain
  - Live-attenuated vaccine
  - Available for use under IND in limited quantity
    - Used to protect researchers, laboratorians working with tularemia
  - Prevents typhoidal forms
  - Ameliorates ulceroglandular disease
  - Administered by scarification (similar to vaccinia)
- Antibiotics
  - None licensed for use before exposure

Tularemia LVS Vaccine (IND)
USAMRIID Experience Since 1958

- USSR: > 1,000,000 vaccinees
- Obtained in 1954 from USSR
  - Further purified
- U.S. > 5,400 IND vaccine recipients
- > 250 aerosol vaccine recipients
- Some oral vaccinees
- > 300 human challenges

Post-Exposure Prophylaxis

- Not advised for likely natural exposures
  - Tick bite, rabbit or other animal exposures
- Not recommended for close contacts of tularemia patients
- Recommended after aerosol exposure
  - Ideally started within 24 hours of exposure
  - Continue for at least 14 days
  - No documented evidence of human-to-human transmission
- Antibiotic regimens
  - Doxycycline 100 mg PO bid, or
  - Ciprofloxacin 500mg PO bid

Decontamination

- Lack of information on survival of intentionally dispersed particles
  - Suspect very low risk with typical environmental counter-effects
  - Suspect very limited risk of secondary dispersal
- When concerned about environmental risk
  - Examples: wet, cool, low UV exposure conditions; lab spill)
  - 10% bleach solution for 10-minute contact time, then 70% alcohol solution
  - May follow with soap and water for remaining contamination
- Persons with direct exposure to dry or liquid aerosols should wash body and clothing with soap and water

-Working Group on Civilian Biodefense, 2001 (JAMA)

Tularemia – Key Points

- Natural and intentional infection possible
  - Suspect intentional if:
    - Clustering of cases in an urban setting
    - Type A outside North America
    - No difference in susceptibility by age, gender
    - Outbreak appears to have secondary transmission, or massive compressed point source
- Abrupt onset of non-specific symptoms
- Painful ulcers, no eschar
- Pneumonia more common after aerosol exposure (typhoidal)
  - Pneumonia often seen on X-ray but not clinically

Questions?

USAMRIID

Bacterial Threat Agents: Plague

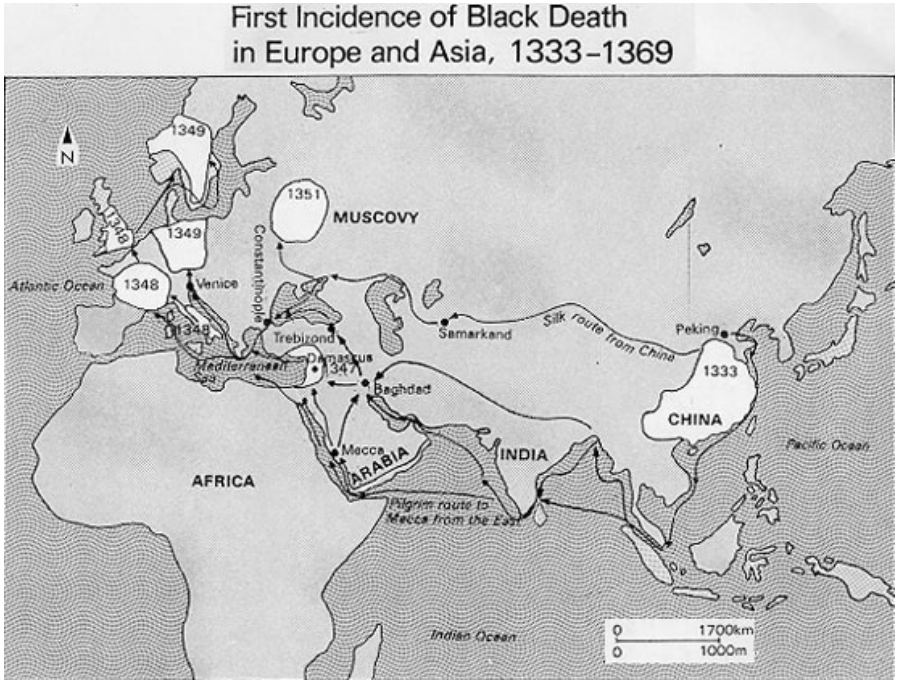
COL Zygmunt F. Dembek, MS
PhD, MS, MPH
USAMRIID, Ft Detrick, MD
May 2008

Lesson Objectives

- Describe the natural epidemiology of plague
- Identify the organism that causes plague and its basic microbiology and pathophysiology
- Distinguish the three clinical forms of plague
- Summarize the clinical management of plague
- Describe mechanisms to prevent disease and/or transmission of plague

Plague History

- ~200 million deaths
- Biblical (I Samuel)
  - 1320 BC, Philistines
- Major Pandemics
  - 541 AD - Plague of Justinian
  - 1346 AD - 'Black Death'
  - 1894 AD - Modern Pandemic



Plague
Biological Warfare

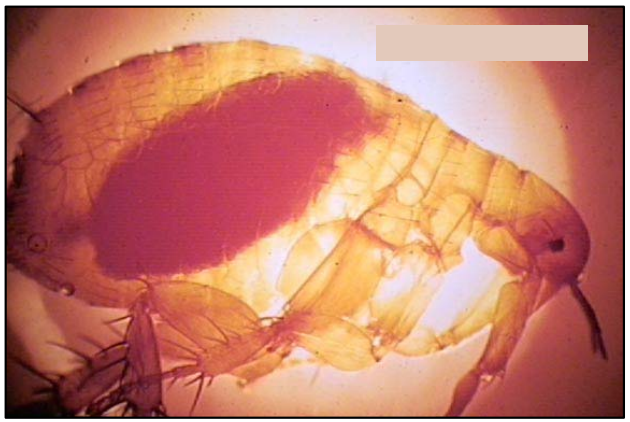
- WWII
  - China: Ningpo, Oct 1940; Changteh, Nov 1941
  - Japanese planes released rice and wheat grains, “strange particles” mixed with fleas
  - Caused hundreds of bubonic deaths
  - No excessive rodent die-off preceding human cases

- Cold War
  - Soviet Union
  - Defecting microbiologists report genetically engineered, highly lethal, antibiotic resistant forms under development

Plague Epidemiology

- Reservoir:
  - Mammals, >200 species
    - Historically, the black rat
    - Rats, squirrels, prairie dogs, cats

- Vector:
  - Flea, >80 species
    - Historically, the oriental rat flea
    - Bacteria grow, block gut
    - Flea is ‘starving’ with full belly
    - Feeding frenzy, regurgitates, inoculates host while trying to feed



Known mammalian reservoirs of plague (United States)



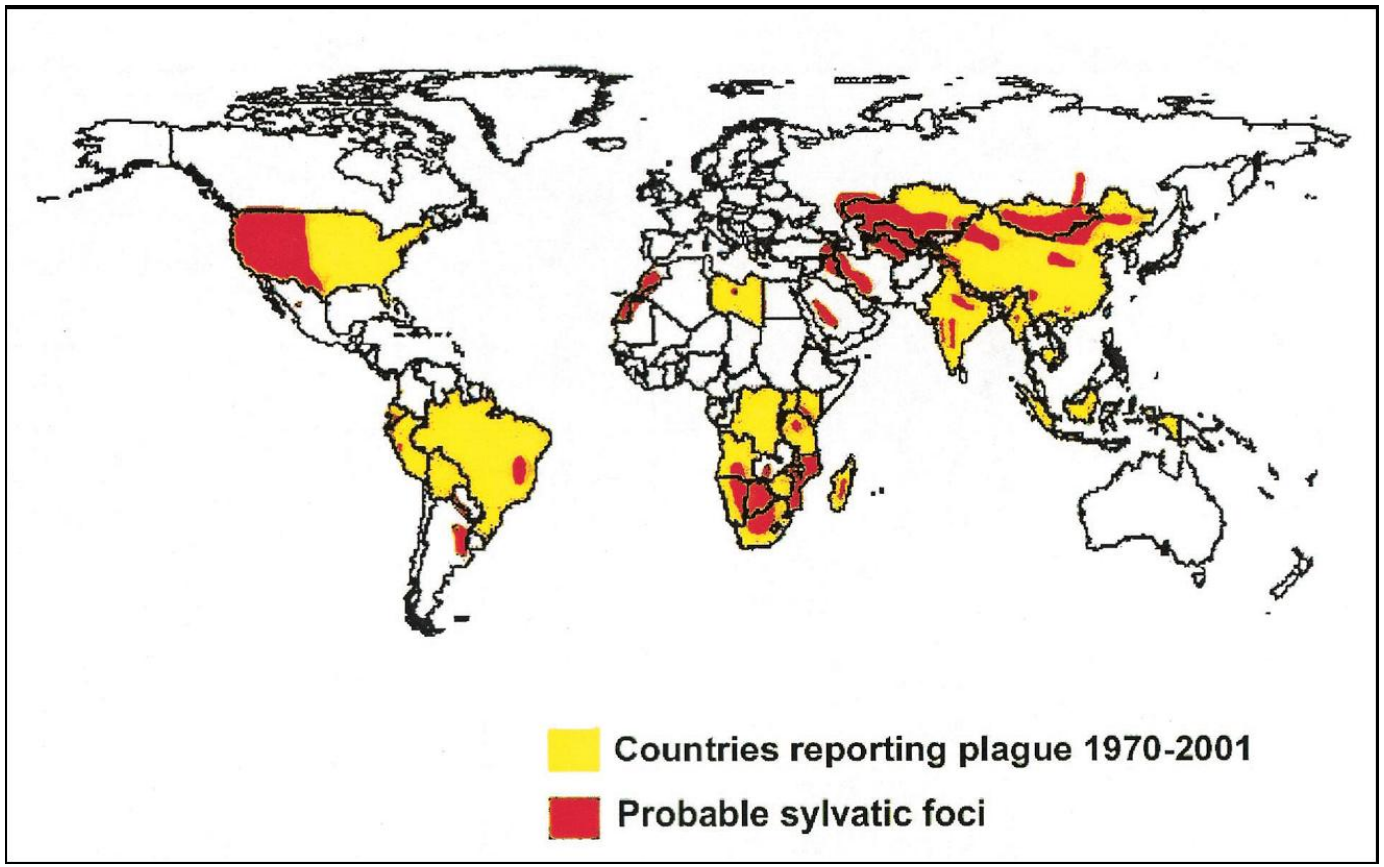






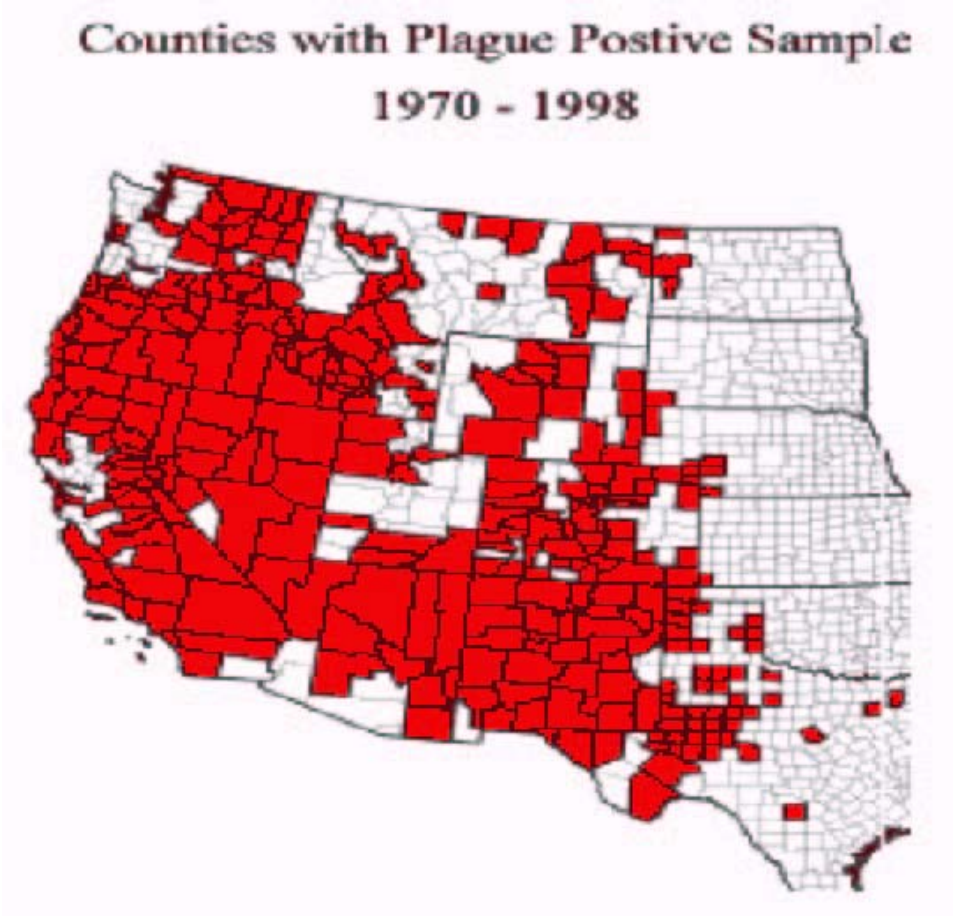
Plague Epidemiology Worldwide

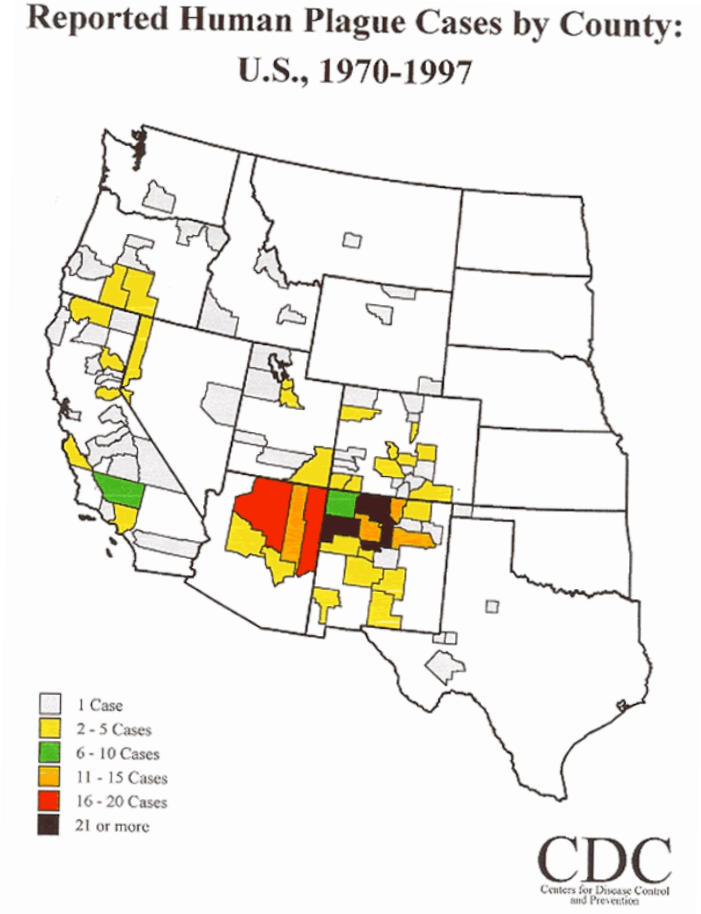
- Approximately 2,500 cases reported annually
- Most cases occur in underdeveloped countries
- Case fatality rate: 8-10% (all forms, 1987-2001)



Plague Epidemiology
United States

- Annual incidence: Range 1-40 cases , Mean 15
- About 500 cases since 1950; 125 from 1987-2001
- Vast majority originate from desert Southwest

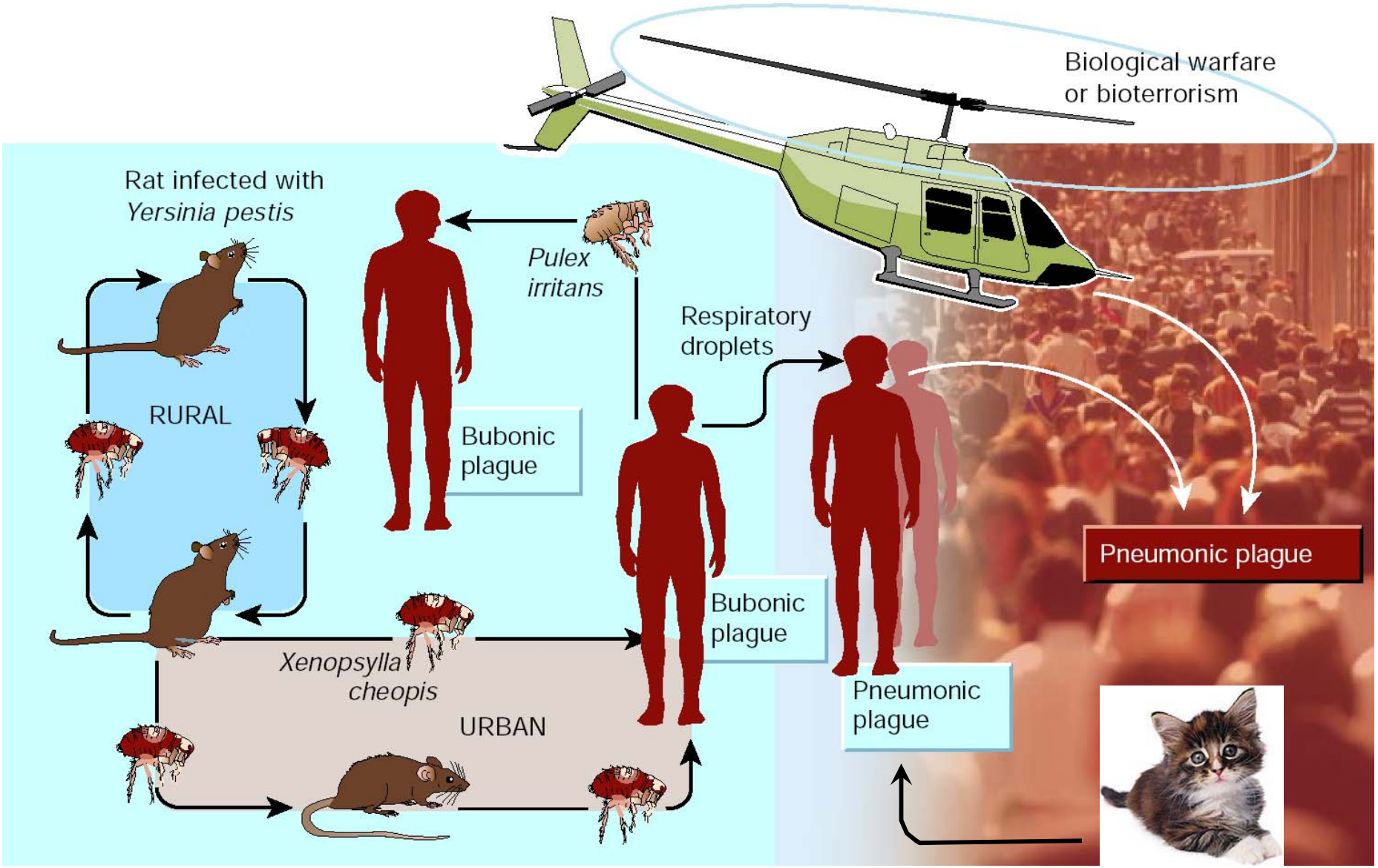




Plague Epidemiology
Risk Factors

- U.S. risk factors
  - <20 years old
  - Close contact with rats
  - Close contact with feline and canine rat predators
  - Rodent harborage and food sources in the vicinity of the home
  - Seasonal (May – October):
    - Fleas and rodents most active
    - People outdoors more

Plague
Modes of Transmissions

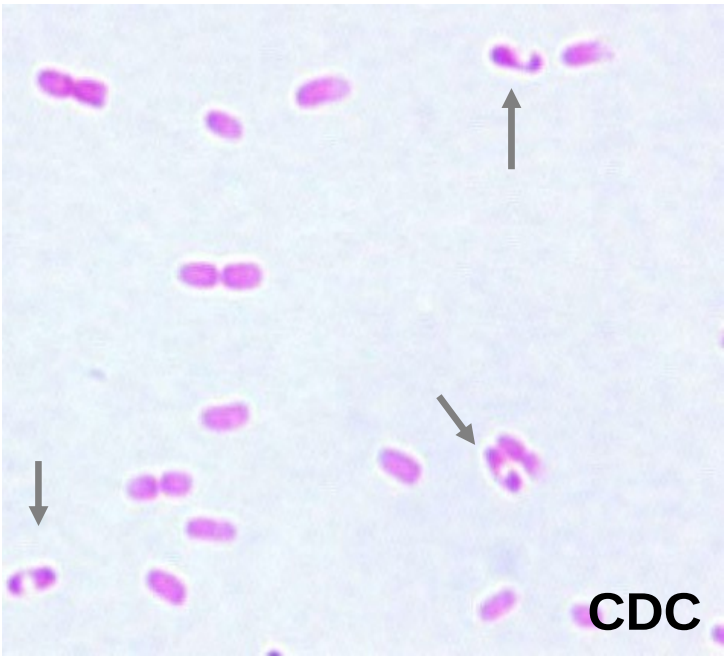


Modified from NATURE|VOL 413 | 4 OCTOBER 2001 |www.nature.com

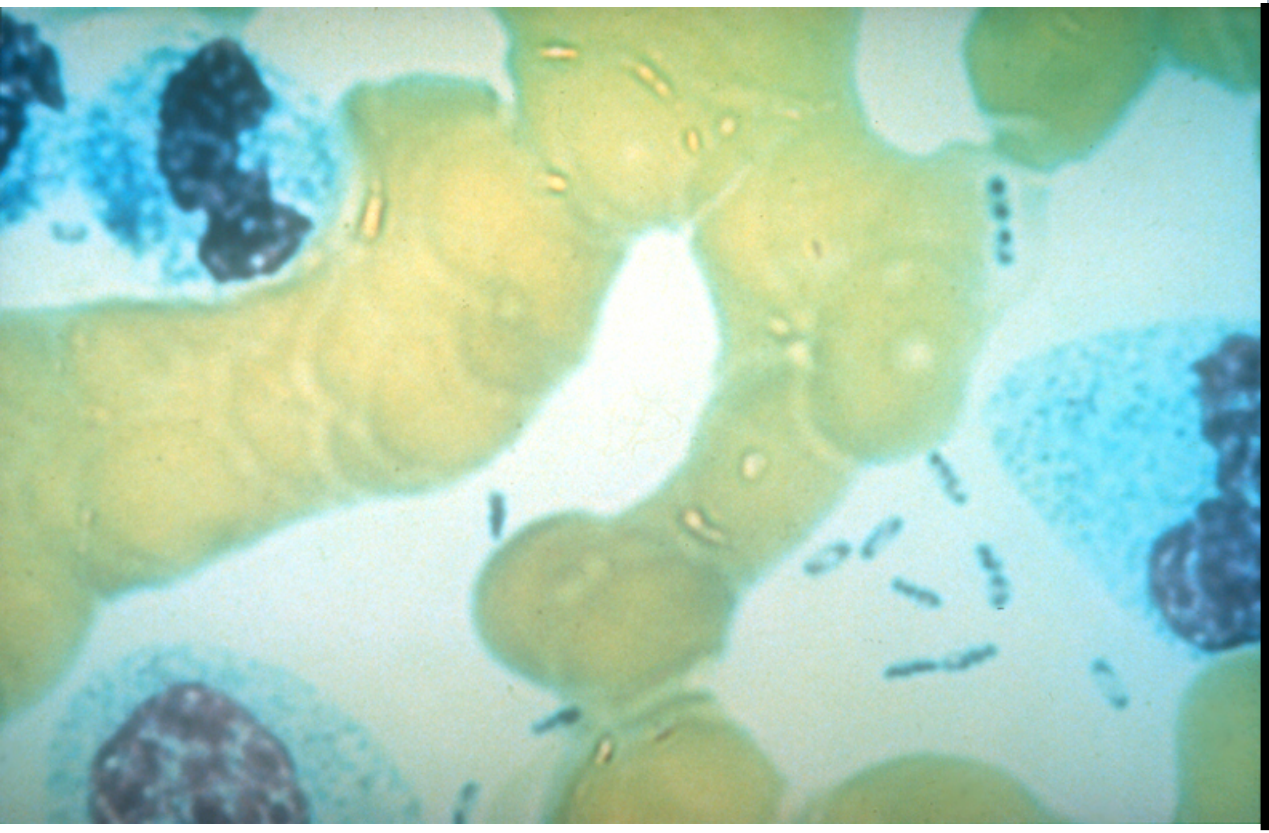
Yersinia pestis

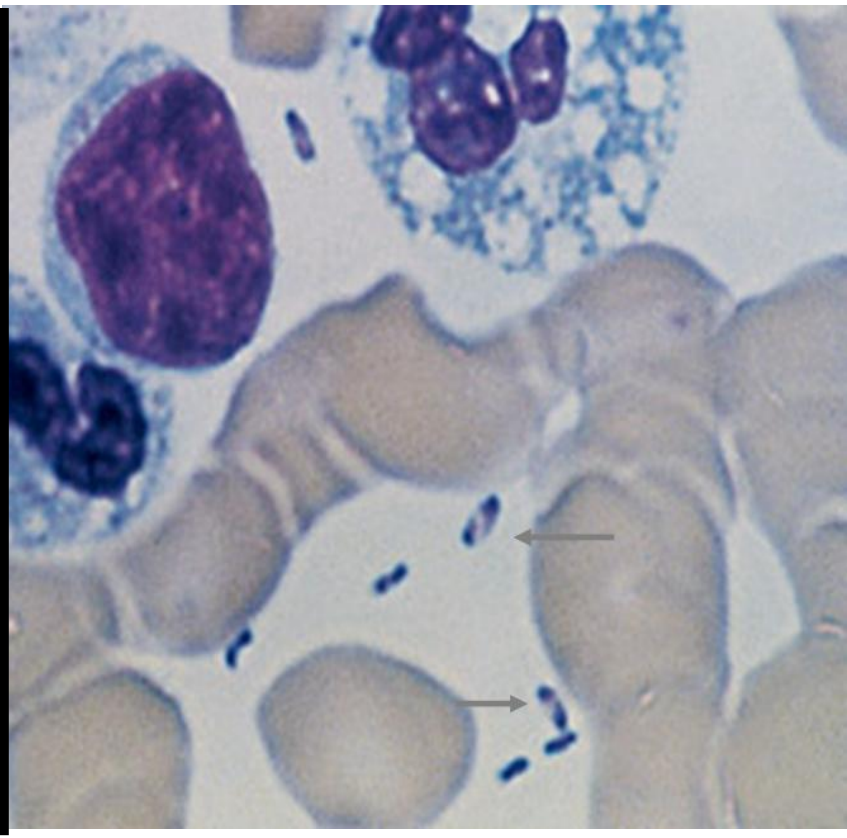
- Family *Enterobacteraceae*
  - Gram-negative, non-motile bacillus
  - Bipolar “safety-pin” staining
- Facultative intracellular pathogen
- Proliferates inside mononuclear phagocytic cells
- Virulence factors
  - Enable organism to evade host immune response
  - Promote lethality

Yersin: courtesy Pasteur Research Centre



Plague
Blood Smears





Plague
Clinical Presentation

- Bubonic
- Septicemic
- Pneumonic

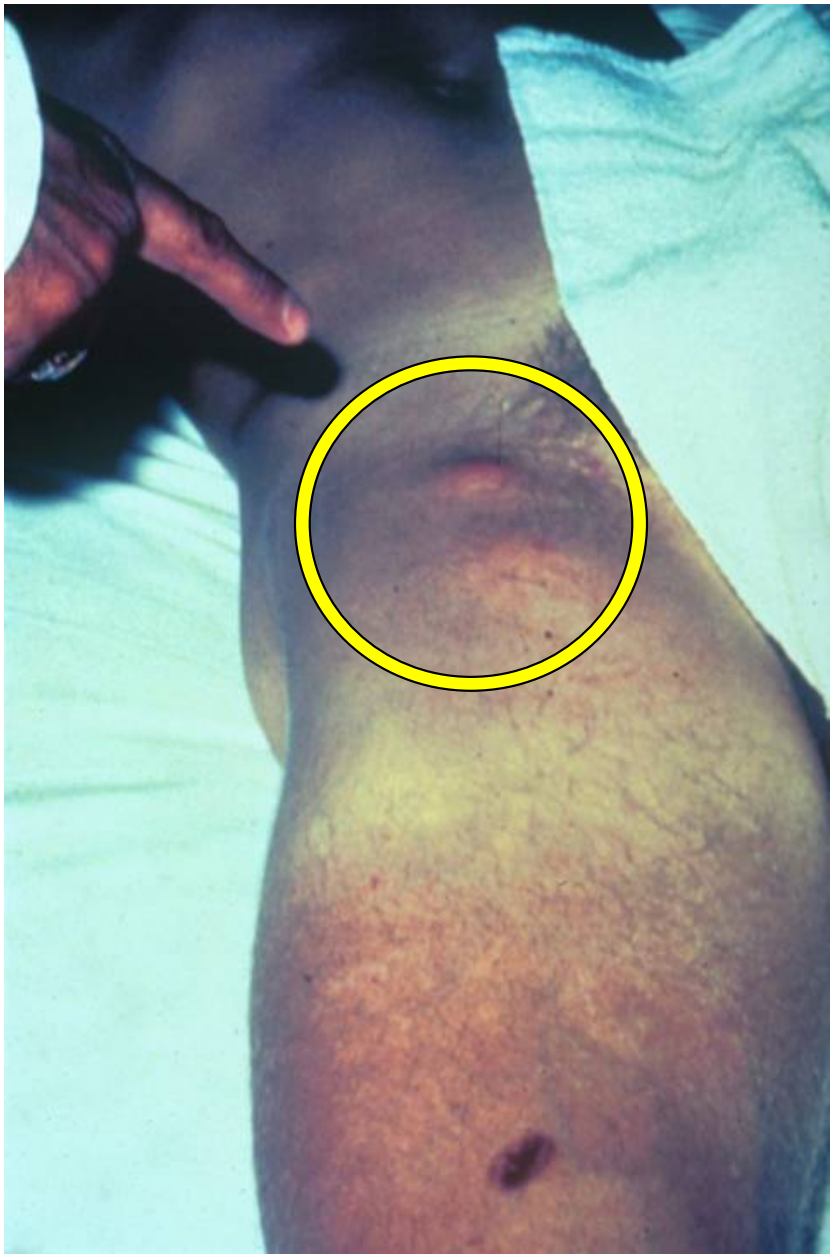
Bubonic Plague

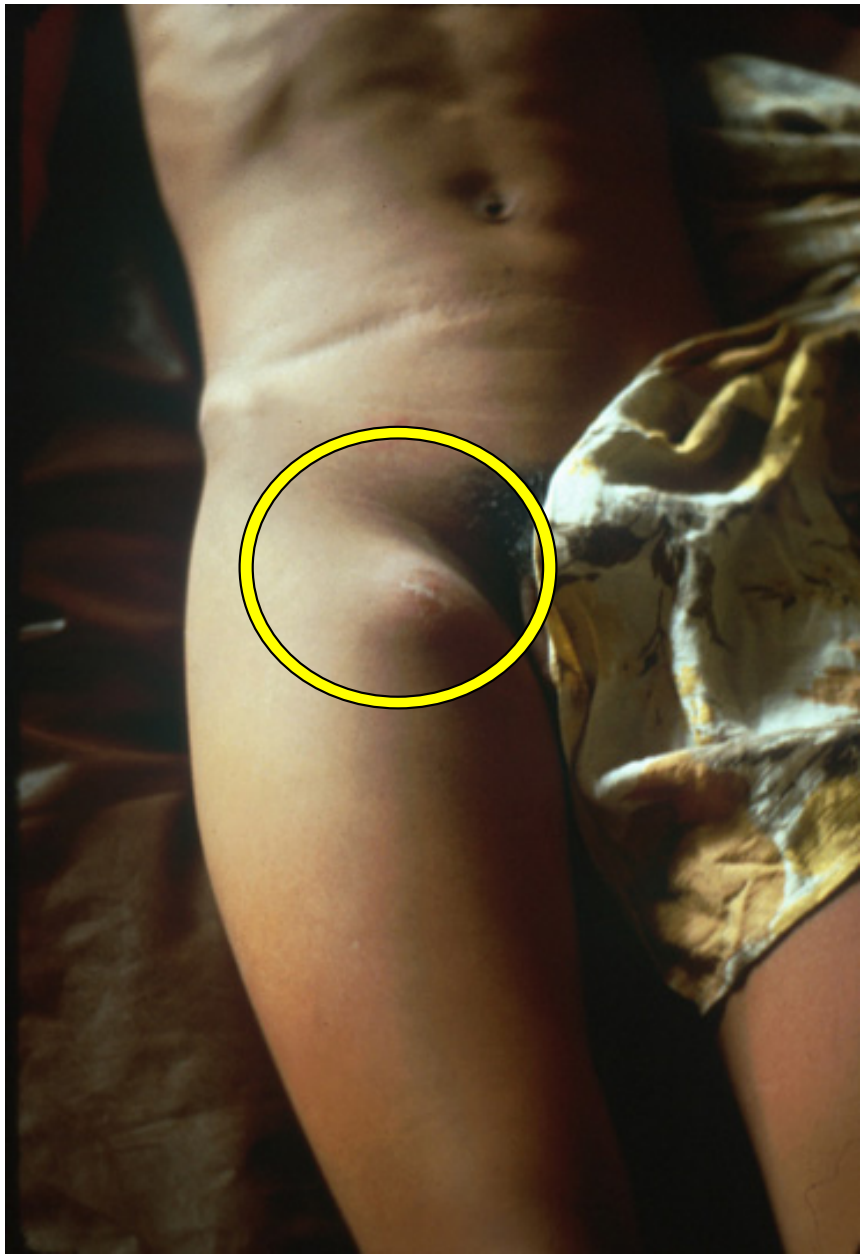
- Incubation 2-8 days (mode 3-5 days)
- Sudden onset of flu-like syndrome
  - Fever up to 40ºC (104ºF)
  - Malaise (75%), chills (40%), headache (20-85%), altered mentation (26-38%), N/V (25-49%)
  - Abdominal pain (50%)
- Bubo develops within 24 hours
  - Swollen, infected lymph node
  - Very painful, but rarely suppurates
  - Range 1-10cm size

Bubonic Plague

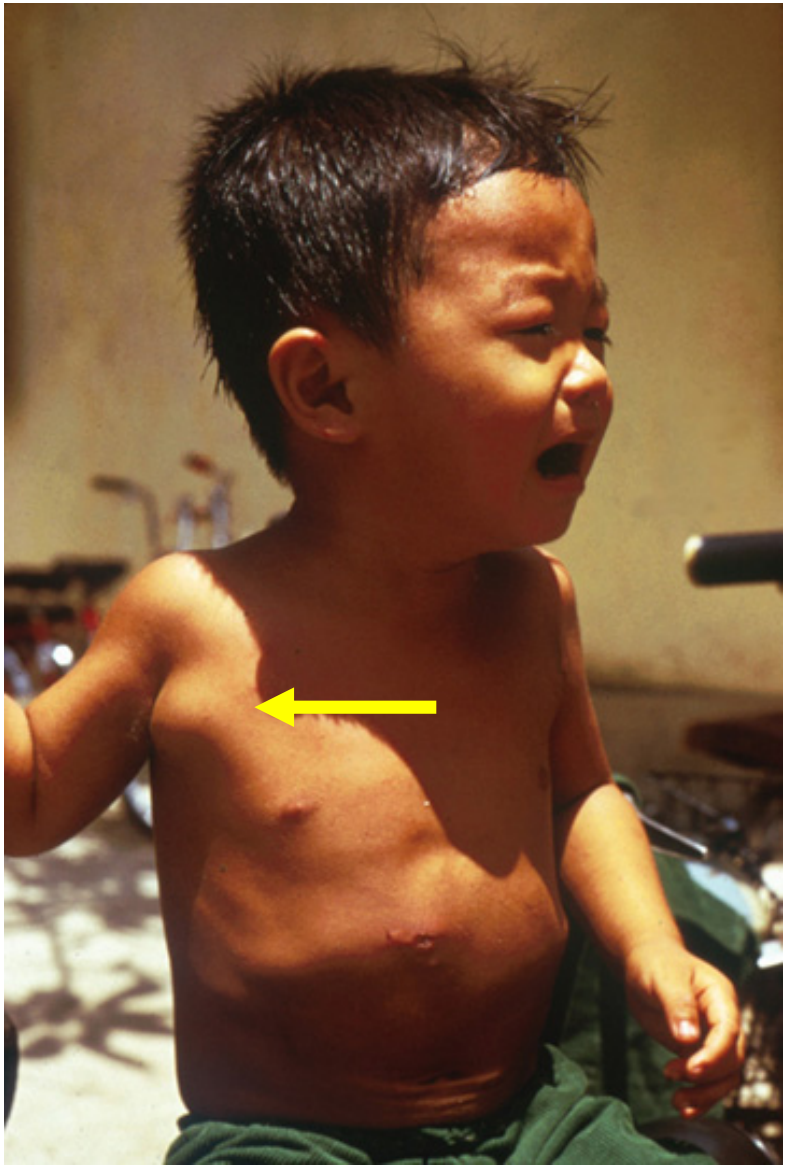
- Buboes
  - Femoral > inguinal > axillary, cervical
  - Any lymph nodes can be involved
- Other findings
  - Papule, vesicle, eschar, or pustule = Flea bite (25%)
  - Tender palpable liver and/or spleen
  - Acute abdomen (due to intra-abdominal node buboes)
  - Complications: Secondary septicemic or pneumonic plague, plague meningitis
- Mortality
  - 60% if untreated, <5% with prompt therapy

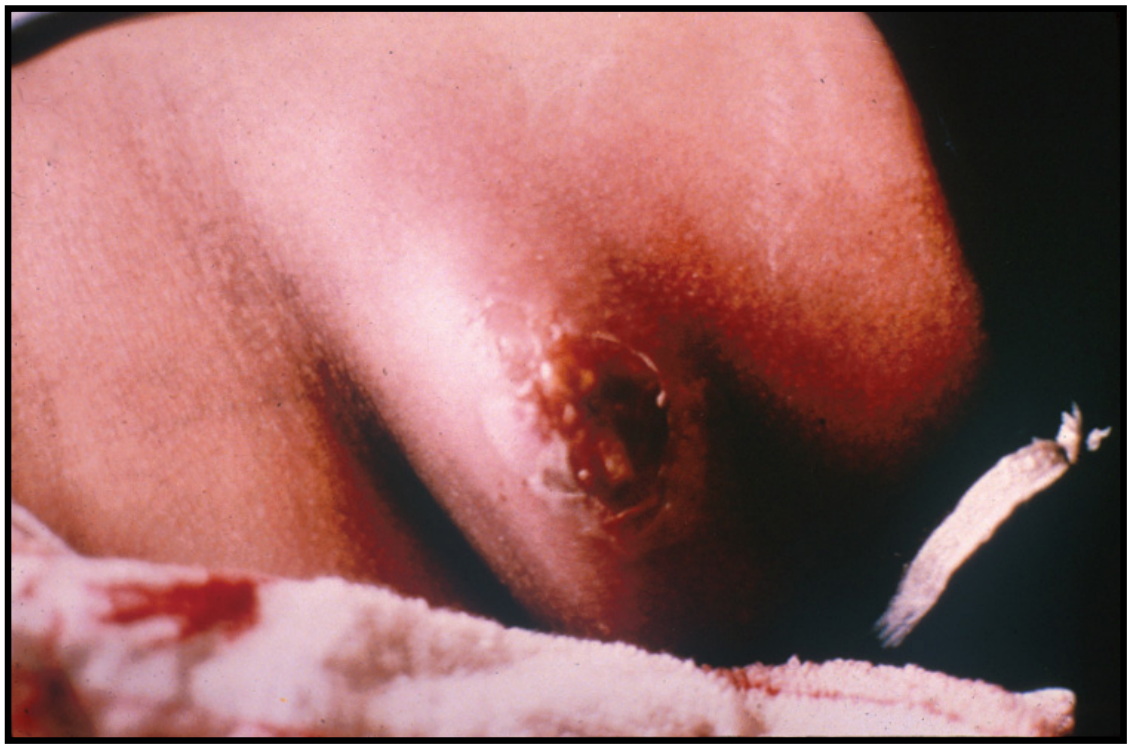
Bubonic Plague
Femoral node buboes





Bubonic Plague
Axillary bubo & Bite site



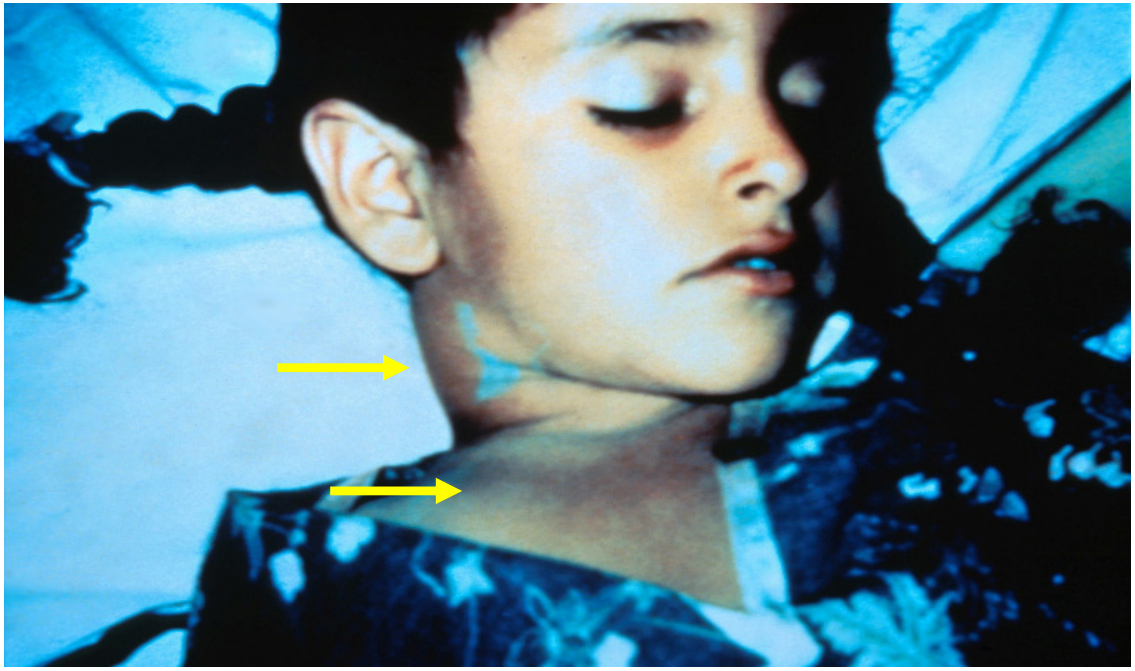


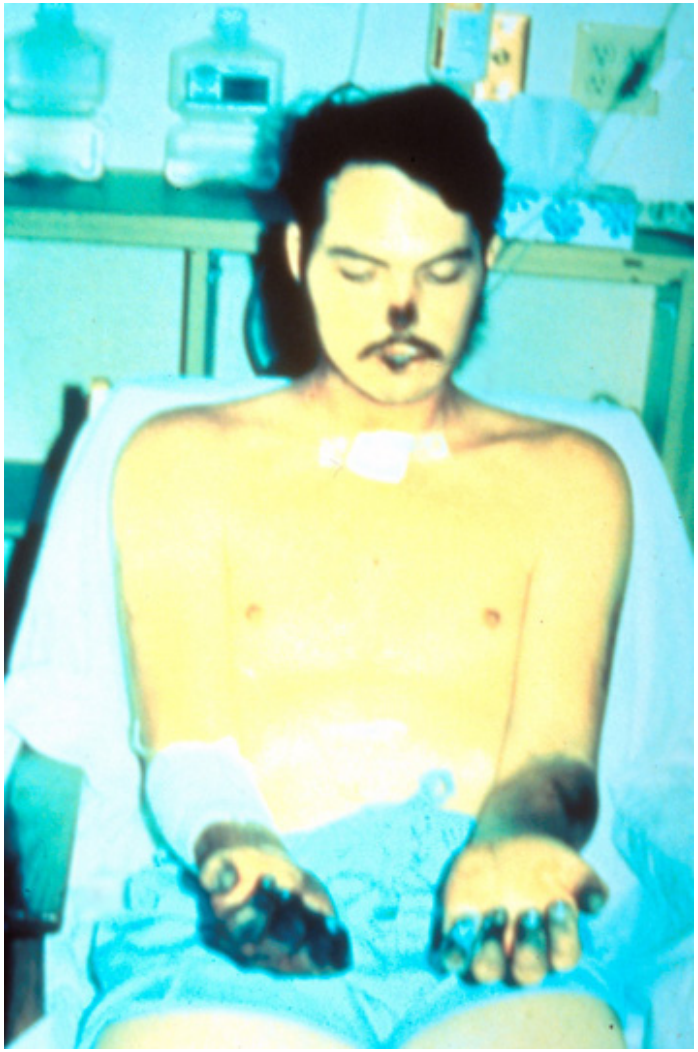
Septicemic Plague

- Secondary extension of bubonic form
  - ~25% of all bubonic forms progress
  - High density bacteremia; rapid multiplication in blood
- Primary cases possible
  - Absence of lymphadenopathy and pneumonia
- Symptoms:
  - Gram negative septicemia
    - High fever, chills, nausea, vomiting, diarrhea
    - Hypotension, tachycardia, tachypnea
  - Thrombosis in small, acral vessels
    - Purpura, necrosis, gangrene, DIC

Septicemic Plague

Adenopathy, purpura

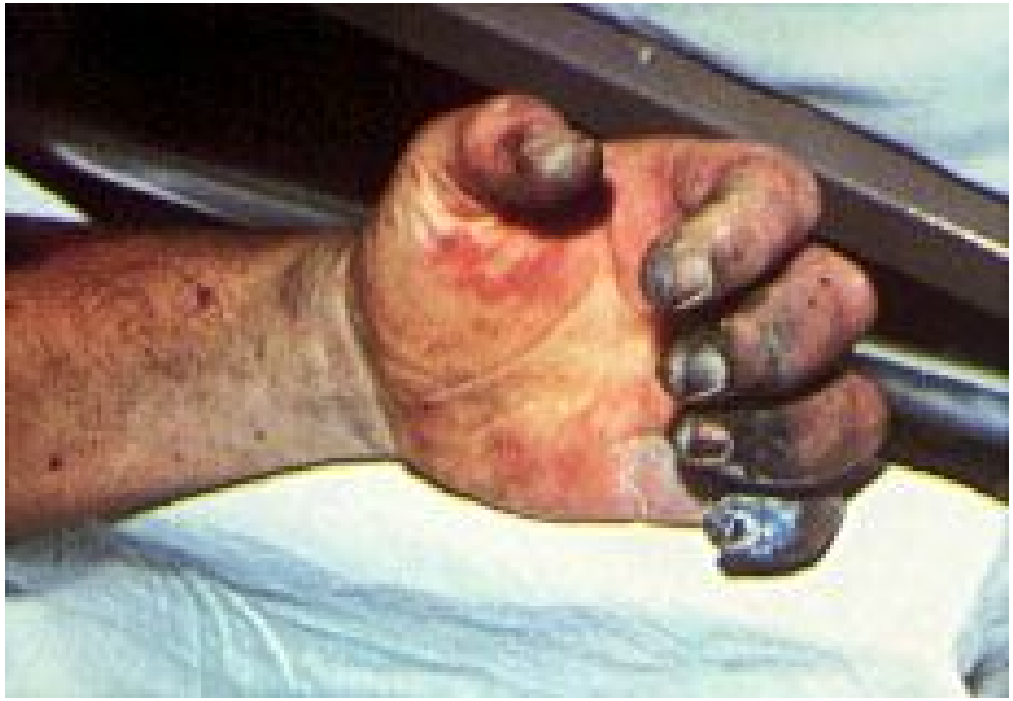


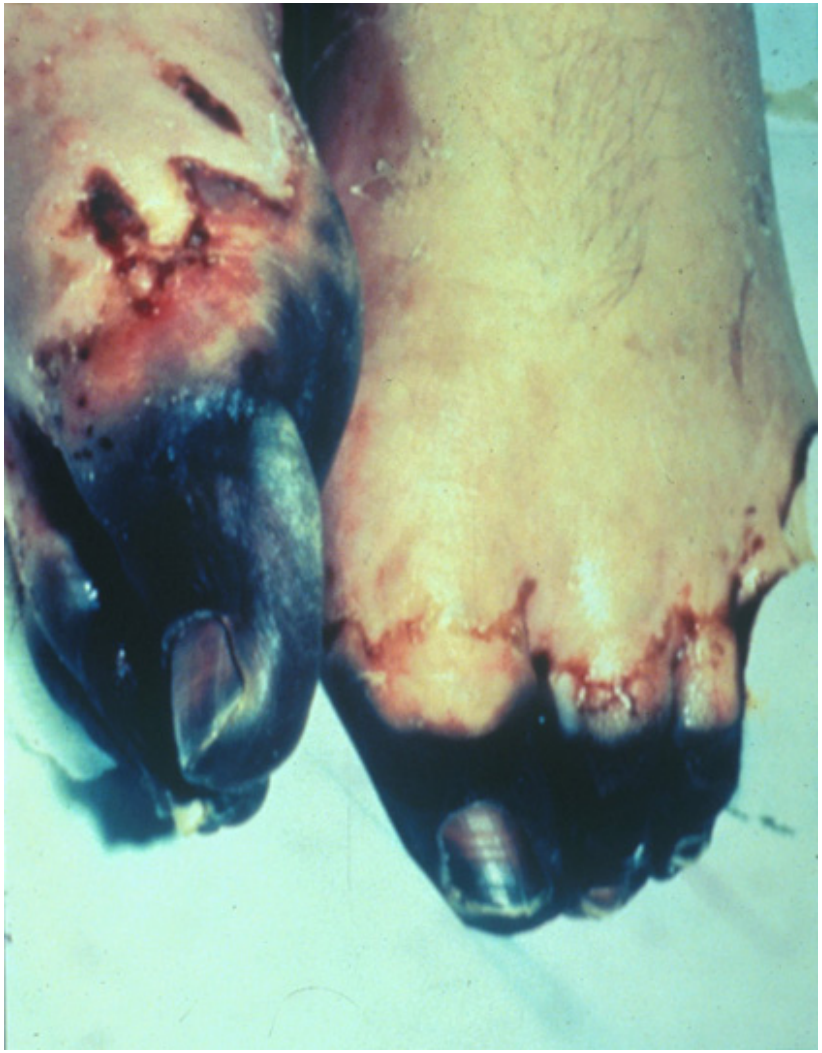


Gangrene resulting from DIC

Photos courtesy of CDC

Septicemic Plague





Pneumonic Plague

- Primary or secondary
- Incubation: 1-6 days (Mean: 2-3 days)
- Acute onset
  - High fever, chills, malaise +/- lymphadenopathy
- Fulminant illness
  - Rapidly advancing tachypnea, dyspnea, hypoxia, chest pain, cough, hemoptysis
  - Cough with bloody sputum (hemoptysis) within 24 hrs
  - Sputum – often purulent, may be blood-tinged or grossly hemorrhagic
- GI symptoms are often present

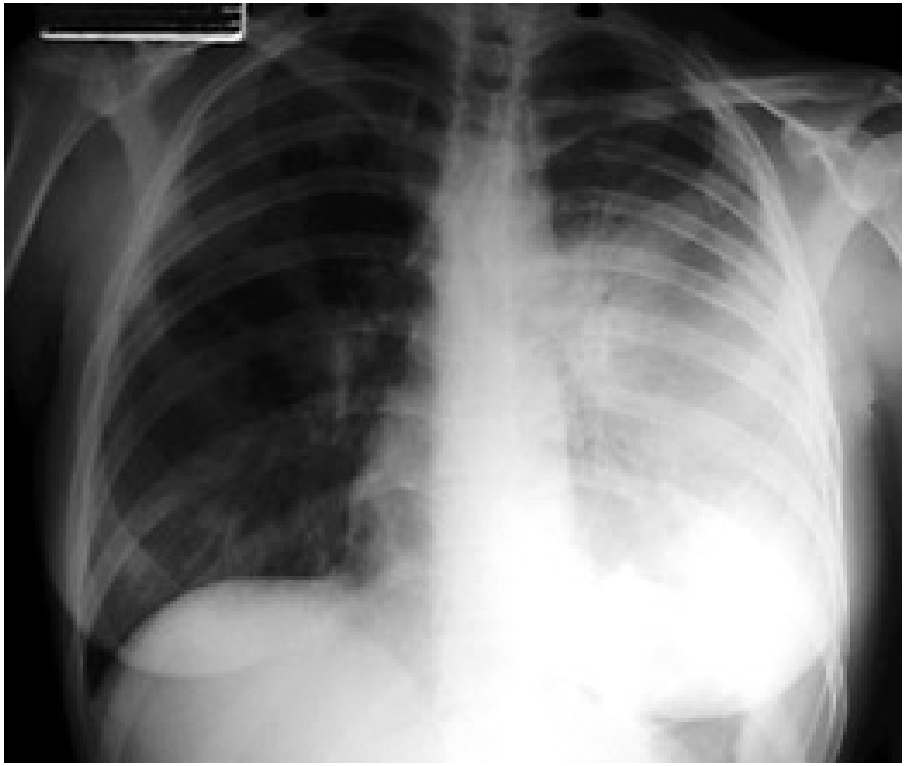
Pneumonic Plague

- Rapidly fatal
  - Respiratory failure, circulatory collapse, bleeding diathesis
  - Most fatal unless treated within 18-24 hrs of infection

- Contagious!! (Respiratory droplet)

Primary Pneumonic Plague

- Chest radiography variable
  - Patchy consolidated bronchopneumonia common
  - Cavities or confluent consolidation also reported
  - Findings may be more impressive than exam would indicate
  - Photo: Lobar consolidation in left lower/mid lung fields



CDC

Plague
Diagnosis

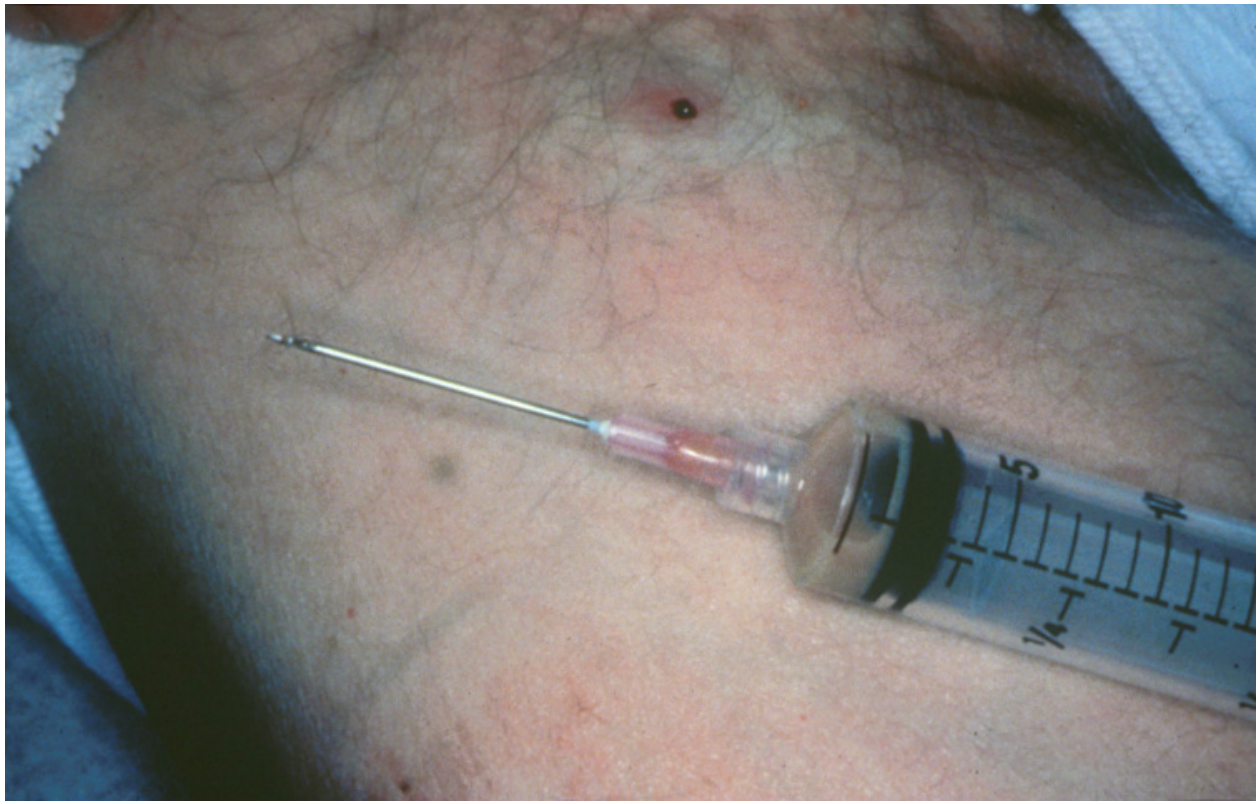
- Acute febrile illness, patient recently in plague endemic zone or known epizootic outbreak
  - Think plague
- Acute rapidly progressing respiratory febrile illness, coughing up blood
  - Think pneumonic plague
  - Investigate for natural vs intentional source
- Bubo aspirates, blood, sputum or CSF
  - Staining: Gram, Wright-Giemsa, Wayson's, DFA
  - Culture
    - BHI broth
    - Agars: sheep blood, chocolate , or MacConkey

Pneumonic Plague Diagnosis (continued)

- Antigen capture assays
- Antibody serology
  - ELISA: IgM & IgG
    - Can differentiate early infection from previous vaccination
    - Presumptive
  - Passive hemagglutination test (PHA): capsular F1-Ag
    - Acute or convalescent sera
    - Single titer 1:16 presumptive, 1:128 diagnostic
  - Direct Fluorescent Antibody (DFA): capsular F1-Ag
    - Very specific staining for use on smears of fluids or cultures

Lymph Node Aspiration

- May alleviate pain
- May aid diagnosis
- Incision and drainage not recommended

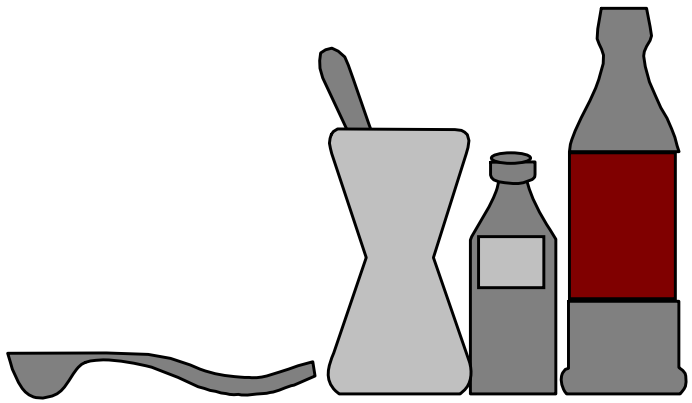


Treatment of Plague (Adult)
All Forms

- Parenteral antibiotics recommended initially
  - Streptomycin (old favorite) 1gm IM bid, or
  - Gentamicin 5 mg/kg IV daily, or 2mg/kg loading dose then 1.7 mg/kg IM or IV q8h, or
  - Doxycycline 100 mg IV q12h or 200 mg IV daily, or
  - Ciprofloxacin 400 mg IV q12h
- Switch to oral antibiotics after appropriate clinical improvement
- Duration of Rx: 10-14 days

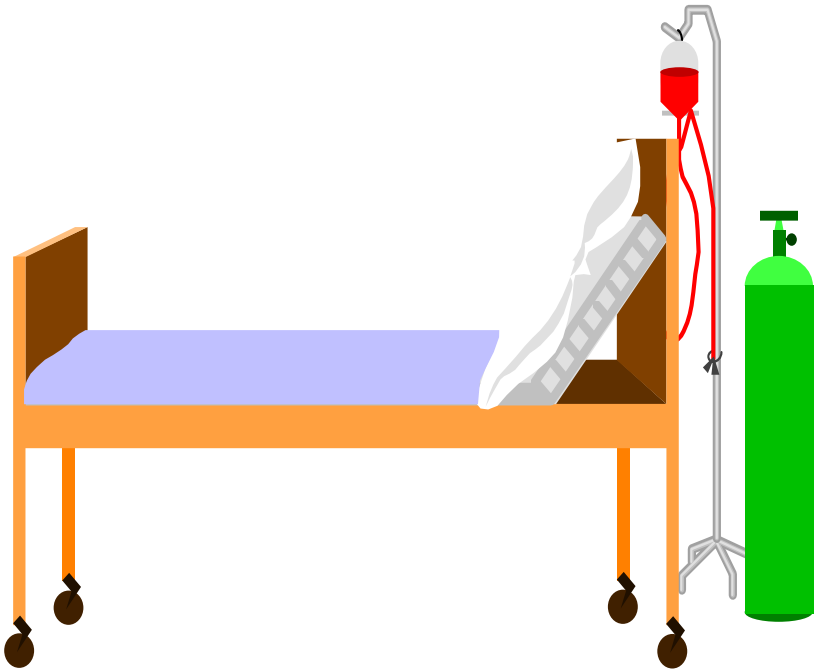
Treatment of Plague Meningitis

- Antibiotic therapy - Meningitis
  - Chloramphenicol - Treatment of choice
    - Adults: 25 mg/kg IV loading dose then 15 mg/kg IV q6h
    - Pediatric: same, except do not use in children < 2 years of age
    - Adjust dose to maintain adequate serum levels
    - Best tissue penetration
    - Best in hypotension
  - Alternatives
    - Streptomycin or Gentamicin



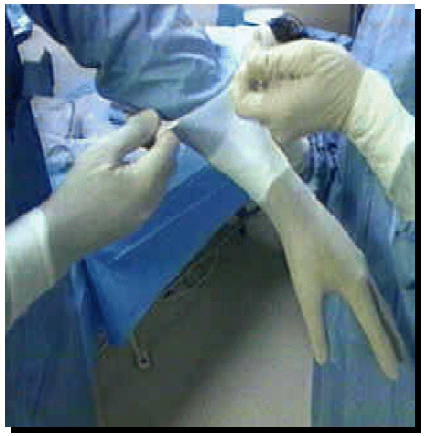
Treatment of Plague

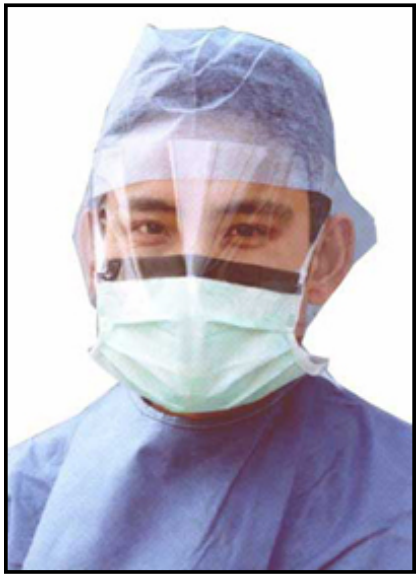
- Common supportive therapy
  - I.V. crystalloids
  - Hemodynamic monitoring
  - Supplemental oxygen
- Rarely needed
  - Heparin
  - Pressor agents



Plague
Infection Control

- Standard precautions PLUS:
- Suspect pneumonic plague:
  - Droplet precautions
  - Until 48-72 hrs of appropriate antibiotics
- Confirmed pneumonic plague:
  - Droplet precautions
  - Until sputum cultures negative
- Aspirate (do not “I & D”) bubo!





MMWR 1996;45:RR-14

Plague
Approach to Contacts & Control

- Promptly report all suspected cases to public health authorities (all forms)
- Bubonic
  - Evaluate for symptoms/signs, “fever watch”
  - If flea source, use topical insecticide and environmental controls
  - Chemoprophylaxis NOT indicated for asymptomatic bubonic plague contacts
- Pneumonic
  - Surveillance, chemoprophylaxis for 1 week

Plague
Post-exposure Prophylaxis

Indications	Duration of Treatment	Antibiotics
Face to face contacts (≤2 meters) of pneumonic case	7 days	Preferred: Doxycycline 100 mg orally BID Alternatives: Ciprofloxacin 500mg orally BID Chloramphenicol 25mg/kg orally QID Others: Other tetracyclines, fluoroquinolones TMP/SMX if susceptibility tests allow
Suspected exposure to plague aerosol	Duration of risk of exposure plus 7 days	

Plague Prevention & Control

- Minimize contact with rodents
  - Rat-proof dwellings in endemic areas
  - Store food and water in rodent-proof containers
  - Appropriate storage and disposal of garbage
  - Avoid rodent burrows
  - Do not handle rodents
- Minimize contact with fleas
  - Shoes and garments to cover legs
  - Repellents and insecticide
  - Treat dogs and cats in endemic areas periodically with insecticide

Plague Prevention & Control

- Public education and personal protective measures
- Quarantine
  - Plague is one of only three WHO reportable diseases
  - Countries required to report plague to WHO in 24 hrs

Plague Prevention & Control - Vaccines

- Plague Vaccine U.S.P. – developed for US Army in 1942
  - Formalin-killed live vaccine previously used in laboratory workers and extensively in military serving in Vietnam
  - Manufacture discontinued (1999) – Still licensed, may be produced in other countries
  - Did not protect against respiratory exposure (pneumonic plague)
  - Unfriendly dose schedule
    - Three doses (1, 0.2, and 0.2mL) series at 0, 1-3 month, and six month
    - Additional booster every 1-2 years
  - No utility in combating epidemic disease
    - Modern improvements in hygiene, sanitation
    - Availability of effective prophylactic antibiotics

Plague Prevention & Control - Vaccine Research

- Research ongoing for vaccines effective against respiratory exposure
  - Candidates:
- Two new plague vaccine candidates the utilize the F1 and V antigens of *Y. pestis* have been developed
- F1-V a recombinant fusion protein expressing the F1 and V antigens was developed by Army scientists at USAMRIID
- A similar vaccine developed at Porton Down (U.K.) and derived from the USAMRIID recombinant clones is a recombinant protein based vaccine, consisting of two separate proteins
- F1-V has been shown to protect African green monkeys from pneumonic plague
- Both of these vaccines are in clinical trials and one may be selected for further development as a human vaccine candidate against plague

Plague Prevention & Control - Vaccine Research

- Novel plague vaccine delivery methods thus far studied include:
  - Microencapsulation via the nasal route
  - Recombinant live, attenuated *Salmonella* spp. as a delivery mechanism
  - Aerosolization

Key Points - Plague

- Natural epi – zoonosis, flea vector, mammal reservoir
- Bubonic plague has a bubo!
- Septicemic plague causes clots in distal vessels
  - "Black Death"
- Pneumonic plague may be primary or secondary
  - Hemoptysis - Y. pestis can cause extensive, fulminant pneumonia with bloody sputum in an otherwise healthy person (usually within 24 hours)
  - No buboes - consider intentional release, particularly if large number cases or no idenitifiable natural exposure
- Contagious – use respiratory (droplet) precautions

Questions?

USAMRIID

Food and Waterborne Terrorism and Agroterrorism

COL Zygmunt F. Dembek, MS

PhD, MS, MPH

USAMRIID, Fort Detrick, MD

May 2008

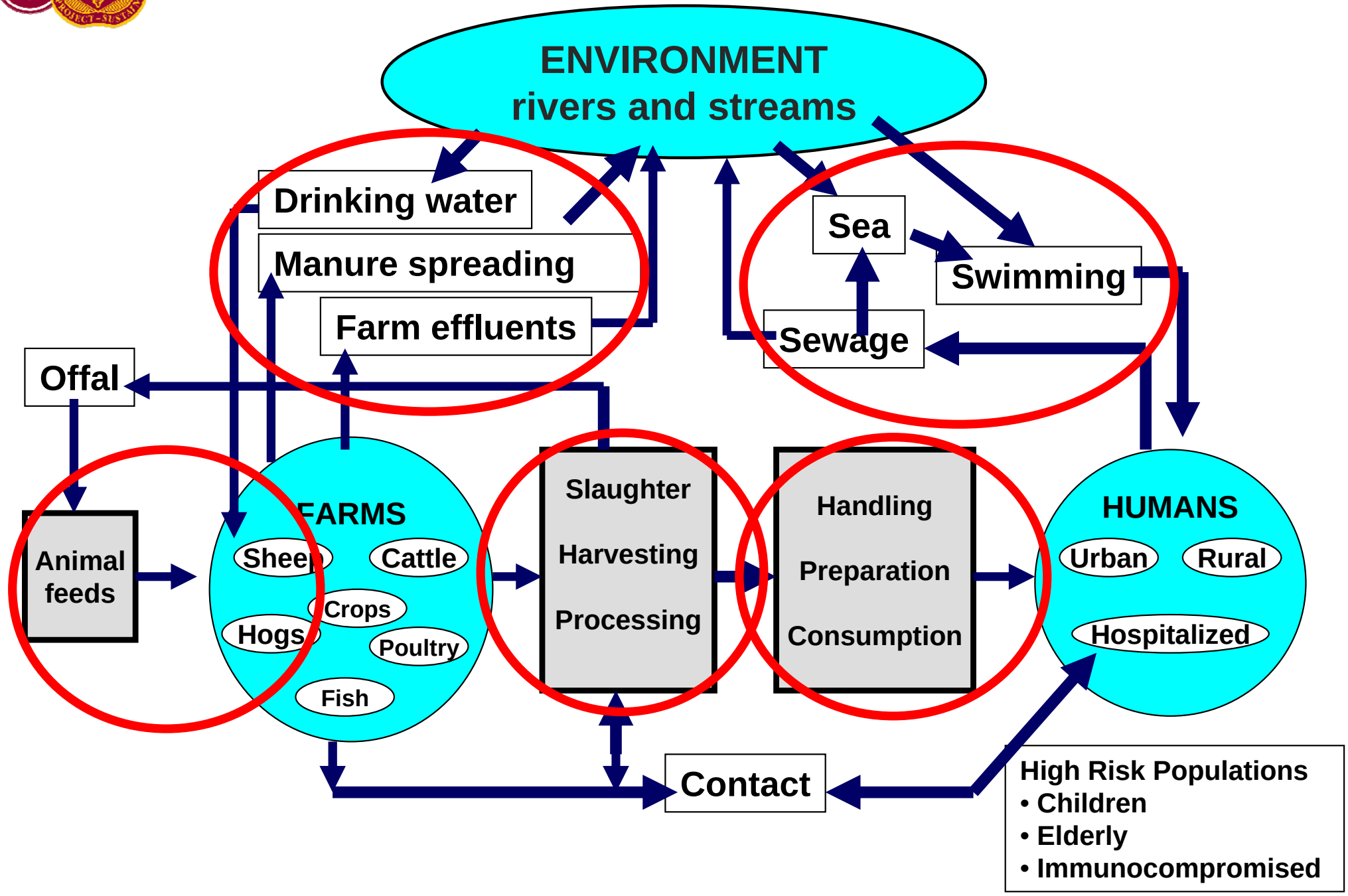
Current News Excerpts…

- FBI terrorist warning: Plots to use nicotine and solanine to poison food, water and beverages
- WHO warning: Terrorist groups threatening to contaminate food supplies
- Food supply vulnerable to terrorist attacks as government can’t ensure processing plant security
- Agents held in plot to poison water supply that serves troops in Middle East
- Worldwide alert: 4 men suspected to be involved in plot to poison food and water supplies

Main Points / Overview

- Ingestion works as a route of entry
  - Contaminated food and water
  - Recreational waters
- Simple, successful
- Increased threat, chatter
- US Agriculture vulnerable → agro-terrorism
- Vulnerabilities
- Risk Reduction

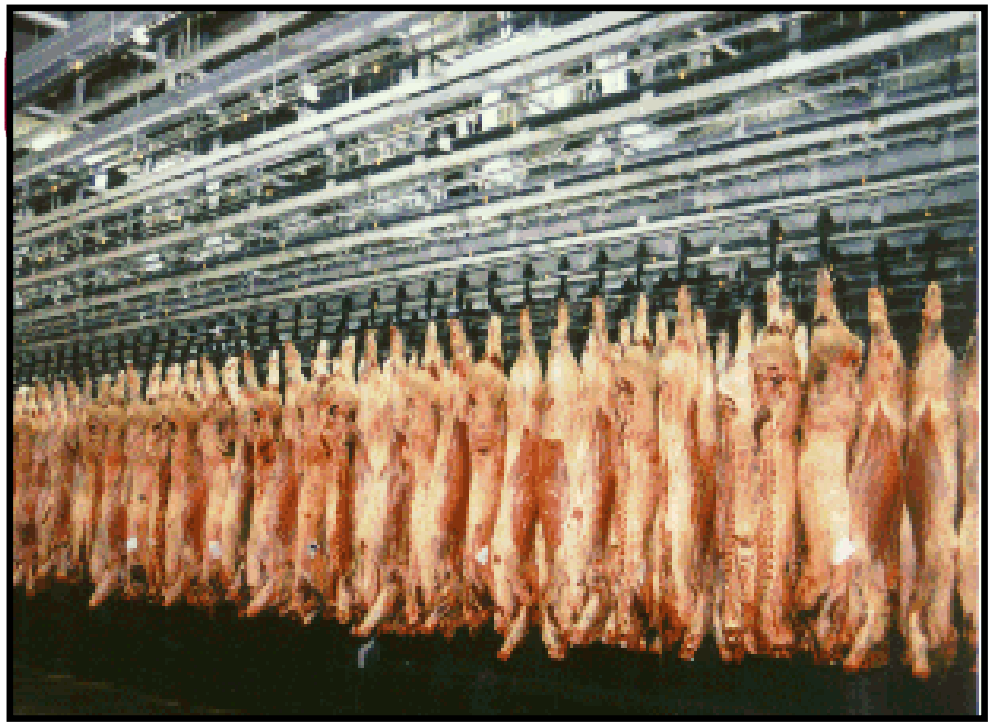
Contamination of the Food Supply

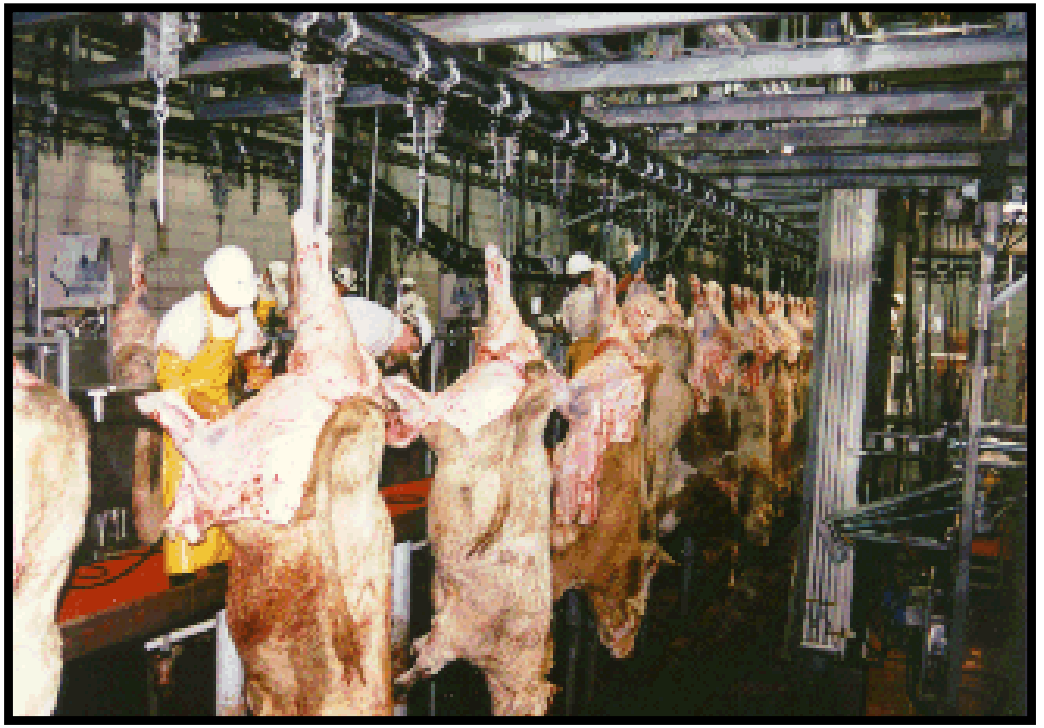


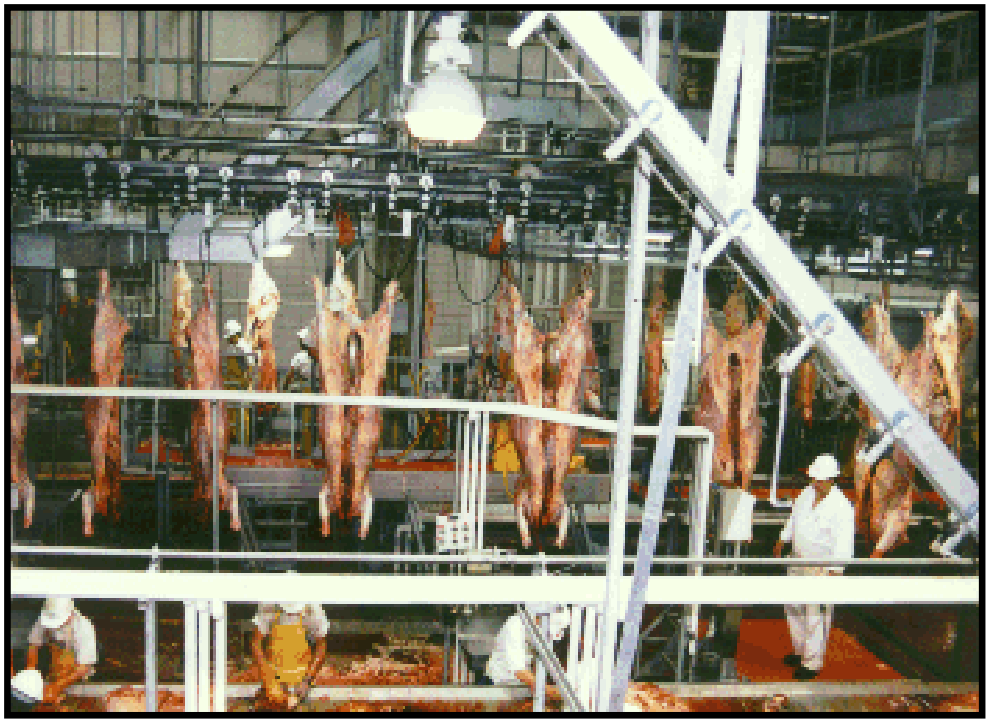
Factors: Economy

Agribusiness = Big Business

- $1 Trillion in economic activity
- 2.8 Million workers
- $60 Billion net farm income
- +$12 Billion to balance of trade
  - $3.5 Billion - Cattle/beef
  - $2 Billion - Poultry
  - $1 Billion - Swine/pork





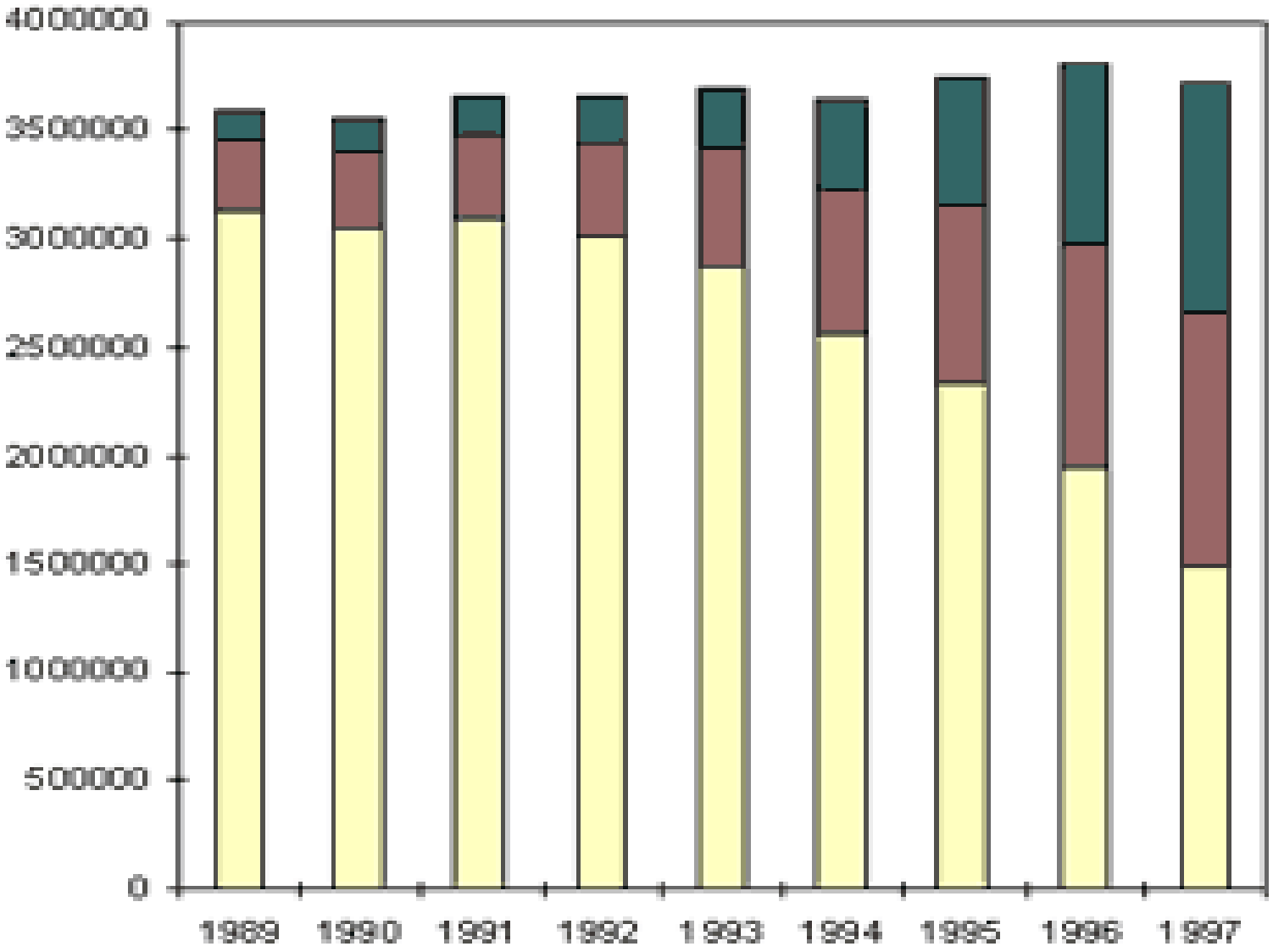


Factors: Imports

- Annual imports – approximately:
  - 1.9 million cattle
  - 700,000 swine
  - 28 million birds
  - 15.3 million tons of grains
  - 18 million tons of fruits and vegetables
  - 56.3 million tons of wine and malt beverages!
- Smuggled birds, meats – being addressed
  - DHHS, DHS, USDA, FDA
  - Longer quarantines, more testing, ‘tighter’ borders

Factors: CAFOs

Increase in Concentrated Animal Feeding Operations

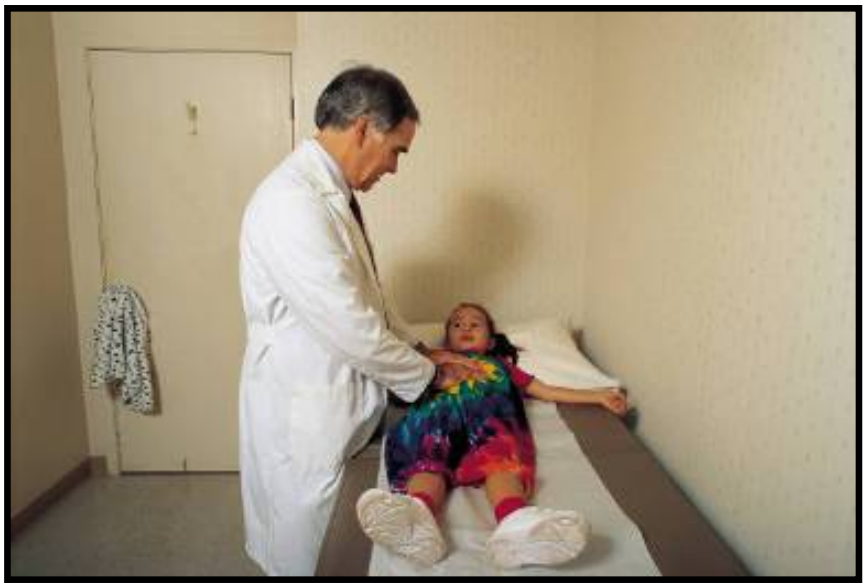


Fewer feedlots

More animals per operation

Factors: Susceptibility

- More than 200 known diseases transmissible through food, water
- Agents: virus, bacteria, toxin, metal, prion, chemical, parasite
- 1million deaths globally
- $3-4 billion in US alone



Naturally Occurring Foodborne Illness

- Listeriosis
- Marine toxin (PSP, etc.)
- *Salmonella* sp.
- *Shigella* spp.
- Trichinellosis
- *Vibrio* spp.
- Viral
  - Noroviruses*
  - Rotavirus
  - Adenovirus
  - Astrovirus
- Amebiasis*
- *Blastocystis hominis*
- Botulinum neurotoxin
- Bovine Spongiform Encephalopathy
- Creutzfeldt-Jakob Disease
- *Campylobacter* spp.
- Cryptosporidiosis*
- Cyclospora infection*
- *Escherichia coli*
- Giardiasis*

*Most common agents associated with US waterborne infections

Higher Impact Foodborne Illnesses (US)

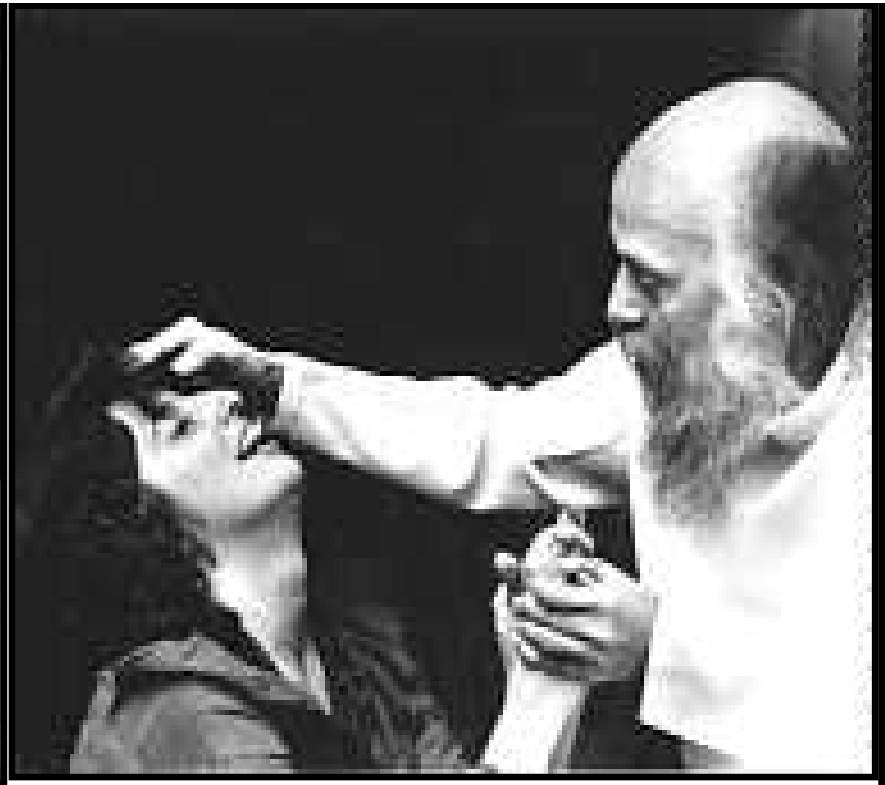
- *Campylobacter* spp.
  - > 1 million/yr, 10 % hospitalized
- *Salmonella* spp.
  - > 1 million/yr, 22 % hospitalized, 1 % CFR
  - Multiple drug resistant strains
- *E. coli* (EHEC and ETEC)
  - > 40,000/yr, 30% hospitalized, 1 % CFR
- *Listeria monocytogenes*
  - 2000/yr, 90 % hospitalized, 20 % CFR

FBD/WBD attraction to a ‘would-be’ terrorist: It’s easy AND it works!

- Ingestion as a route of entry has been successful – unintentional and intentional
- Among reported Biocrime, Biowarfare and Bioterrorism events, ingestion as a route of entry comprised:
  - 17 % of events
  - 91 % of casualties
  - 50 % of deaths

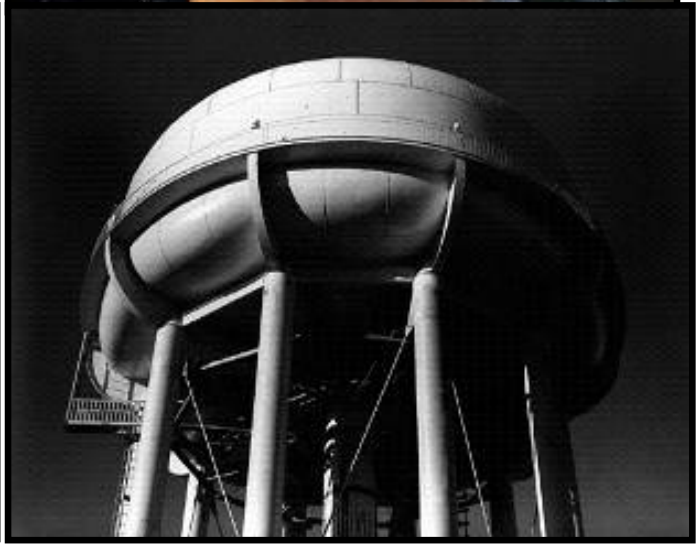
Agro-terrorism: Kenya, Mau Mau poison steers with toxic plant (1954)

BioCrime: Shigellosis, Dallas, Texas, 12 hospital employees (1996)



• Bioterrorism: *S. typhimurium* contamination of 10 salad bar restaurants in a small Oregon town (1984)

•751 reported cases



Potable Water

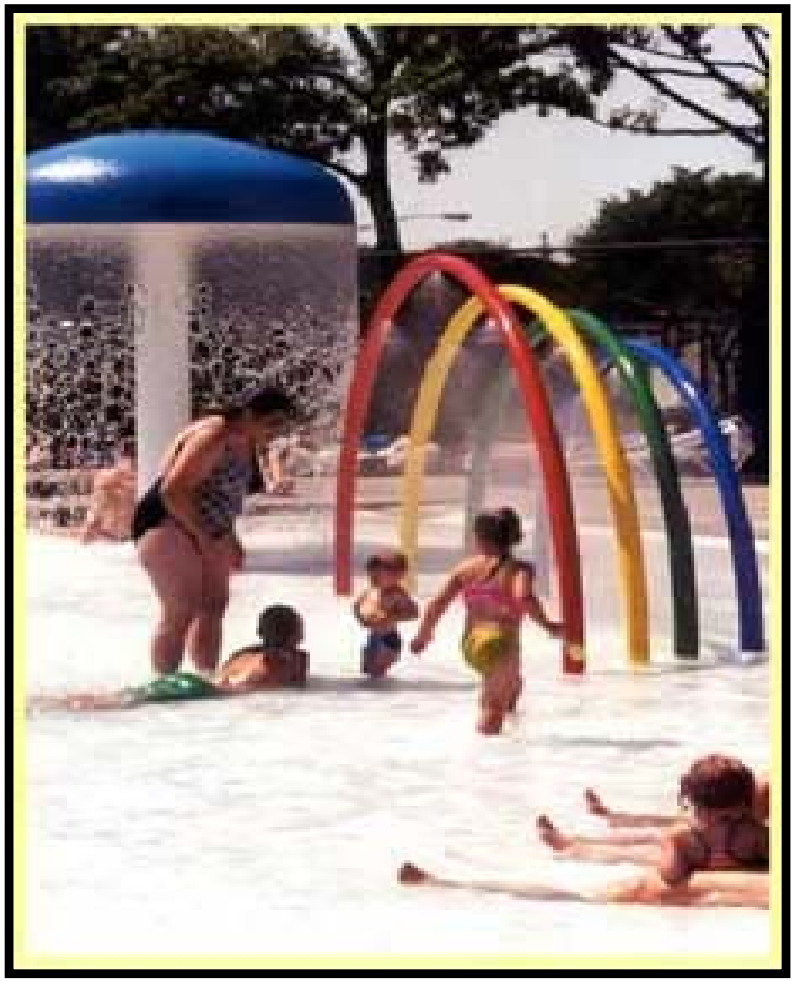
- Effective water treatment methods
  - Exceptions: Toxins, *Cyclospora* sp., Cryptosporidium, Norovirus, anthrax spores
- Significant contamination following attack is unlikely - dilution reduces toxic exposure risk
- Most home / industrial water is not used for consumption
- Potential risk is contamination near end-user after treatment; chlorination no longer effective

‘Classic’ Unintentional Outbreak

- Milwaukee WI, USA spring of 1993
- Outbreak of acute watery diarrhea, abdominal cramps, fever, and vomiting
- 403,000 people affected
- 39% of population
- Cryptosporidium oocysts passed through filtration system of one of city’s water treatment plants

Recreational Water

“Fecal Accident Response: Recommendations for Pool Staff What do you do when you find poop in the pool?”



http://www.cdc.gov/healthyswimming/fecalacc.htm

Interactive Water Fountain Outbreak

- Volusia County FL, USA summer of 1999
- Outbreak of diarrhea, abdominal cramps
- 38 people affected, all attended beachside park from August 15 –September 2
- 8 y.o. median age, used ‘interactive’ water fountain
- Infected with *Shigella sonnei* and *Cryptosporidium parvum*

Recreational Water Illnesses (RWIs)

What are they?

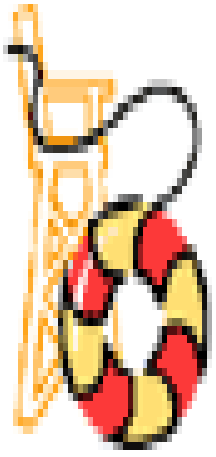
How are they spread?

Why doesn't chlorine kill them?

Where are they found?

Who is most likely to get ill?

How can we prevent them?



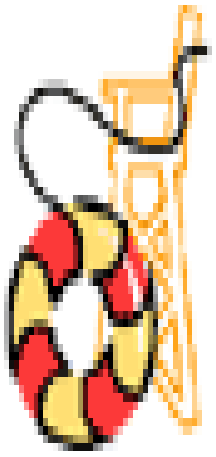
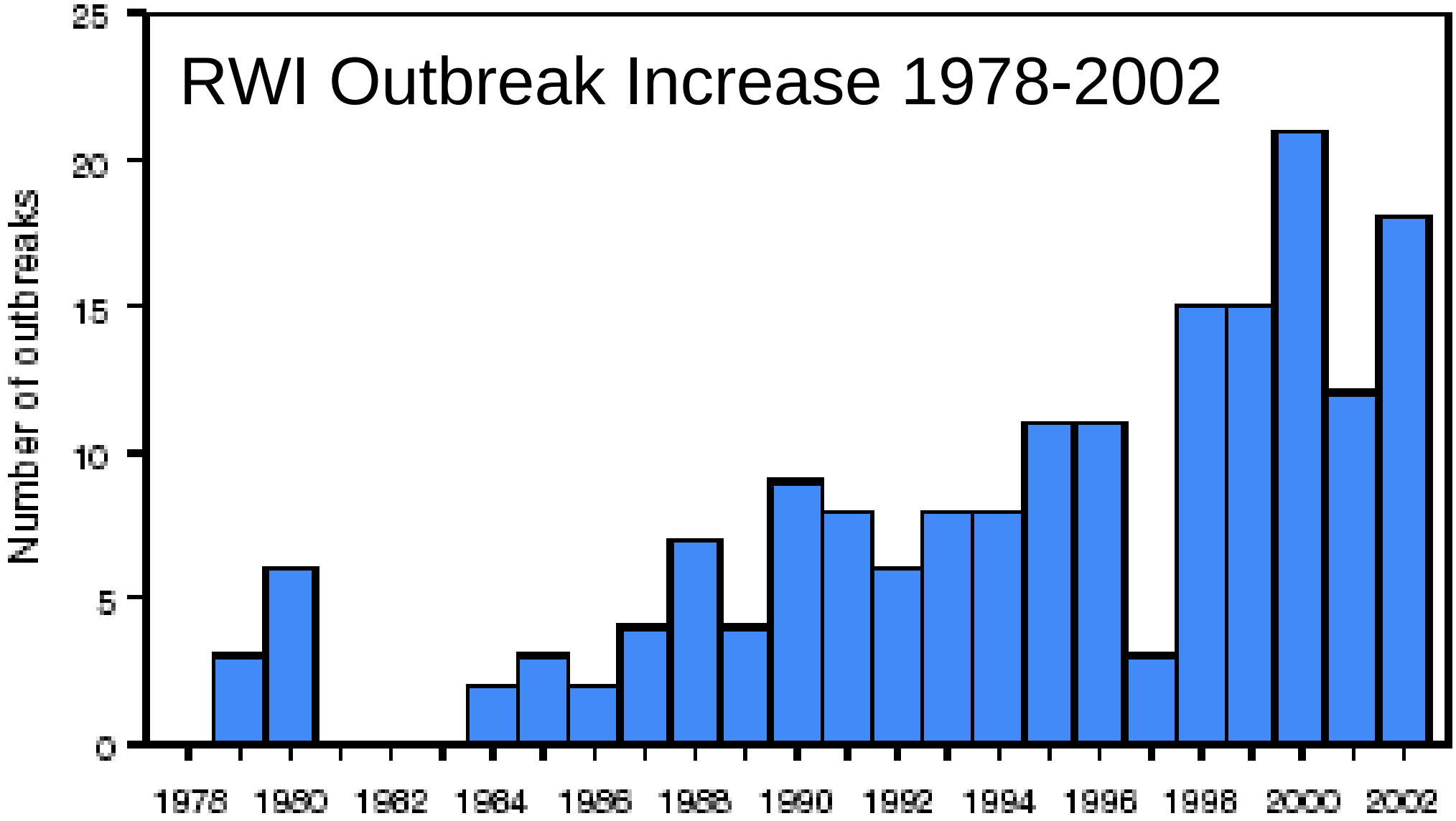
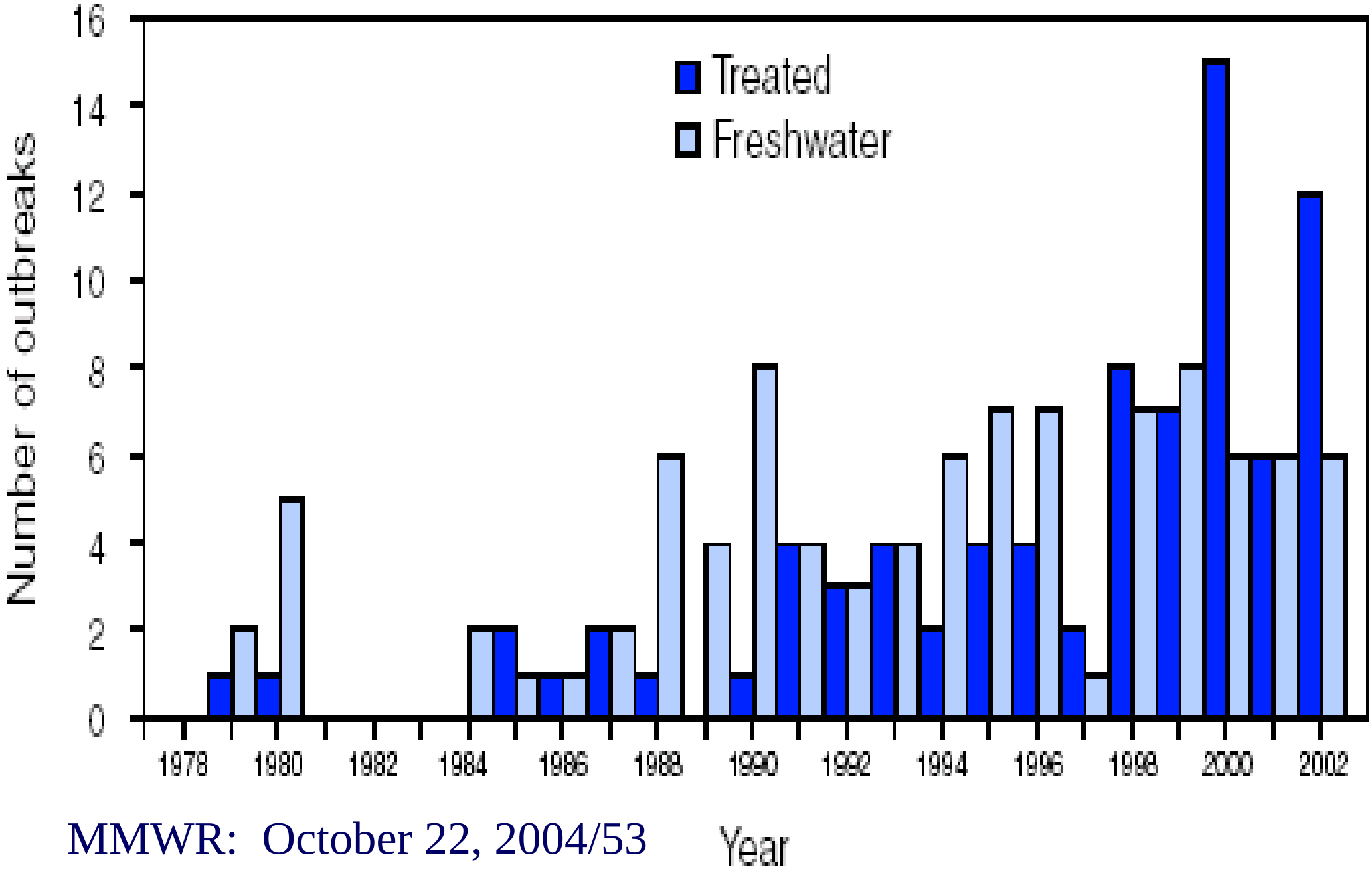


FIGURE 5. Number of waterborne-disease outbreaks of gastroenteritis (n = 176) associated with recreational water, by year — United States, 1978–2002



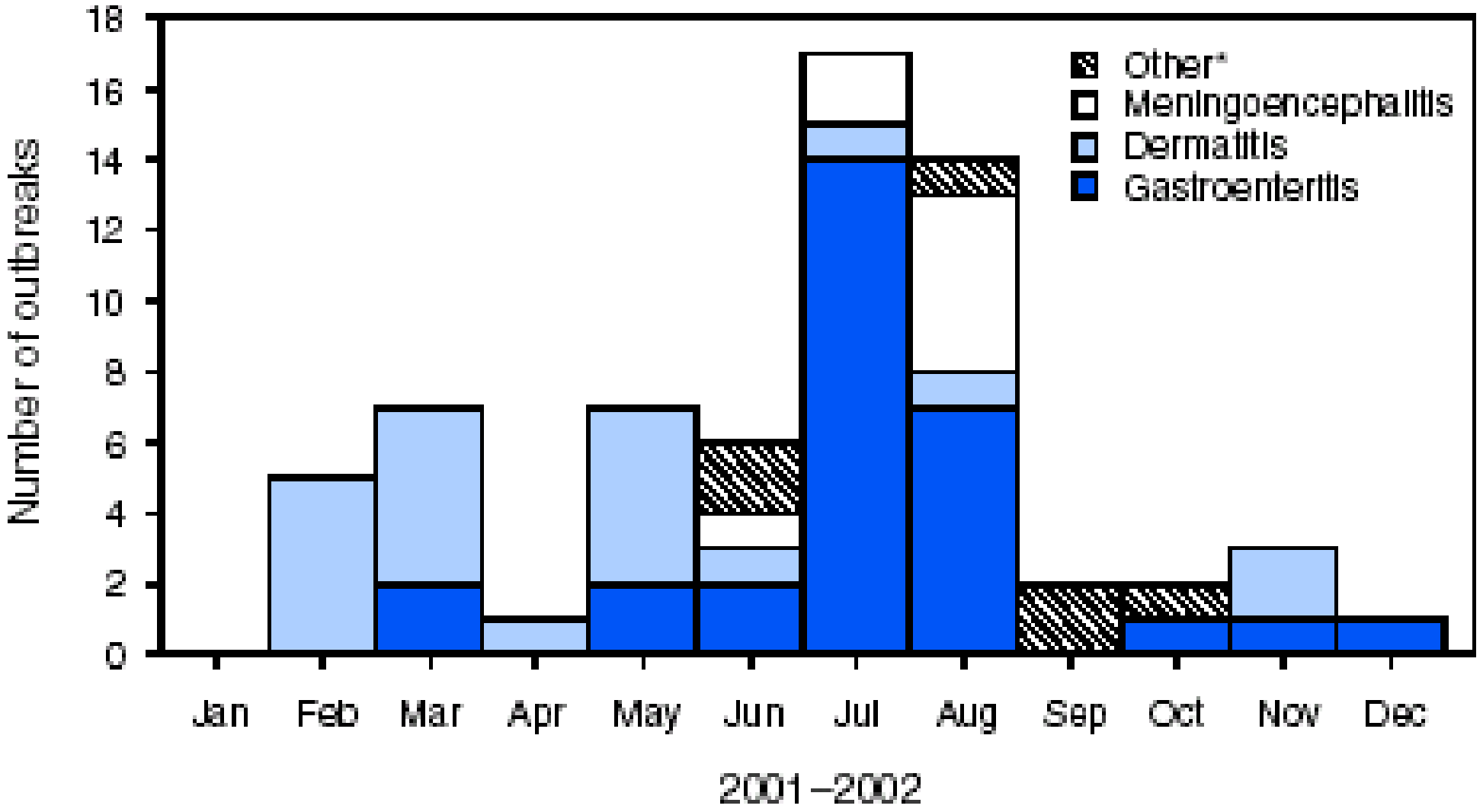
MMWR: October 22, 2004/53

FIGURE 6. Number of waterborne-disease outbreaks of gastroenteritis (n = 176) associated with recreational water, by water type — United States, 1978–2002



MMWR: October 22, 2004/53

FIGURE 2. Number of waterborne-disease outbreaks (n = 65) associated with recreational water, by illness and month — United States, 2001–2002



* Acute respiratory illness, Pontiac fever, or chemical exposure.

Factors: Effect

- 'Mad Cow' effect on economy, beef consumption
  - 40% drop in meat exports
  - "Deficit in Trade Tops $43 Billion" Jan 04
    - Higher oil prices and beef exports were blamed
- Cryptosporidiosis in Milwaukee, WI
  - 39% of population
- Odwalla / E. coli
- Fast food restaurants / E. coli
- Cruises / Norovirus

- Menu
- CANTINA
- Locations
- Special Offers
- Chi-Chi's Kids
- Contact Chi-Chi's
- Employment Opportunities
- Corporate Information
- Press Release
- Nutrition

http://www.chi-chis.com/

We would like to thank all of our loyal customers of the past 27 years and with a tear in our eye, say

¡Adios!

Risk?

- Increased threat –makes news regularly
- Increased preparedness attention, but still little information … spread the word!
- Among CBRNE events ‘E’ most common
- U.S. food supply is safest in the world
  - Continuously emerging mandated prevention strategies
- … … … Risks still exist

Threat Assessment

- Ready-to-Eat foods
  - Raw, or contaminated after cooking
- Use of Toxins
  - Survive cooking
  - ‘Easier’ than culture
- Location
  - Homeland vs abroad

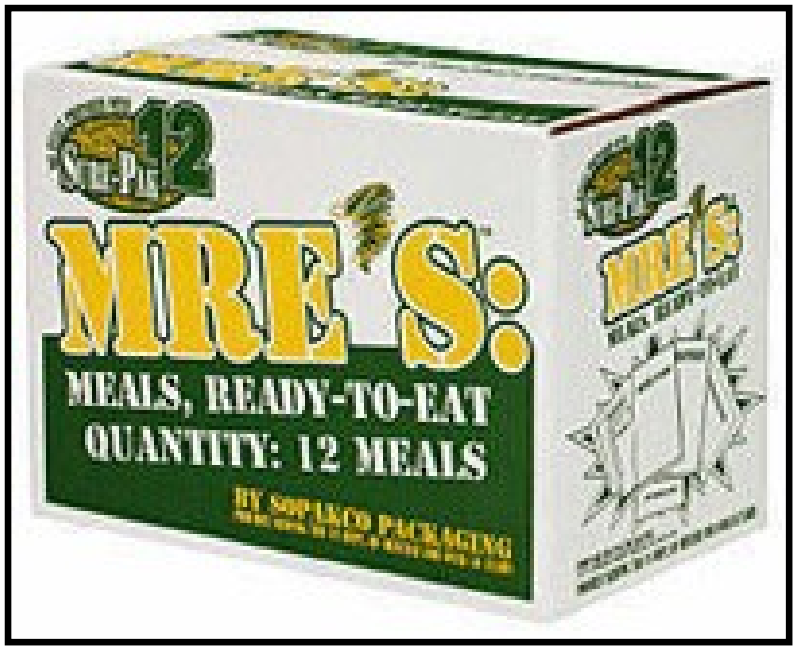
Threat Assessment

- Foods used to feed deployed troops could be attacked before delivery to receiving point

  MRE = PDS

  … pretty darned safe!

- Recent event …



Threat Assessment

- Centralized facilities for process / storage
  - –Attack one facility - could affect millions
- Imported foods attacked before arrival
- Livestock carriers
- Crops, livestock not in constant view

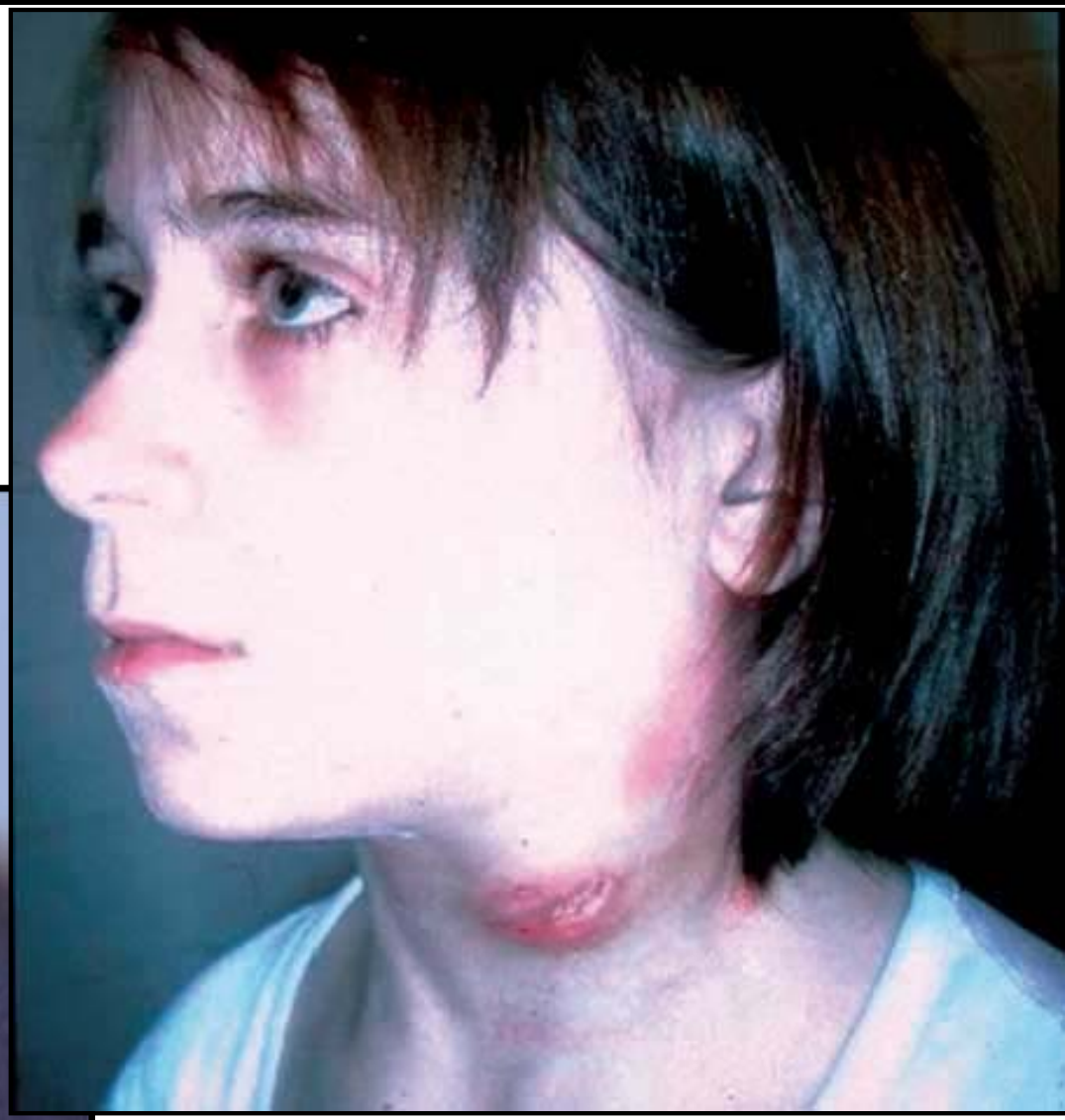
Attack with Cat ‘A’ Agents

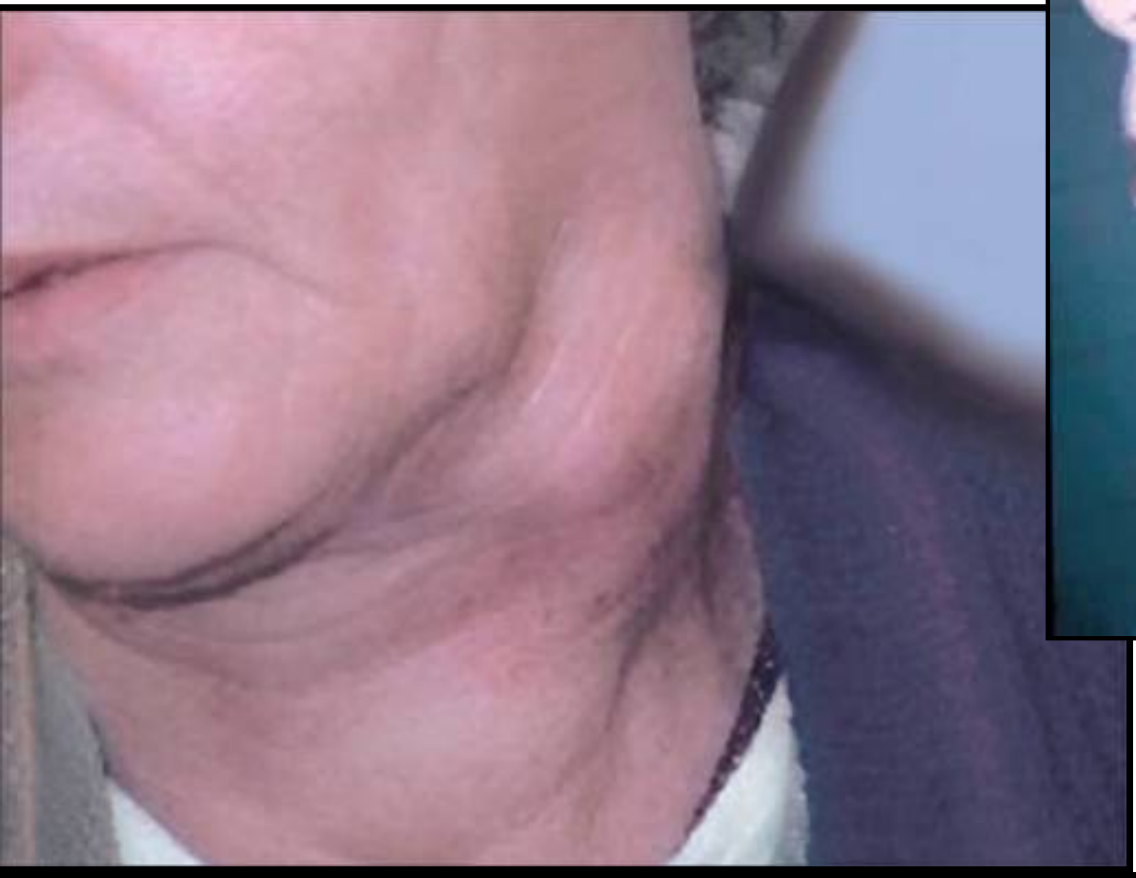
- Outbreak with significant proportion of GI, laryngeal, oropharyngeal forms of disease
  - Anthrax: gastrointestinal, oropharyngeal, septic
  - Plague: pharyngitis, laryngitis, GI symptoms
  - Tularemia: ulceroglandular neck, pharyngeal, GI
  - Ricin: gastrointestinal vs respiratory
  - ‘Classic’ forms are also possible
- Botulism: cluster patterns
  - Common food versus common aerosol source

Foodborne Plague?

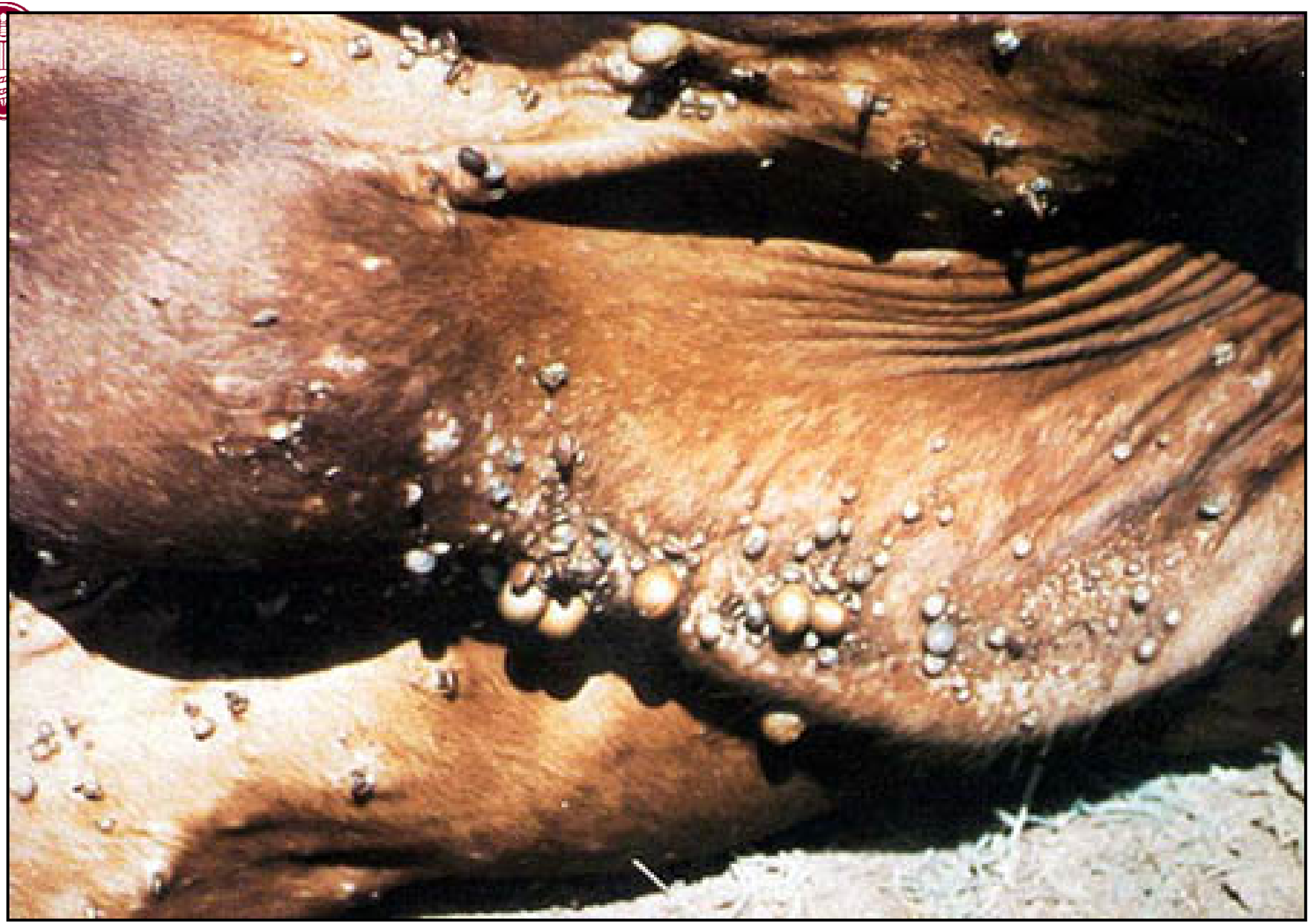
Tularemia

Tularemia Outbreak Investigation in Kosovo *EID* 2002;8,1 p 70





Tularemia as a Biological Weapon *JAMA.* 2001;285: p 2767



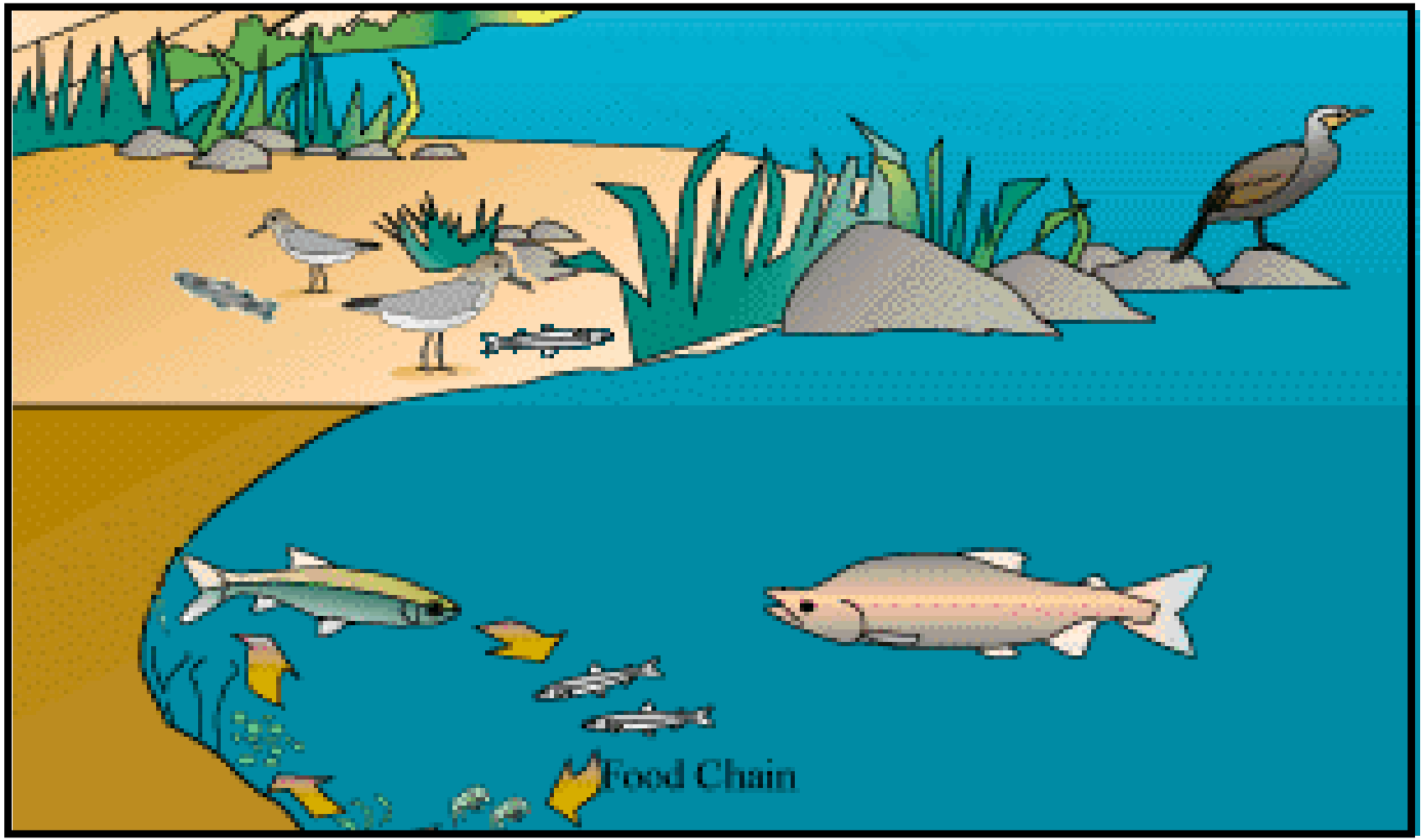
Outbreak? Look for tick-infested sheep, dog

Natural (unintentional) Infections

- Livestock problem
  - Silage, hay, grass clippings, dead animals in feed
  - Several sensational, devastating outbreaks
  - 1998 CA: 427 Holstein cattle dead in 2 days
    - Traced to one bale of hay with BoNT + dead cat
- Type C, E wildlife problem (occasionally human)
  - Great Lakes, Florida, California, UK, Canada, Greece
  - Fish, birds, others through food chain
  - Tens of thousands of wildlife deaths annually

"Can I go in first?"

Type C and E Lake-associated



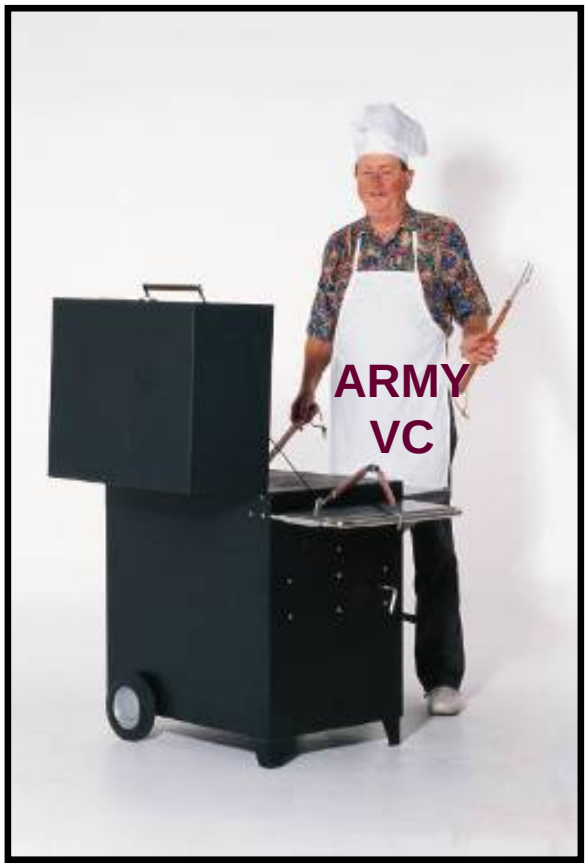

Ontario
Quebec
Superior
Minnesota
Wisconsin
Michigan
Huron
Michigan
Ontario
New York
Erie
Pennsylvania
Illinois
Indiana
Ohio
Image © GLIN

BoNT

Ricin ingestion

How to Decrease Vulnerability?

- Food safety from farm to fork
  - Domestic, deployed
  - Trusted sources*
  - Watch RTE foods
  - Traceback
- Index of suspicion for providers
  - Know background noise
  - Partner with Public Health
  - Rapid reporting
- Resources



Resources: LRN / Diagnostic

Every agent has lab info via CDC BT webpage

- http://www.bt.cdc.gov/agent/agentlist.asp
- http://www.bt.cdc.gov/labissues/
  - "Specimen Selection" table is outstanding:
  - http://www.bt.cdc.gov/documents/PPTResponse/table2specimenselection.pdf

Resources: Food Specific

Diagnosis and Management of Foodborne Illnesses: A Primer for Physicians – online:

- http://www.ama-assn.org/ama/pub/category/3629.html
- http://www.foodsafety.gov
- http://www.fsis.usda.gov/OA/consedu.htm


Questions?

USAMRIID

Toxins: Characteristics and Implications for Medical Defense

Lesson Objectives

- Identify the major differences between biological and chemical agents.
- Evaluate the potential exposure scenarios to include potency, availability, stability, and impact of toxins.
- Communicate important considerations for diagnosis, prophylaxis, and therapy of toxin exposure.
- Identify the epidemiology, clinical features, and medical management of botulinum neurotoxins, SEB, ricin, and cyanobacterial toxins.

Toxins

- Products of living organisms which produce adverse clinical effects on humans, animals, or plants

- Differ from chemical agents
  - *Source*
  - *Physical Characteristics*

Toxins vs. Chemical Agents

Toxins

- Natural origin
- Production difficult
  - An art
- None volatile
- More toxic than many chemicals
- Few dermally active

Chemicals

- Man-made
- Production difficult
  - Industrial
- Many volatile
- Less toxic than many toxins
- All dermally active

Toxins vs. Chemical Agents

Toxins	*Chemicals*
• Legitimate medical use	• Use only as weapons
• Odorless and tasteless	• Odor and taste
• Diverse toxic effects	• Fewer types of effects
• Effective immunogens	• Poor immunogens
• Aerosol delivery	• Mist/Droplet delivery

What Must We Protect Against?

Scenarios for use:

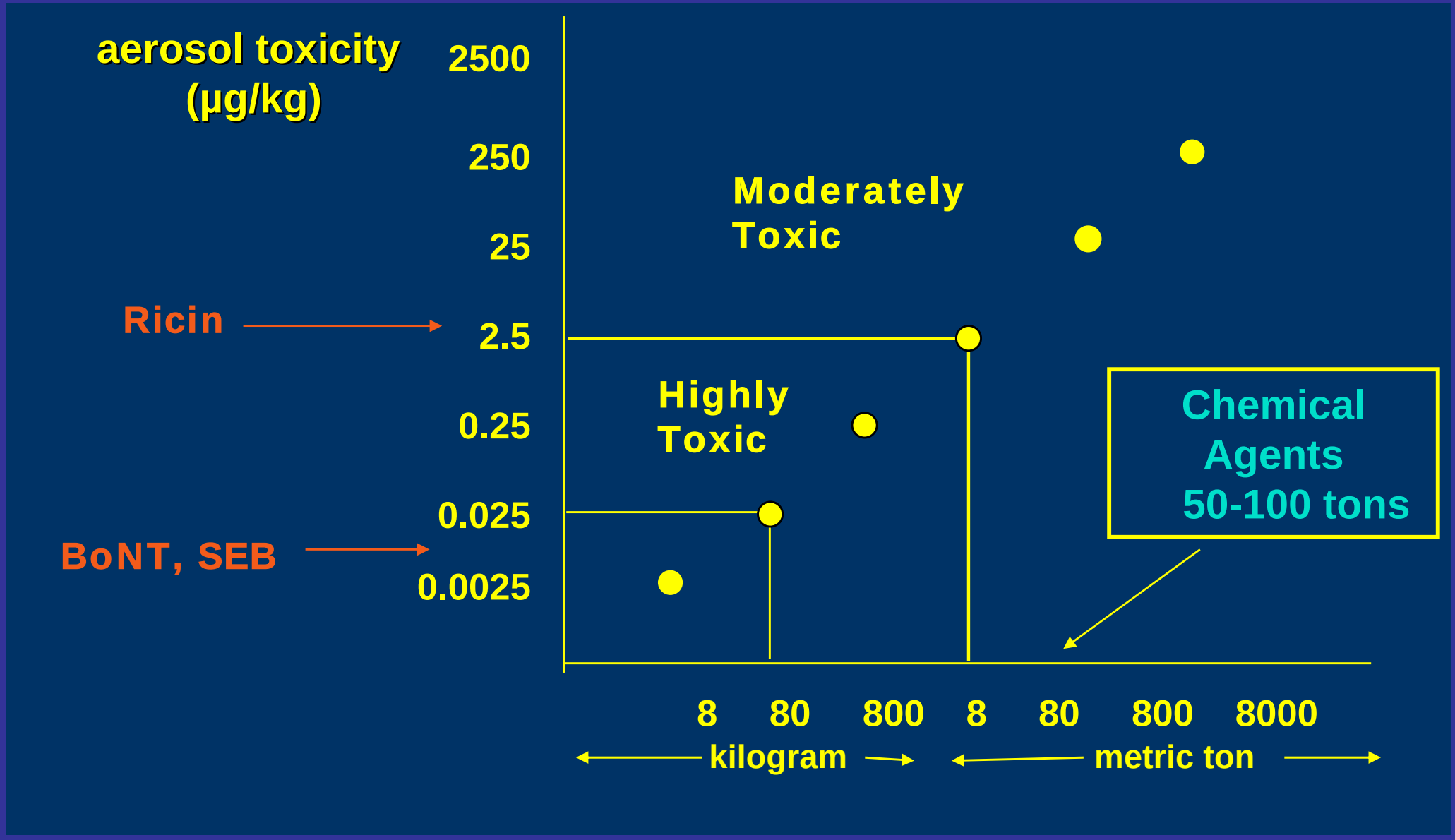
Open air line or point source delivery

Limited air delivery applications

Limited delivery in water supplies

Direct application (assassination)

Toxicity Limitations



Comparative Lethality of Toxins and Chemical Agents in Mice (ug/kg)

Botulinum toxin, SEB (human)	0.01- 0.02
C. perfringens toxins	0.1-3.0
Ricin	2.0-5.0
VX	15.0
SEB (monkey)	25.0-30.0
Soman, sarin	60.0-100.0
T-2	1000.0

Availability

- Plant Toxins
- Bacterial Toxins
- Marine Toxins
- Peptide Toxins

Stability

- UV light
- Water
- Bacterial decomposition

Medical Defense Against Toxins

Prophylaxis

- *Physical protection*
- *Active immunization*

Treatment

- *Diagnostics*
- *Passive immunotherapy*
- *Chemotherapy*
- *Supportive care*

Diagnosis

- Consider toxins

  May be mixed with other agents or chemicals

- Epidemiology

  Tightly clustered cases

- Lab specimens

  Blood - clot, spin, and freeze if possible

  Skin and nasal swabs, urine, feces

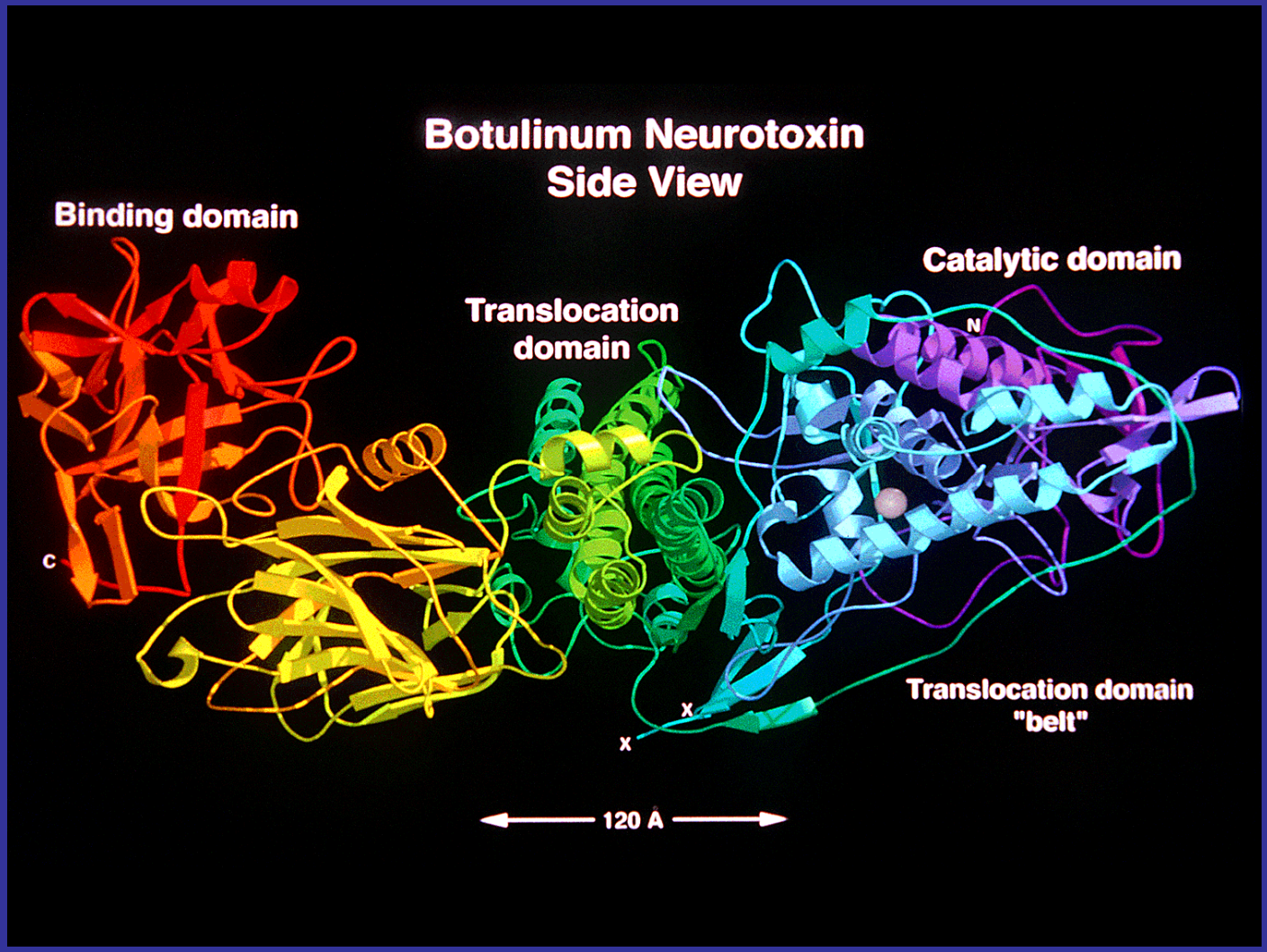
Prophylaxis and Therapy

- Prevention:
  Physical protection
  Vaccination
- Decontamination:
  Not a major problem (non-volatile aerosols)
  Risk to health-care providers minimal
- Don’t assume all casualties = mortalities
- Symptomatic care useful for some intoxications

Toxins of Concern

- Botulinum Toxin
- Staphylococcus Enterotoxin B
- Ricin
- Cyanotoxins

Botulinum Toxin



Botulinum Toxins: History

- Produced by *Clostridium botulinum*
  Gram-positive anaerobic bacillus
- Numerous cases of botulism related to improper food preparation and canning
- Toxicity Allows Variable Application
  LD50 = 1-2 ng/kg (i.v)
  Aerosol slightly less toxic (10-13 ng/kg)
- Significant threat to U.S. forces
  Easily produced
  Weaponized by several countries

Botulinum Toxins: Epidemiology

- Food-borne (24 / yr)
  - *Incubation 12 - 36 hours*
  - *Types A, B, E*
    - *Type A - highest mortality*
    - *Type B - lowest mortality*
- Infant (71 / yr)
  - *Recognized since 1975*
  - *Age 3 weeks to 8 months*
  - *Nearly all serotype A*

Botulinum Toxins: Epidemiology

- Wound (3 / yr)
  - *Types A and B*
  - *Incubation 4-18 days*
  - *Typically in young boys; single case per outbreak*
  - *Black tar heroin use*
- Inhalation
  - *Incubation 24 - 36 hours*
  - *Does not occur naturally*

Botulinum Toxin: Characteristics

- Seven related toxins (serotypes A to G)
- Most potent naturally-occurring toxins known
- Molecular weight approximately 150 Kd
- 2 polypeptide subunits

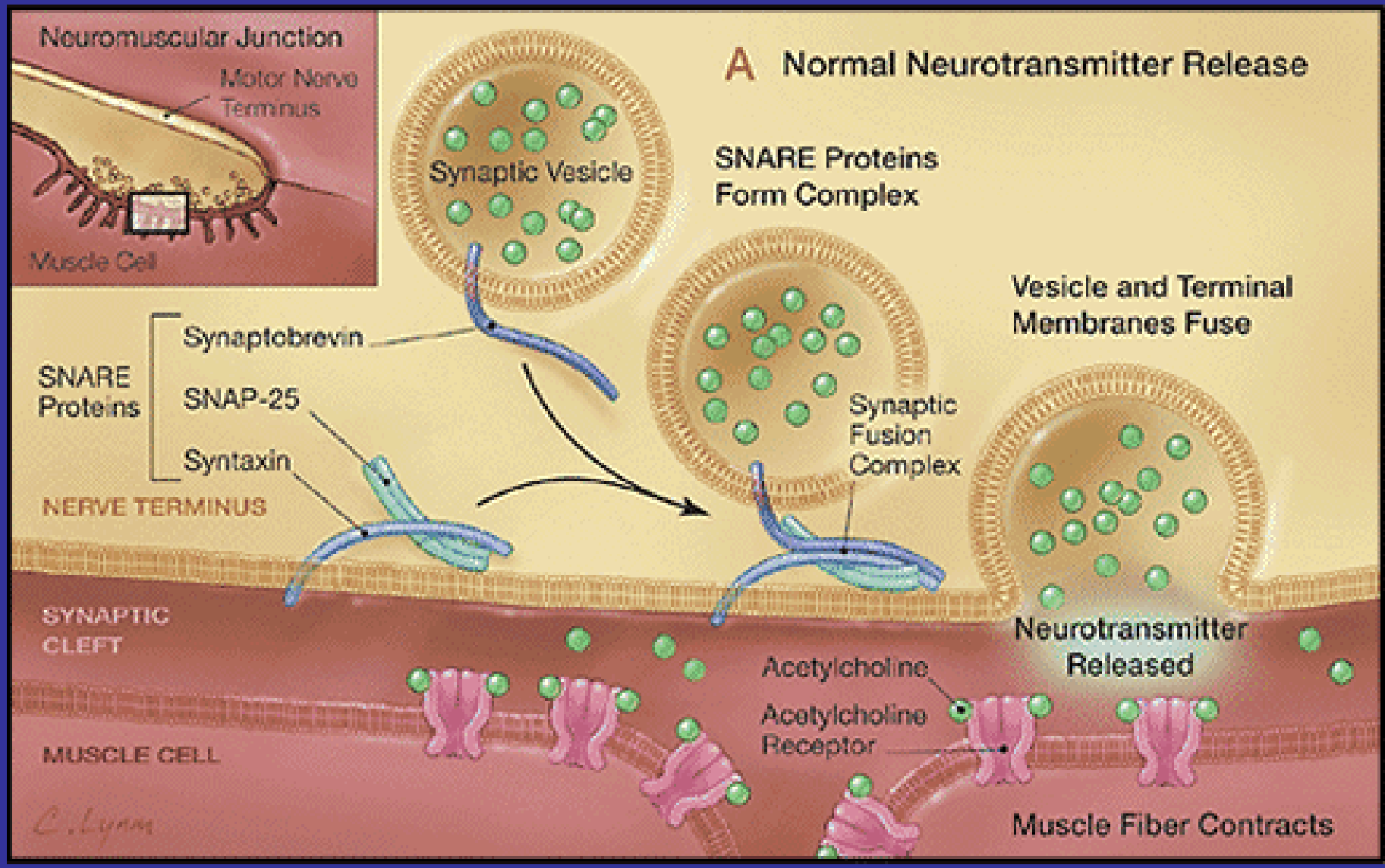
  A chain – exerts cytotoxic effect in the cell

  B chain – binds to axons of motor neurons

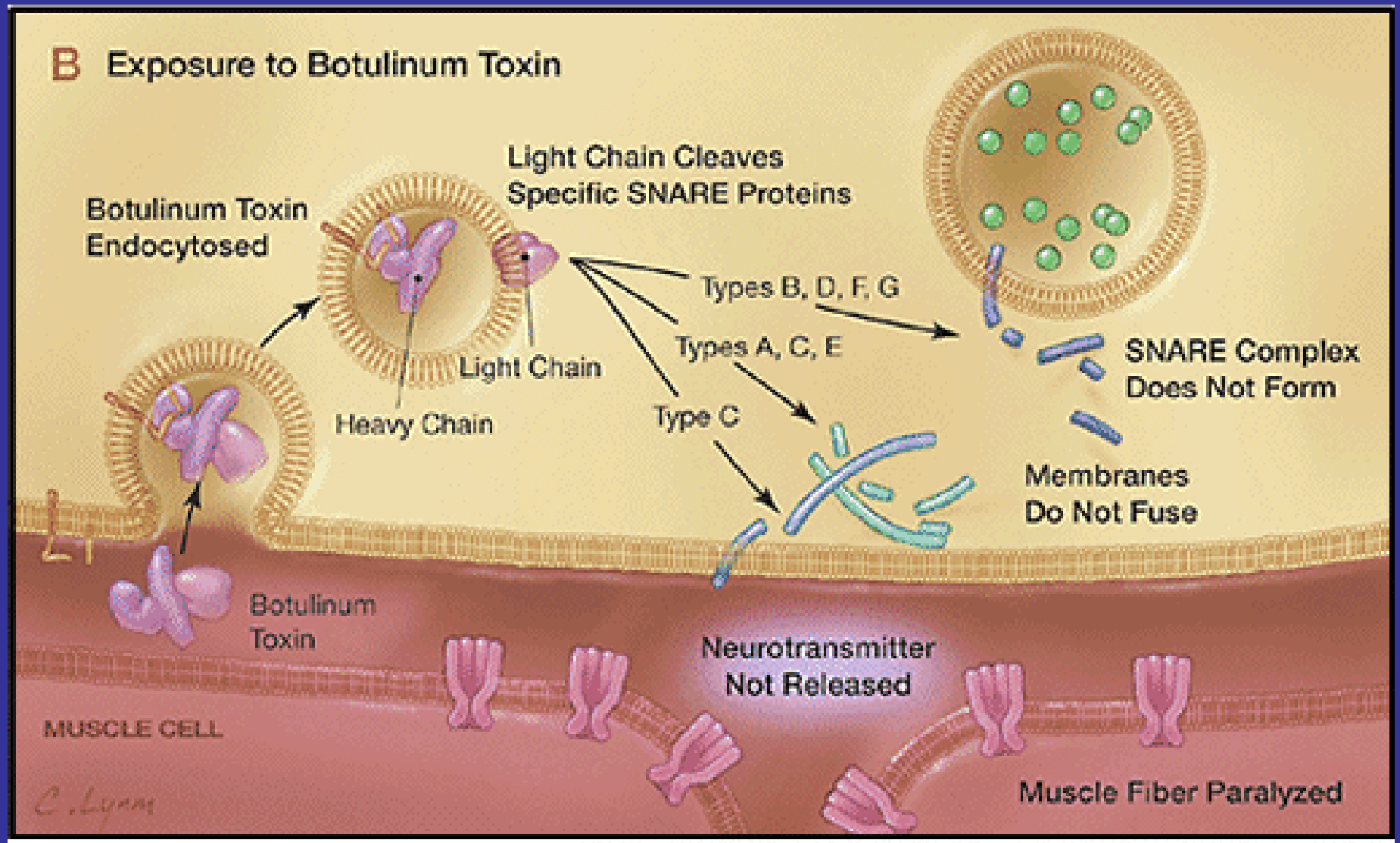
Botulinum Toxins: Mechanism of Action

- Enters pre-synaptic nerve terminal
- Prevents release of acetylcholine at the NMJ
  - *Flaccid paralysis*
  - *Anticholinergic toxidrome*

Botulinum Toxins: Mechanism of Action

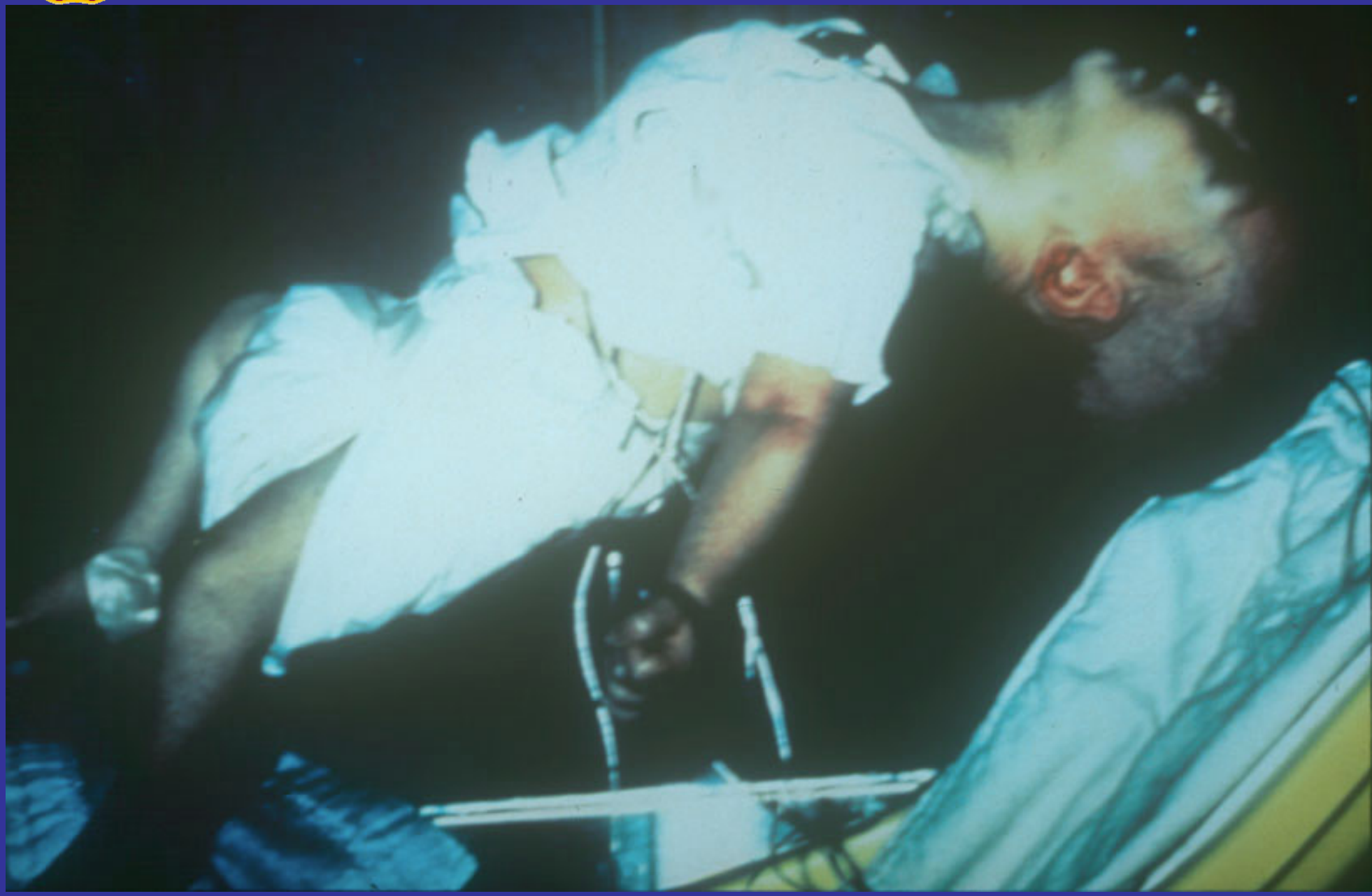


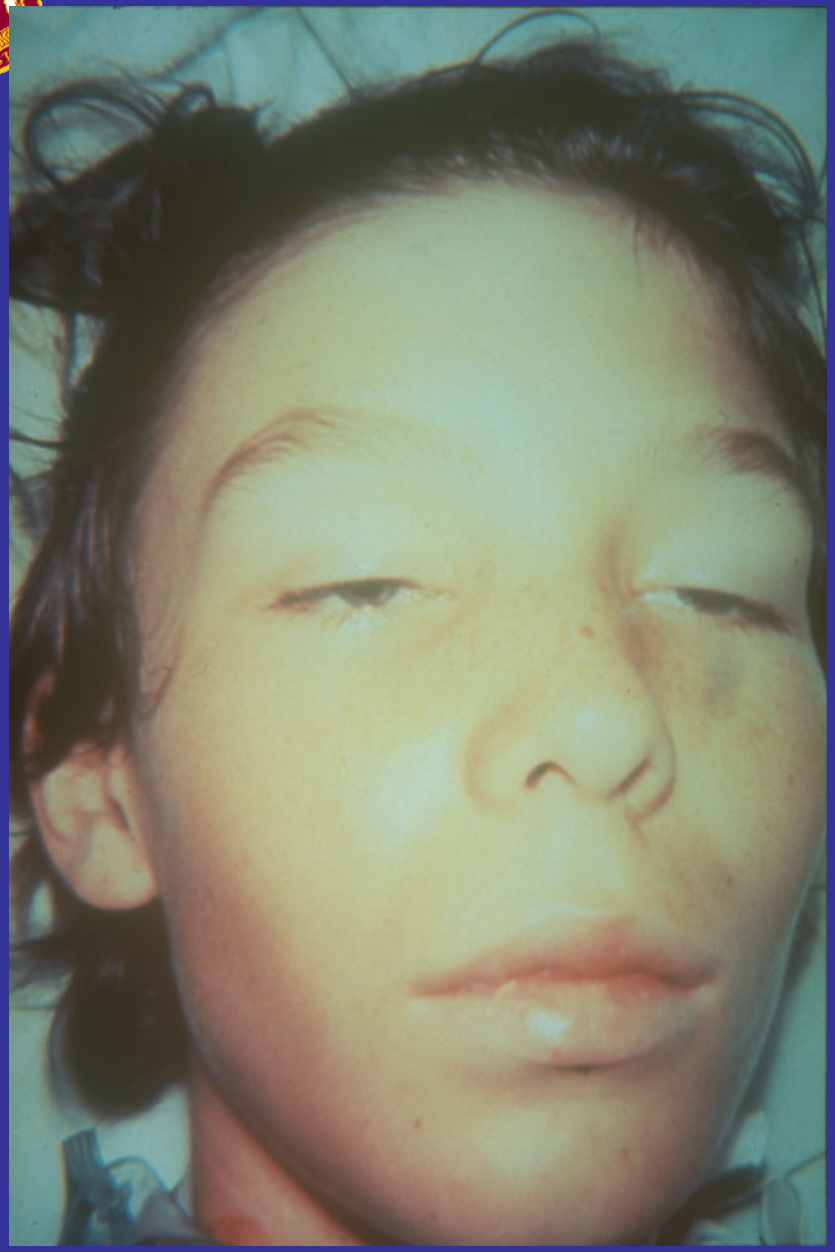
Botulinum Toxins: Mechanism of Action

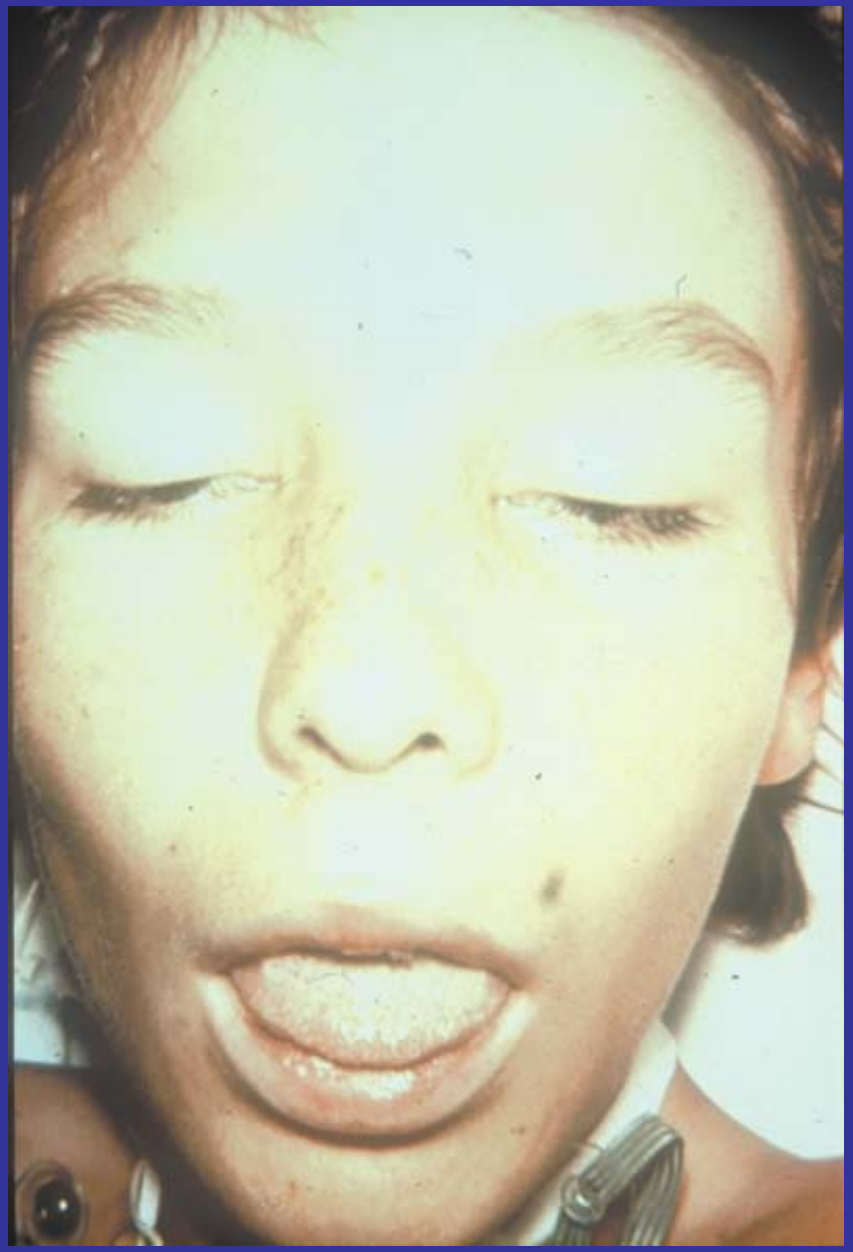


Botulism: Clinical Features

- Latent period: 24 - 36 hours after inhalation
- Symmetrical descending flaccid paralysis
  - *Cranial nerves affected first: blurred vision, diplopia, ptosis, and photophobia*
  - *Bulbar nerve dysfunction: dysarthria, dysphonia, and dysphagia*
  - *Skeletal muscle paralysis: may lead to respiratory failure*
- Inhalational botulism similar to food-borne







Botulism: Diagnosis

- Clinical features
  - *Alert and afebrile*
  - *Symmetric descending flaccid paralysis*
- May need to rule out other diseases
  - *Cerebrospinal fluid normal*
  - *Electromyography*

Botulism: Diagnosis

- Mouse bioassay of serum or stool
  Traditionally used
- Detection of the toxin
  Immunoassay – most sensitive
  Serum, gastric aspirates, stool, or respiratory secretions
- Survivors typically do not develop antibodies

Botulism: Medical Management

- Anti-toxin
- Intubation
- Ventilatory assistance
- Intensive supportive care

Botulism: Medical Management

- Respiratory failure is most serious complication and cause of death
- Mortality rate < 5%
  ~60% before 1950
- Recovery may be prolonged
  May take up to 3 months to improve
  May take up to a year to fully recover

Botulism: Medical Management

- Anti-toxin

  Neutralizes circulating toxin only

  As antitoxin is delayed, treatment becomes less effective

- Early detection and diagnosis essential to successful therapy!

Botulinum Anti-toxin

- Heptavalent de-speciated USAMRIID product

  Provided significant protection when given 24 hrs after aerosol challenge

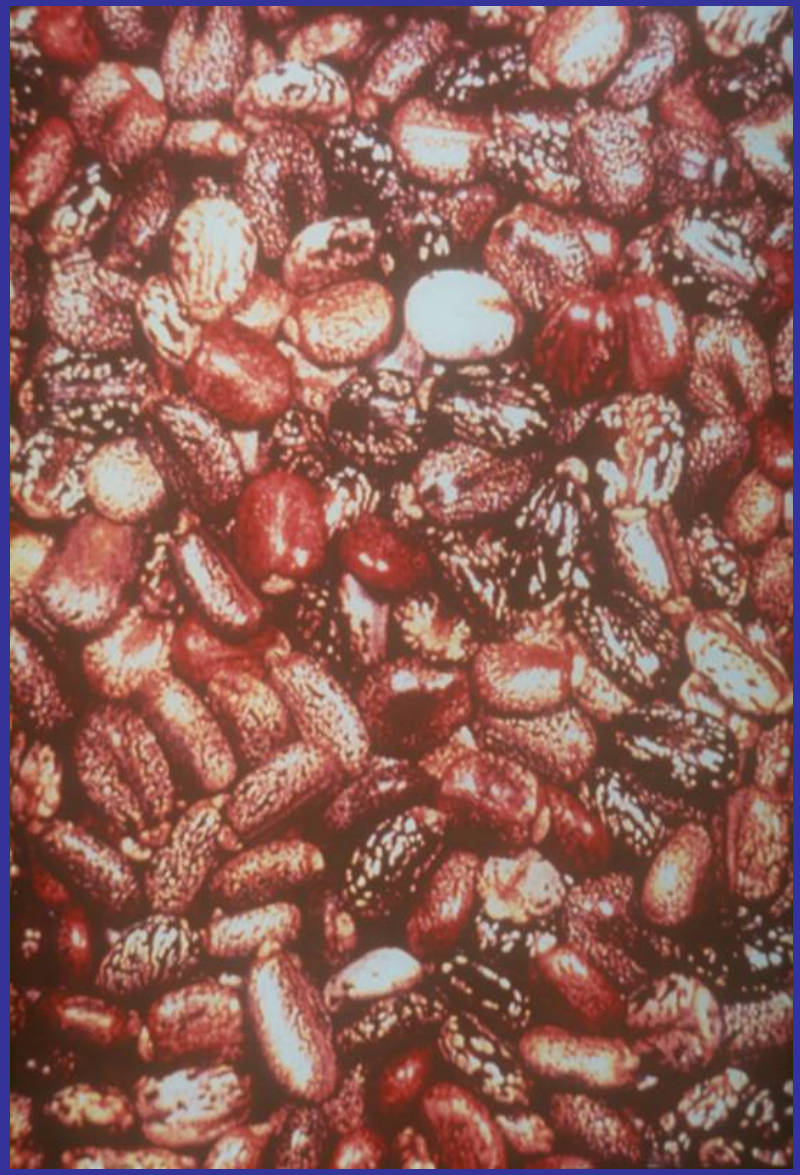
  Did not protect against lethality if treatment was delayed until the onset of clinical signs

Botulinum Toxin: Prophylaxis

Botulinum toxoid vaccine

- *IND status*
- *Pentavalent*
  - *Serotypes A, B, C, D, and E*
- *Primary series - 0, 2, and 12 weeks with 1 year booster*
  - *Protective titer in >90%*
- *Monkeys given 2 doses (0 and 2 weeks) were protected against aerosol inhalation challenge*

Ricin



Ricin: History

Ricinus communis -
Castor bean

One million tons
processed annually

Waste mash
~3-5% ricin

Ricin: Characteristics

- Globular glycoprotein
  Molecular weight 66,000
- 2 polypeptide chains
  A chain – active chain
  B chain – binding chain
- Marginal toxicity limits application
  3- 6 µg/kg LD50
  (vs. 1-3 ng/kg for BoNT LD50 and SEB ED50)

Ricin: Mechanism of Action

- Inhibits cellular protein synthesis
  - Leads to local necrosis
  - Systemic uptake leads to vascular leak syndrome
- Clinical features dependant on route of administration

Ricin: Clinical Features

Inhalation

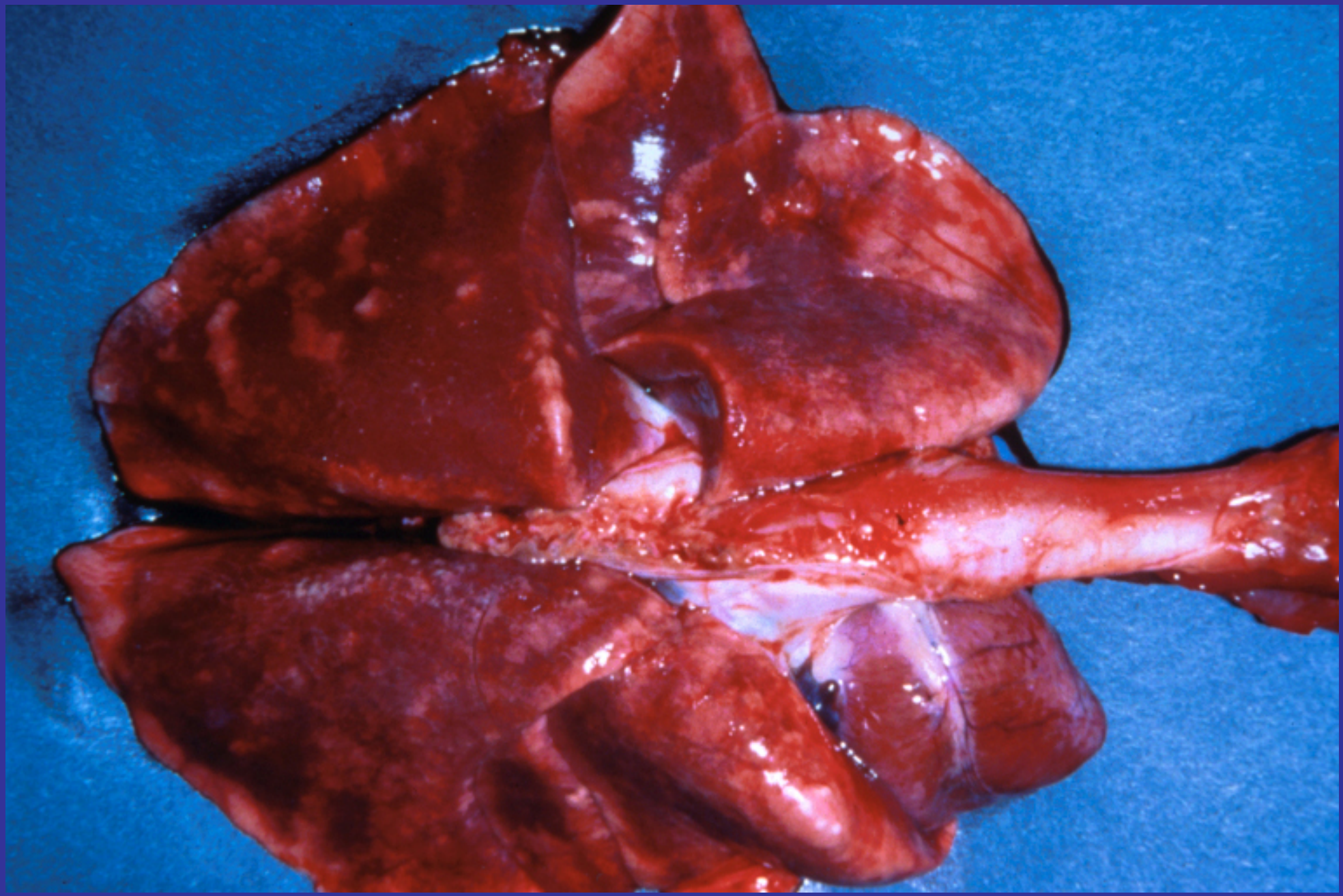
Latency period 8 - 24 hrs (dose-dependent)

Fever, chest tightness, cough, dyspnea, nausea

Hypothermia, cyanosis, and massive pulmonary edema

Necrosis of airways

Death in 36-48 hr



Ricin: Clinical Features

Ingestion

Latency period of a few hours

Nausea, vomiting, abdominal cramps

Severe diarrhea, GI hemorrhage, and vascular collapse

Necrosis of liver, spleen, kidneys, lymph nodes

Death by 3rd day or later

Ricin: Diagnosis

- Aerosol exposure
  Swab sample from nasal mucosa
  Swab <24 hr after exposure
- Immunoassays of blood (theoretical)
  Ricin bound and internalized within hours
- Immunohistochemical techniques
  Direct analysis of tissue

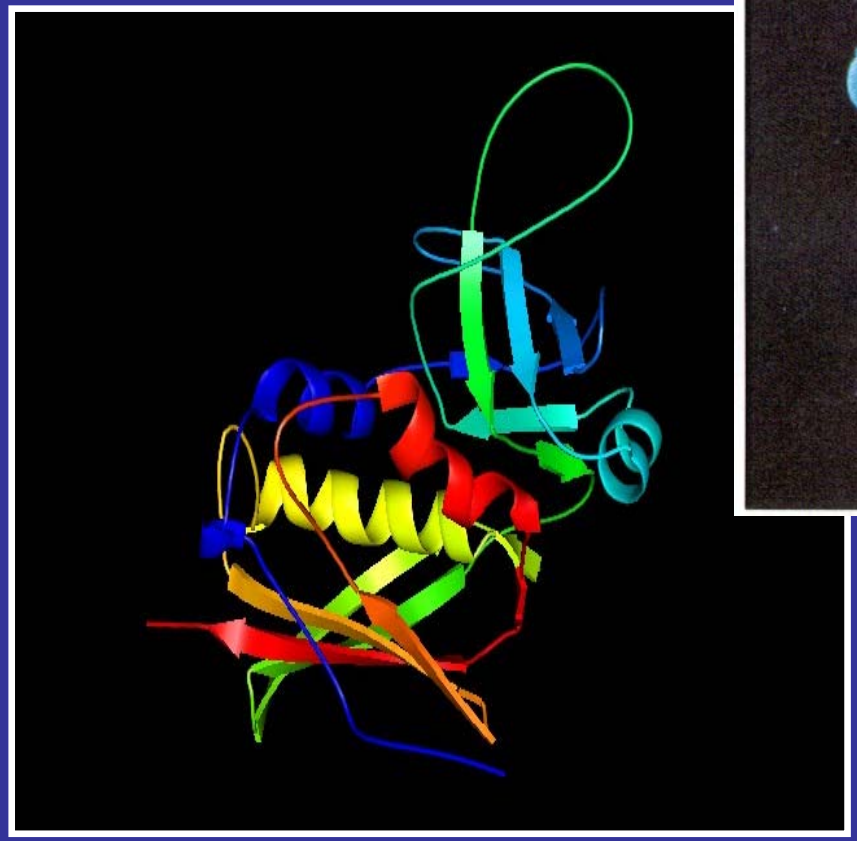
Ricin: Medical Management

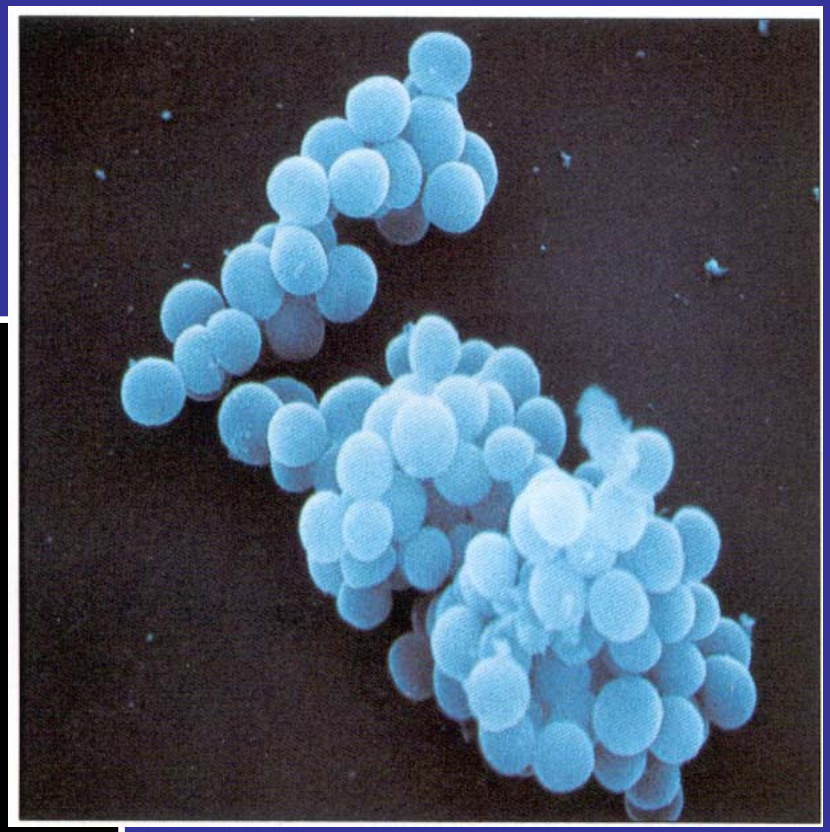
- Supportive care based upon route of exposure
- Inhalation exposure:
  Aggressive airway management
  Monitor fluid balance and hemodynamics
- Oral intoxication:
  Gastric lavage, cathartics, IV fluids and electrolyte replacement

Ricin: Prophylaxis

- Physical protection:
  - *Respiratory protection is critical*
  - *Ricin is not dermally active*
- No vaccine available for human use
  - *Recombinant vaccine candidate approved by FDA for human clinical trials*

Staphylococcal Enterotoxin B (SEB)





SEB: History

- *Staphylococcus aureus* toxins:
  - SEB
  - Related exotoxins
    - SEA, SEC1, SEC2, SEC3, SED, SEE, SEH, TSST-1
- Pyrogenic toxin causing food poisoning
  - *Different clinical syndrome when inhaled than when ingested*
- Weaponized by the U.S.
- *Significant morbidity with aerosol attack*

SEB: Characteristics

- SEB is both lethal and incapacitating
  - *LD50 = 20 ng/kg*
  - *ED50 = 0.4 ng/kg incapacitation*
  - *Effective military weapon*
- Extreme toxicity allows variable application
  - *Open-air weapon*
- Easily produced

SEB: Mechanism of Action

- Bacterial superantigen
  - *Binds to MHC class II receptors on antigen presenting cells (APCs)*
  - *Stimulates T-cell proliferation*
  - *Massive cytokine release*
- Intense inflammatory response results in:
  - *Tissue injury*
  - *T-cell anergy*
  - *Apoptosis*

SEB: Clinical Features

- Severely incapacitating illness
- Rapid onset

  3-4 hrs
- Modest duration
- Fever, chills, myalgia and headache

  Latency period 8-20 hr

  Fever of 103^{o}-106^{o} F

  Duration 1-3 days

SEB: Clinical Features

- Respiratory signs and symptoms
  - *Nonproductive cough*
  - *Dyspnea with moist inspiratory and expiratory rales in severe cases*
  - *Substernal pleuritic chest pain*
- Gastrointestinal symptoms
  - *Nausea, anorexia, vomiting*
  - *No diarrhea*

SEB: Diagnosis

- Clinical features

  Symptoms plateau early

- Epidemiology

- Laboratory identification

  Immunoassay

  Serum, urine, respiratory secretions, and nasal swabs

SEB: Medical Management

- Treatment limited to supportive care
- No specific antitoxin available for human use

SEB: Prophylaxis

- Vaccine not available for human use
- Vaccine candidate tested in monkeys

  Recombinant SE vaccine

  Pending transition to advanced development

USAMRIID

Microcystins and Other Cyanotoxins (Blue Green Algal* Toxins)

** Not really*

Cyanobacterial toxins:

Hepatotoxins

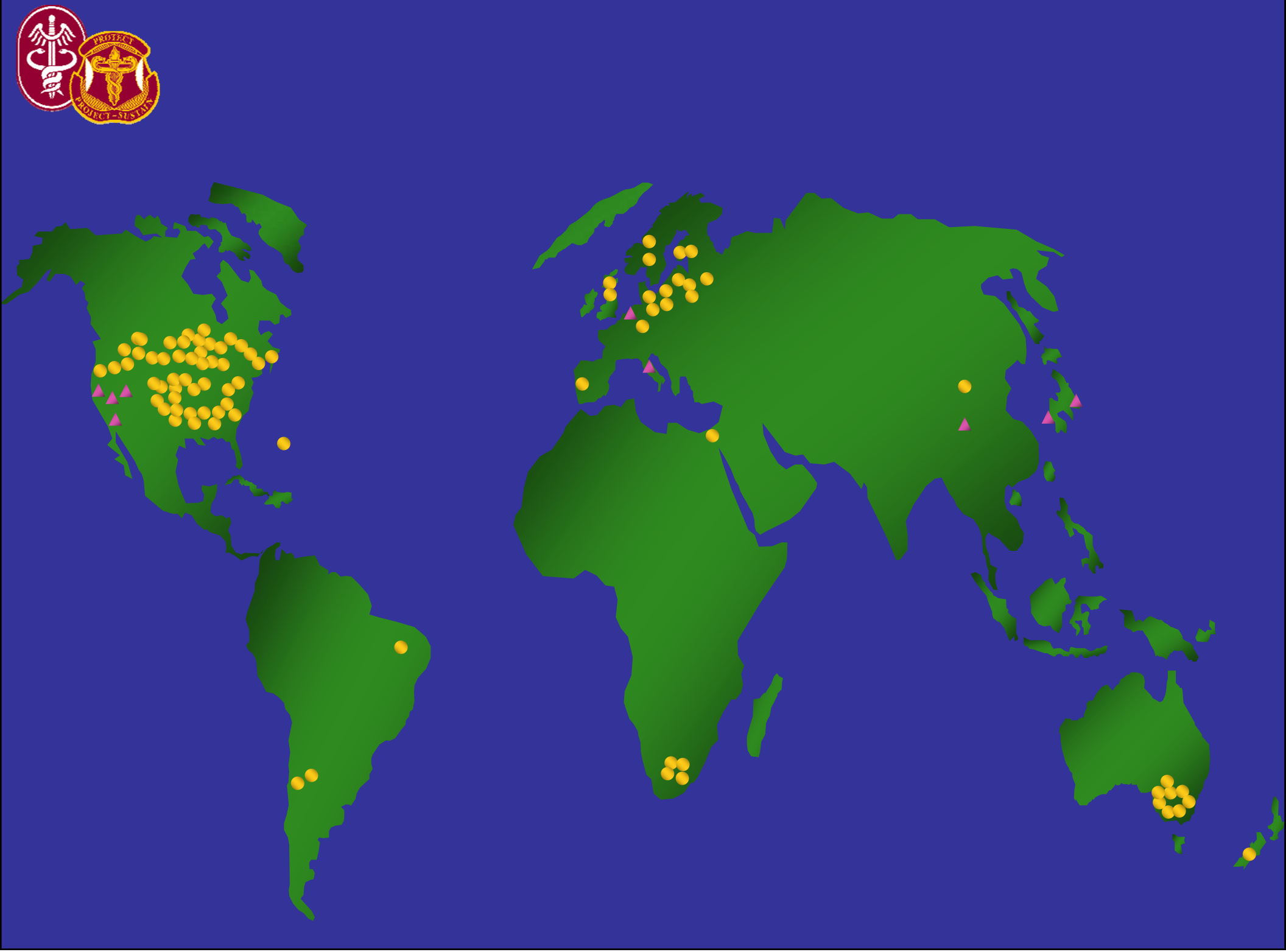
- microcystins (*Mycrocystis, Oscillatoria, Anabaena)*
- nodularins (*Nodularia)*
- cylindrospermopsins *(Cylindrospermopsis)*

Neurotoxins

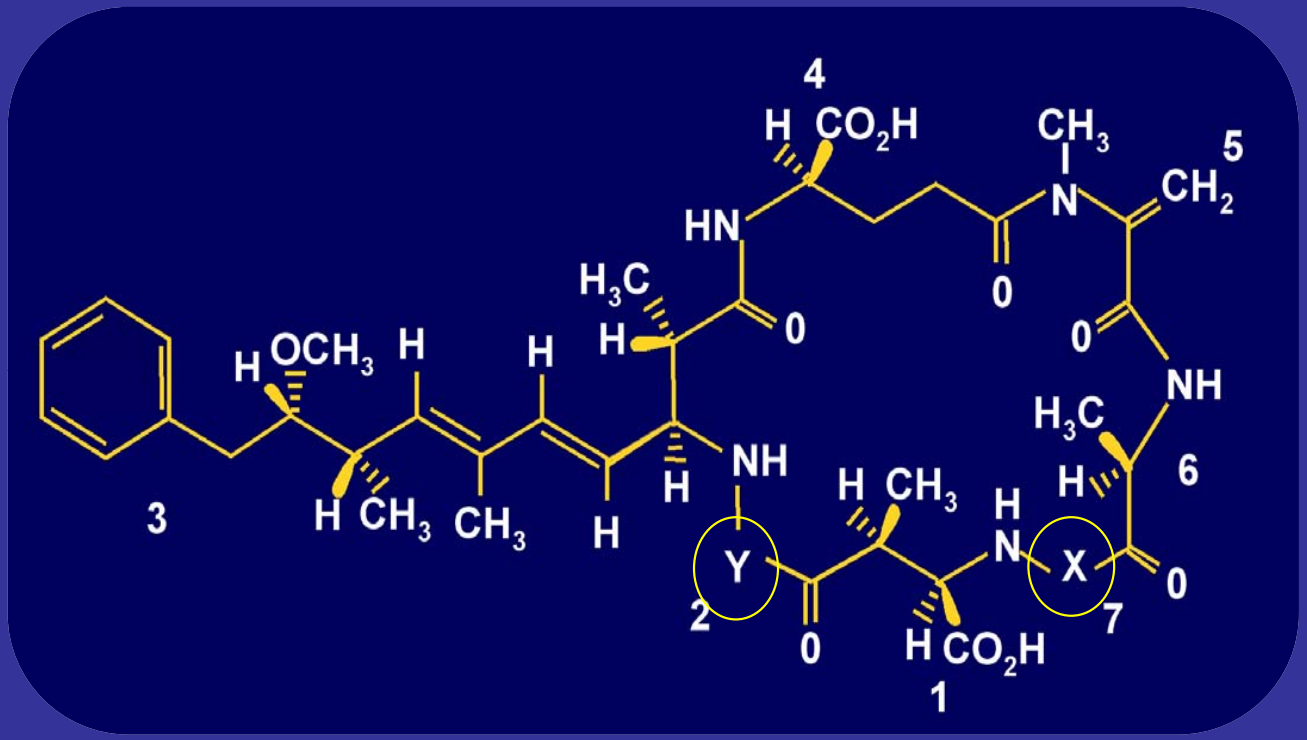
- anatoxin-a *(Anabaena, Aphanizomenon, Cylindrospermopsin, Oscillatoria)*
- anatoxin a(s) *(Anabaena)*
- saxitoxins (*Aphanizomenon, Anabaena, Lyngbya)*
- **BMAA –** Guam neurogenerative disease

"Acute and chronic hazards presented by these toxins to human and animal health are now ranked among the most urgent and serious problems associated with surface water supplies all over the world."

Takei, A. and K. Harada In: *Seafood and Freshwater Toxins,* (L. Botana, editor) Marcel Dekker, 2000


PROJECT
PROJECT - SUSTAIN

Microcystins



1 = methyl aspartate
2 = (variable)
3 = adda
4 = D-Glu
5 = methyl dehydroalanine
6 = D-Ala
7 = (variable)

	AMINO ACID	
	X	Y
Microcystin-LR	Leu	Arg
Microcystin-RR	Arg	Arg
Microcystin-YR	Tyr	Arg

In vivo Effects: Animals

Initially:

- Vomiting, diarrhea, weakness, heavy respiration

At higher doses:

- Disruption of lobular and sinusoidal architecture
- Pooling of blood in the liver
- Hemorrhagic shock

No primary toxic effects at any other organs

LD_{50} (mouse ip) = 50-100 ug/kg (LR)

Human Intoxications

- Known since 1878, usually from ingestion of surface waters
- Less toxic orally (~11 mg/kg in mice)
- Symptoms include:
  - Loss of appetite
  - Vomiting, diarrhea
  - Stupor
  - Convulsions
  - Loss of consciousness
  - Death

Caruaru, Brazil 1996

- 76 Brazilian patients died after receiving hemodialysis using water from a reservoir containing a massive growth of cyanobacteria (and 19.5 ug/L MYC-LR, -YR, -AR)

- Symptoms included myalgia, weakness, nausea, tenderness around the liver and a range of neurological symptoms.

- "Caruaru Syndrome" is characterized by:

extreme hepatomegaly
ecchymosis
metrorrhagia
hyperbilirubinemia
cholestasis
jaundice
epistaxis (nosebleed)
elevated transaminases
hypertriglyceridemia
cytoplasmic vacuolization
liver cell deformities, apoptosis, and necrosis

Mechanism of Action

Uptake through bile acid transporters

Specific inhibitors of protein phosphatases 1 and 2A (PP1 and PP2A)

- hyperphosphorylation of cytoskeletal proteins

  (microtubules, intermediate filaments, actin microfilaments)
- abnormal intracellular redistribution of these proteins
- extensive cellular deformation leads to altered hepatic architecture, cell death

A possible cancer connection?

Mycrocystins are potent tumor promotors, because of the importance of PPs in cell division and proliferation

Epidemiological studies in China show a strong correlation between liver cancer and use of surface waters contaminated with cyanobacteria

Medical Management

- Symptomatic care only
- Prevention:

  Prevent exposure to contaminated surface waters

Detection

- Immunoassays:

  Easily detected in the pg/mL range – natural occurrence is ng/ml range

  Cross-reactivity among congeners is variable and problematic

- Analytical methods

  HPLC and LC-MS methods have been developed for identification in pM range

- Biological methods

  Based upon PP inhibitory activity – sensitive to ng/ml

Should we be concerned?

- Widely available
  - worldwide distribution
  - blooms can be very large
- Reasonable toxicity
  - LD50 ~50-100 ug/kg for LR, others mostly less
  - insufficient for BW, but reasonable for BT
  - high casualty numbers not likely
- Not a select agent
  - minimal controls on possession/shipping

Summary

- The toxin threat is real,…..BUT
  - *Potency, availability, stability, and weaponization issues limit employment as BW/BT agents*
  - *Medical countermeasures can confer significant protection*
  - *Research programs are in place to address knowledge gaps*

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Viral Hemorrhagic Fevers

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This presentation developed and previously delivered by LTC (ret) Tom Larsen & LTC Ed Stevens.

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"...it is time to close the book on infectious diseases"

U.S. Surgeon General William H. Stewart, 1969.

Briefing Organization

- Learning Objectives
- Definition
- Etiologic Agents
- Threat Level / Weaponization Potential
- History
- Epidemiology
- Pathogenesis
- Clinical Features
- Diagnosis
- Medical Management
- Prevention / Control

Learning Objectives

- List the agents responsible for causing viral hemorrhagic fever (VHF) and understand their epidemiology
- Recognize the clinical signs elicited by VHF causing agents
- Briefly review containment principles for VHF cases and/or outbreaks in a field setting

Definition

Viral hemorrhagic fever (VHF) is a term historically used to define an acute, *febrile*, multisystemic illness characterized by malaise, myalgia, prostration, and *bleeding diathesis* caused by lipid-enveloped, single-stranded, *RNA viruses* in *Filoviridae*, *Arenaviridae*, *Bunyaviridae*, and *Flaviviridae* families.

Hemorrhagic fever virus (HFV) is a term used to generically identify those agents that cause VHF.

http://0-www.cdc.gov.mill1.sjlibrary.org/ncidod/dvrd/spb/mnpages/dispages/vhf.htm

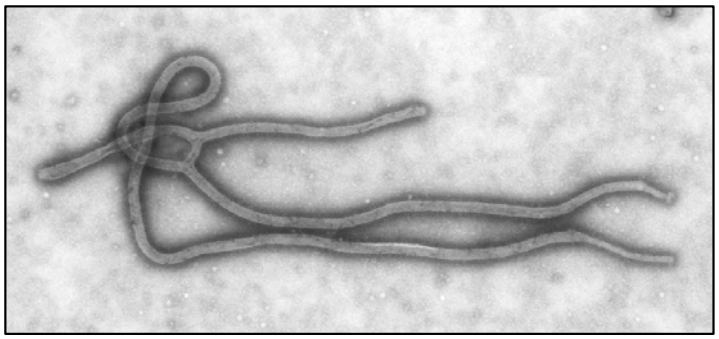
Overview of Etiologic Agents of VHFs

Family	Genus	Species
Filoviridae	*Ebolavirus*	Zaire, *Sudan*, *Ivory Coast*, *Reston*
	Marburgvirus	*Lake Victoria marburgvirus*
Arenaviridae	*Arenavirus*	*Lassa* (“Old World”) *Junin, Machupo, Guanarito, Sabia* (“New World”)
Bunyaviridae	*Nairovirus*	*Crimean-Congo hemorrhagic fever*
	Phlebovirus	*Rift Valley fever*
	Hantavirus	*Hantaan*, *Seoul*, *Puumala*, *Sin Nombre*, etc.
Flaviviridae	*Flavivirus*	*Omsk HF* *Kyasanur forest disease* *Dengue* *Yellow fever*

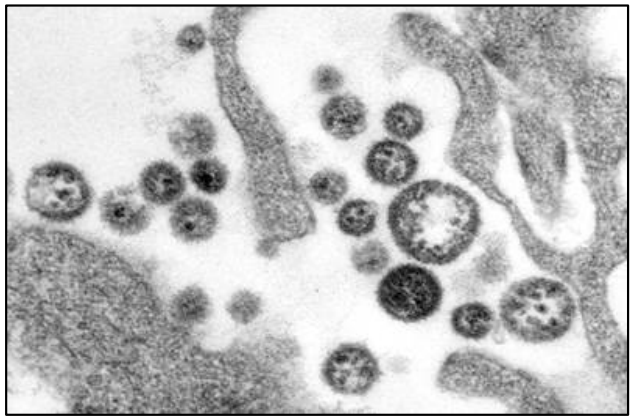
The “Deadly” VHFs

VIRUS	Mortality Rate
Ebola Zaire	*75-90%*
Marburg	*25-90%*
Lassa	15-20% of hospitalized
Crimean-Congo hemorrhagic fever	30%
Rift Valley fever	50% of patients with hemorrhagic form

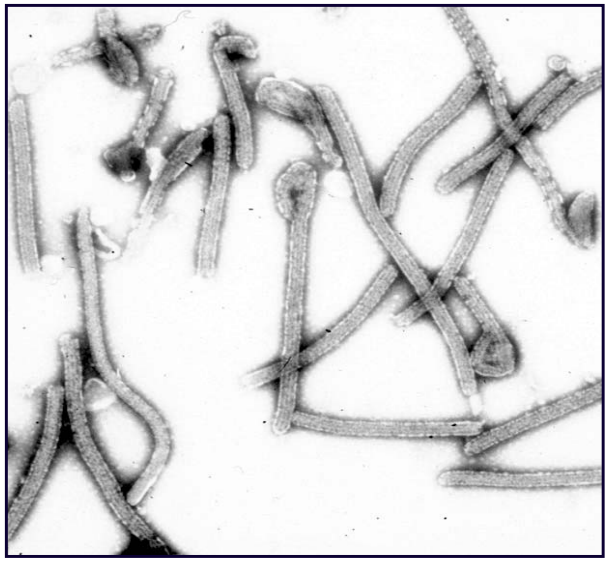
Etiologic Agents



Ebola virus, negative stain
Dr. Sherif Zaki, Pathology, CDC



Arenavirus
Dr. Sherif Zaki, Pathology, CDC



Ebola virus, negative stain
Dr. Tom Geisbert USAMRIID

Overview of Epidemiology of HFVs

Disease (virus)	Distribution	Natural Host/ Vector	Other Sources	Incubation (days)
Ebola HF	Africa, Philippines (ER)	Unknown	Nosocomial, etc.	2-21
Marburg HF	Africa	fruit bat?	Nosocomial, etc.	5-10
Lassa fever	West Africa	Rodent	Nosocomial, etc.	5-16
Argentine HF (*Junin*)	South America	Rodent	Nosocomial	7-14
Bolivian HF (*Machupo*)	South America	Rodent	Nosocomial	9-15
Venezuelan HF (*Guanarito*)	South America	Rodent	Nosocomial	7-14
Brazilian HF (*Sabia*)	South America	Rodent	Nosocomial	7-14
CCHF	Europe, Asia, Africa	Tick	Animal slaughter	3-12
Rift Valley fever	Africa	Mosquito	Animal slaughter	2-6
HFRS/HPS (*Bunyaviridae*)	World-wide	Rodent		9-35
Omsk HF	Soviet Union	Tick		2-9
Kyasanur forest disease	India	Tick		2-9
Dengue HF	Asia, Americas, Africa	Mosquito		3-15
Yellow fever	Africa, tropical America	Mosquito		3-6

Biological Select Agents and Toxins

HHS Select Agents & Toxins

- **Crimean Congo HF**
- **Ebola virus**
- **Marburg virus**
- **Lassa fever virus**
- **New World Arenaviruses (Junin, Machupo, Sabia, Flexal, Guanarito)**
- **Tick-borne encephalitis complex (Central European TBE, Far Eastern TBE, Kyasanur forest disease, Omsk hemorrhagic fever**

HHS & USDA Overlap Select Agents & Toxins

- **Rift Valley fever virus**

http://www.cdc.gov/od/sap/

Relevance?

Why is this important?

Consider…..

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“Mother Nature is the Greatest Bioterrorist Known to Mankind……."

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"……., but not the only one"

Potential of VHF's for Weaponization

- PRO
  - **Many demonstrated as infectious by aerosol transmission**
    - **Exception is Dengue**
  - **Potentially high morbidity and mortality**
  - **Replicate well in cell culture**
    - **Exception are viruses in *Bunyaviridae* (especially CCHF)**
  - **Capability to overwhelm medical resources**
  - **Frightening effects of illness / terror value**

- CON
  - **Lack of treatment or vaccine to protect user's own "troops"**
    - **May not be deterrent for some countries / non-state actors**
  - **Possible entry into local vector / reservoir population**
  - **Stabilizers must be used to enhance viability**

History of Weaponization

- Yellow fever and RVF were weaponized by the U.S. during their offensive program
- Former Soviet Union produced large quantities of Ebola, Marburg, Lassa, Junin, and Machupo
- Yellow fever may have been weaponized by North Koreans
- The Aum Shinrikyo cult unsuccessfully tried to obtain Ebola virus to create biological weapons
- Several studies have demonstrated ability to aerosolize Ebola, Marburg, Lassa, and some of the New World arenaviruses

History - Ebola Virus (filoviridae)

- **Four species of Ebola** each with one or more strains
  - ***Zaire* (ZEBOV), *Sudan* (SEBOV),** *Ivory Coast* (CIEBOV), *Reston* (REBOV)
- First discovered in 1976 with separate outbreaks of **ZEBOV** (318 cases / 88% mortality) & SEBOV (284 cases / 53% mortality)
- Another large outbreak of **ZEBOV** in Kikwit, Democratic Republic of Congo (DRC) in 1995 (315 cases / 81% mortality)
- Another outbreak of SEBOV in Uganda in 2000-2001 (425 cases / 53% mortality)
- **Apr-Nov 2007 Outbreak in Kampungu, Kasai Occidental, DRC.** First major resurgence of EBOV in years confirmed by laboratory analysis in September 2007. Approximately 160 deaths (352 suspected cases) in an 8 month period. Concurrent Shigella and Typhoid outbreak. Outbreak officially declared over on 19 Nov 2007
- **Nov 07-Jan 08 Outbreak in Uganda.** As of 4 Jan 2008 Director General of Health Services issued a statement saying that the cumulative total of Ebola patients stands at 149 with 37 deaths.

History - Marburg Virus (filoviridae)

- **One species (*Lake Victoria marburgvirus*)** with recognized strains such as Musoke, Ravn, Popp, etc.
- First discovered in 1967 in a Marburg, GE laboratory using infected African green monkey tissue; 32 cases with a 21% mortality rate
- Sporadic cases between 1975 and 1987 with low numbers of deaths
- 154 cases in the Democratic Republic of Congo with a fatality rate of 83% between 1998 & 2000
- 324 deaths were reported in Angola between 13 October 2004 & 8 August 2005; epidemic officially over 7 November 2005; large percentage of children affected
- **July 2007 sporadic outbreak; 2 mine workers in Uganda confirmed by CDC (1 death; 1 survivor); reservoir (fruit bat most likely)**

Epidemiology - Filovirus

- **Recent literature suggests a common African fruit bat (*Rousettus aegyptiacus*) is the natural reservoir for marburgvirus**
- Direct contact with blood, secretions, or tissues of humans and NHPs
- Nosocomial contact: Needlestick injuries, contaminated syringes
- Direct contact with the body during burial ceremonies or handling of bodies can play a significant role in transmission
- Mucosal exposure
  - demonstrated in NHPs
- Aerosol
  - Mixed information: demonstrated in NHPs, but outbreaks in Africa have been controlled without respiratory precautions
  - four personnel seroconverted in the Reston outbreak

Towner JS, Pourrut X, Albarino CG, Nkogue CN, Bird BH, et al (2007) Marburg Virus Infection Detected in a Common African Bat. PLos ONE 2(8): e764. doi:10.0371/journal.pone.0000764

History - Arenaviruses

- *Junin* virus (Argentine HF) was found in 1958 in the pampas of Argentina among corn harvesters; it was the first of the HF arenaviruses to be identified

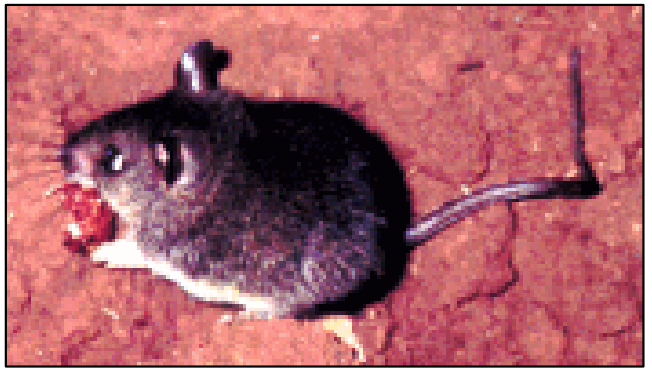
- ***Machupo* virus (Bolivian HF)** was found in 1963 in the savannas of Bolivia
  - March 8, 2007: 6 cases; 2 deaths (dengue outbreak was occurring simultaneously)
  - March 14, 2007: 3 cases; 2 deaths (method of detection unknown)

- *Guanarito* (Venezuelan HF) and *Sabia* (Brazilian HF) were identified later

- *Lassa* virus was found in Nigeria in 1969 (last known outbreak in Liberia in April 2007)

Epidemiology - Arenavirus

- **Natural reservoir includes several species of mice and rats**
- **<u>Direct contact with rodent feces and urine</u>**
- Exposure to rodents caught in agricultural machinery
- Secondary **person-to-person** (blood, sexual contact, urine, pharyngeal secretions) and nosocomial transmission
  - e.g. *Lassa* and *Machupo*
- Contaminated food or water
  - *Lassa*
- Aerosol
  - <u>Natural transmission to humans is via rodent urine and feces</u>
  - Suspected person to person based on one study, but no definitive evidence to date



Mastomys sp. - Lassa reservoir

Lassa Fever Outbreak in Liberia

- Nimba county primarily affected
- Some 21 suspected cases reported -- 13 were confirmed (method unknown)
- 5 suspected fatalities

Source: ProMED Digest V2007 #181

History & Epidemiology
Rift Valley Fever (bunyaviridae)

- First isolated in the Rift Valley, Kenya in 1930 during an investigation into a disease epidemic in sheep

- A zoonotic disease transmitted by several species of **mosquitoes**

- A natural disease in several species of livestock, including sheep, cattle, camels, and goats: **Abortions are common**.

- Humans are infected during epizootics of the disease through **mosquito bites**, handling infected tissues (**animal slaughter**), and possibly through the **ingestion** of raw milk. **Aerosol** transmission has also led to infection in laboratory workers.

Recent Outbreak of Rift Valley Fever in Sudan

- Sketchy history…deaths (unknown) reported along the Nile valley in central Sudan
- Sudan's health ministry sought help from the UN in October 2007.
- As of **15 Jan 2008**, a cumulative total of **698 cases (222 deaths)** reported from 6 states yielding a case fatality rate of **32.4 per cent.**
- no new cases have been reported since 5 Jan 2008

2006-2007 Outbreak of Rift Valley Fever in Kenya
USAMRIID Involvement

- Index human case seen in Garissa District of northeastern Kenya in a patient on November 30, 2006.

- Outbreak associated with **heavy rains leading to explosion of mosquitoes** and increased infections in animals.

- In Kenya, as of 30 Jan 07, 411 suspect cases with 121 deaths (case-fatality rate, 29%). 131 cases laboratory confirmed.
- In Somalia, as of 30 Jan 07, 100 suspect cases with 48 deaths.
- In Tanzania, as of mid-Mar 07, 118 suspect cases with 14 deaths

http://www.who.int/csr/don/archive/disease/rift_valley_fever/en/

2006-2007 Outbreak of Rift Valley Fever in Kenya
USAMRIID Involvement

- As of May 2007, at least 200 people in Muyinga Province, Burundi infected; 1 death

- Likely due to infected meat from Tanzania

- This is the first report of Rift Valley fever cases in Burundi and represents a significant extension westwards of the Rift Valley fever outbreak in East Africa

- As of 04 June 2007, no new outbreaks (at least in Tanzania)

http://www.infoplease.com/atlas/country/burundi.html

2006-2007 Outbreak of Rift Valley Fever in Kenya
USAMRIID Involvement

- **Suspect case** - acute onset of fever (>99.5ºF [>37.5ºC]) with headache or muscle and joint pain since December 1 in a person who had no other known cause of acute febrile illness (e.g., malaria).
- **Probable case** - acute onset of fever in a person with unexplained bleeding (i.e., in stool, vomit, or sputum or from gums, nose, vagina, skin, or eyes), vision deterioration, or altered consciousness.
- **Confirmed case** - suspected or probable case with laboratory confirmation -- serum anti-RVF virus IgM by ELISA or RVF virus RNA by RT-PCR.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5604a3.htm?s_cid=mm5604a3_e

2006-2007 Outbreak of Rift Valley Fever in Kenya
USAMRIID Involvement

- Ban on livestock slaughtering.
- Vaccination of animals with live, attenuated RVF vaccine.
- Prevention messages were disseminated and public meetings were held to spread information rapidly to the community. Village elders, chiefs, and religious leaders were consulted, leading to a district ban on the slaughter of livestock and closure of the livestock market.
- Health-care workers were trained to care for persons suspected to be infected with RVF virus.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5604a3.htm?s_cid=mm5604a3_e

Summary of Initial Response to 2006/2007 RVFV Outbreak in Kenya - USAMRIID Entomology

- 765 mosquito pools processed in country (Kenya) from outbreak areas; 29 RVFV isolates confirmed by RT-PCR (conventional and real-time, virus growth and sequencing)

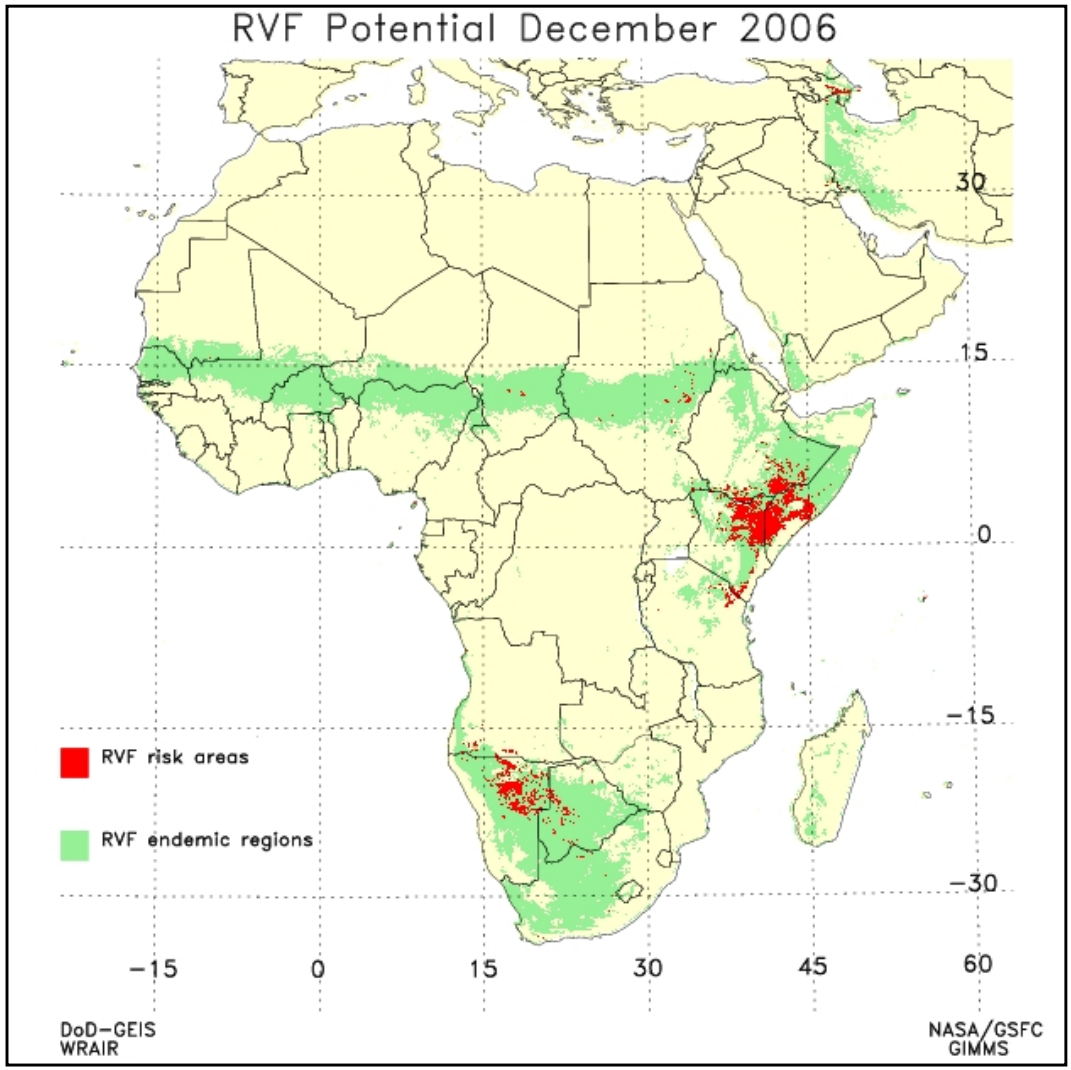
- 16 other virus isolates representing 6 distinct viruses from 717 mosquito pools
  - Ndumu (7)(Alphavirus)
  - Semliki Forest (1) (Alphavirus)
  - Bunyamwera (3) (Bunyavirus)
  - Sindbis (2) (Alphavirus)
  - Babanki (1) (Alphavirus)
  - West Nile virus (2) (Flavivirus)

Courtesy of LTC Monica O'Guinn

Summary of Initial Response to 2006/2007 RVFV Outbreak in Kenya - USAMRIID Entomology

- Mosquito collections started week of Christmas 2006
- NASA and DoD-GEIS predicted the outbreak 5 months prior based on rainfall and “Greenness” of affected areas

(http://www.geis.fhp.osd.mil/GEIS/SurveillanceActivities/RVFWeb/indexRVF.asp)



Courtesy of MAJ Jason Richardson USAMRU-K

Herder hooch

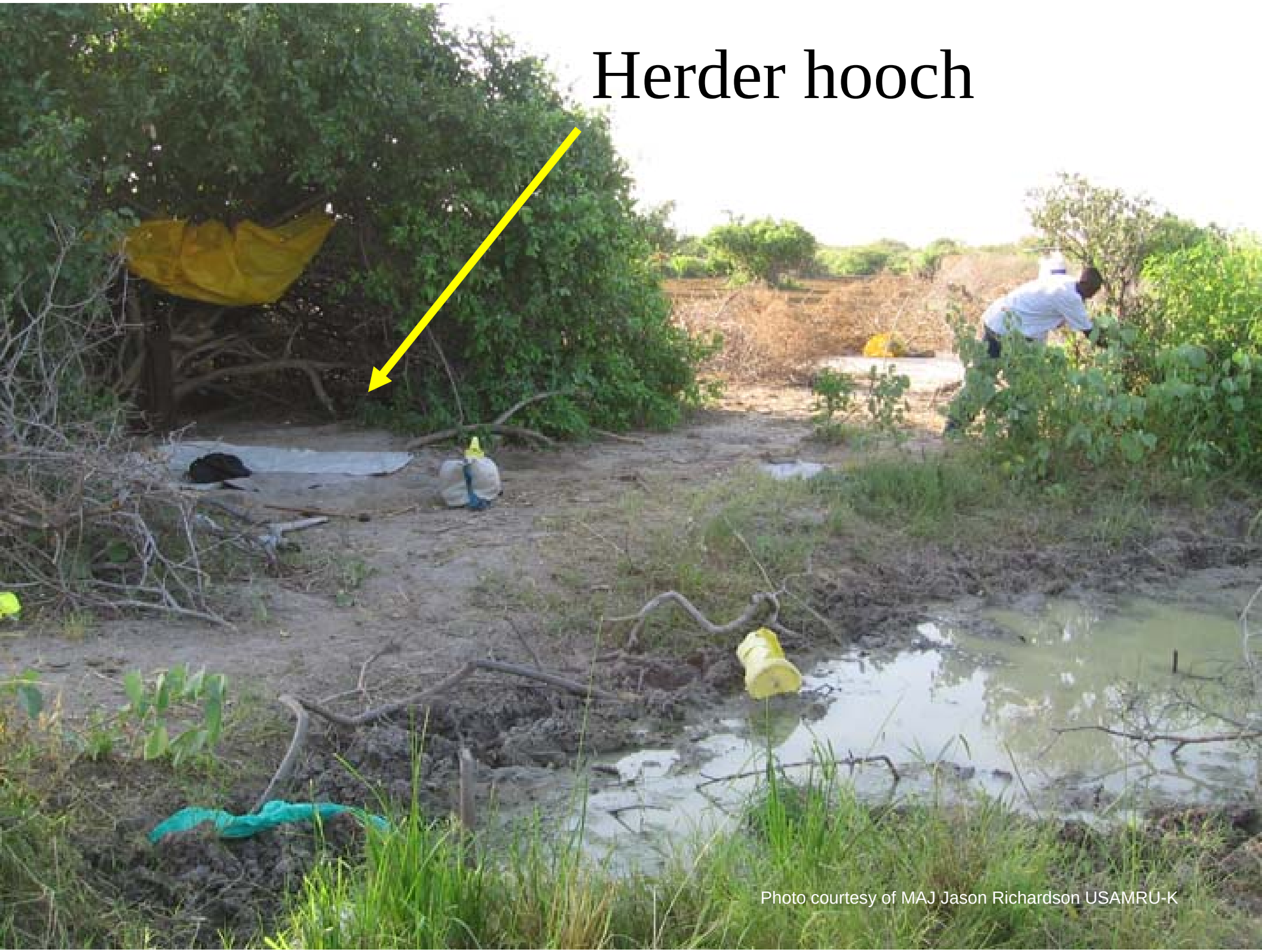
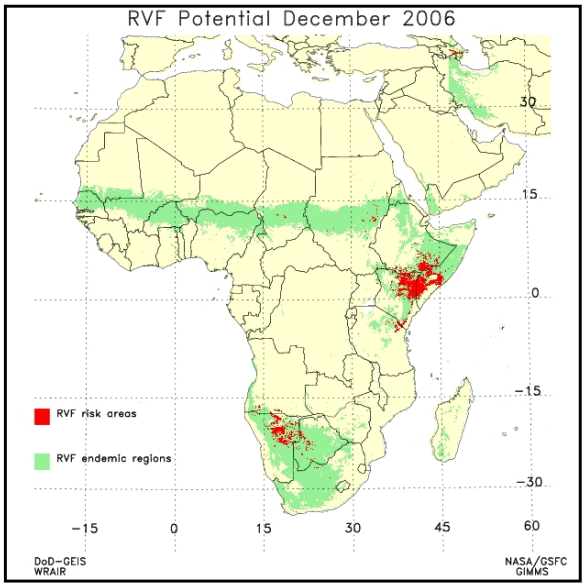


Photo courtesy of MAJ Jason Richardson USAMRU-K


Photo courtesy of MAJ Jason Richardson USAMRU-K


Typical trap for 1 night
Photo courtesy of MAJ Jason Richardson USAMRU-K



RVFV causes significant disease in sheep, cattle, camels, and goats

Photos courtesy of MAJ Jason Richardson USAMRU-K

USAMRIID Deployable PCR Lab – 6 pelican cases – highly mobile

Photo courtesy of LTC Monica O'Guinn

Deployable PCR Lab – Standardized, ready-to-go products

Photo courtesy of LTC Monica O'Guinn

History & Epidemiology
Crimean Congo HF (bunyaviridae)

- First described in Crimea (southern Ukraine, peninsula extends into the Black Sea) in 1944 and called Crimean HF
- In 1969, it was determined that Congo virus was the same virus that caused Crimean HF; therefore, the name was changed to CCHF
- The distribution of CCHF is wide; >30 countries in Africa, Asia, South-East Europe, and the Middle East
- CCHF is a **zoonotic disease that is transmitted by ticks** and infects a wide range of domestic and wild animals
- Humans contract the disease from handling infected livestock (slaughtering), direct contact with blood, or from tick bites
- **CCHF infection is currently (2007) an increased problem throughout Russia and central Asia**

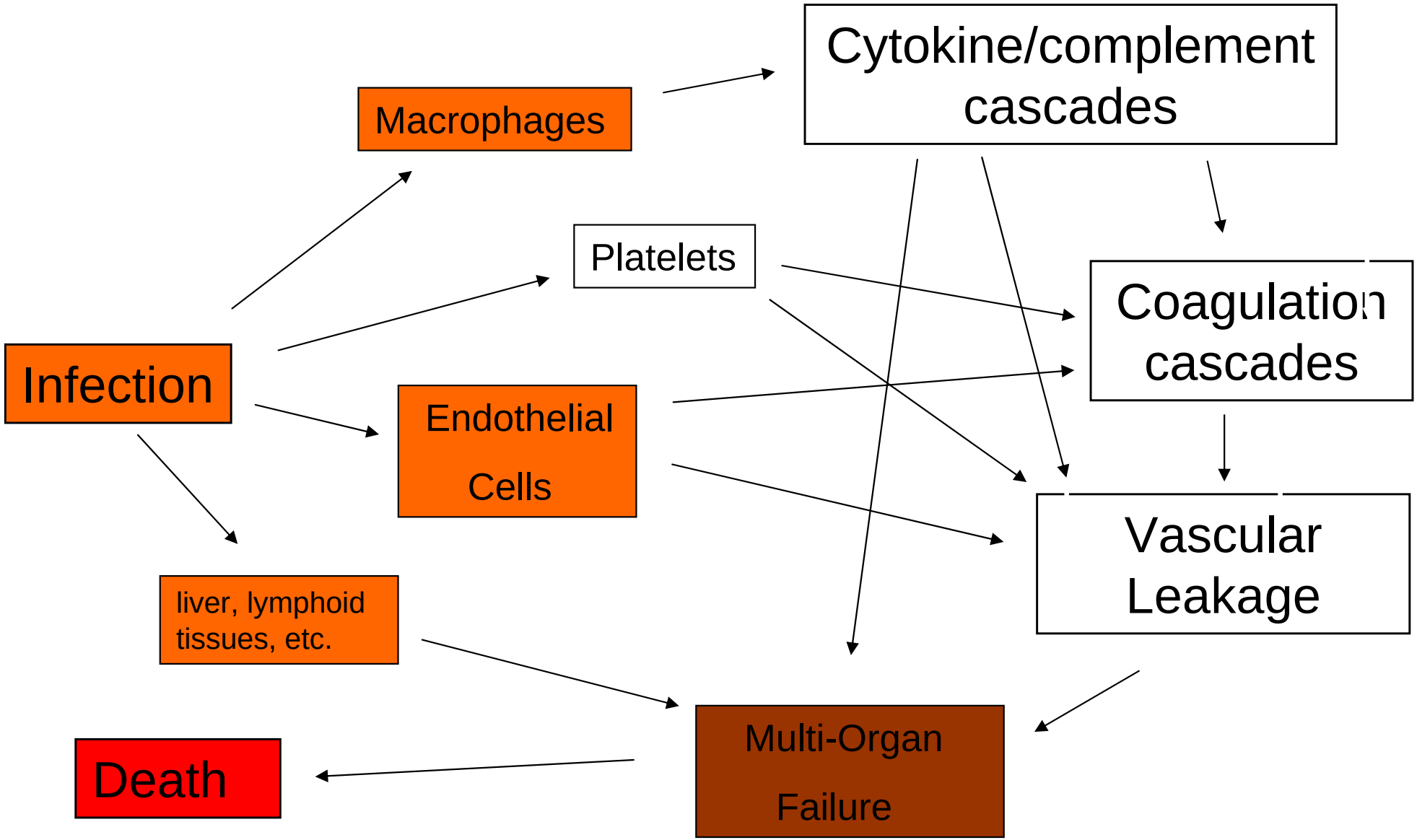
Epidemiology & Clinical Signs Kyasanur Forest Disease & Omsk Hemorrhagic Fever (flaviviridae)

- Tick-borne disease found in India (KFD) and Soviet Union (Omsk)
  - Nosocomial transmission not reported
- Incubation period 3-8 days
- Fever, cough, papulovesicular lesions of the soft palate, hyperemia of trunk & faces w/o rash
- Biphasic course w/ KFD
  - 1st phase similar to OHF, then become afebrile
  - Up to 50% develop meningoencephalitis
- Little is known about the pathogenesis

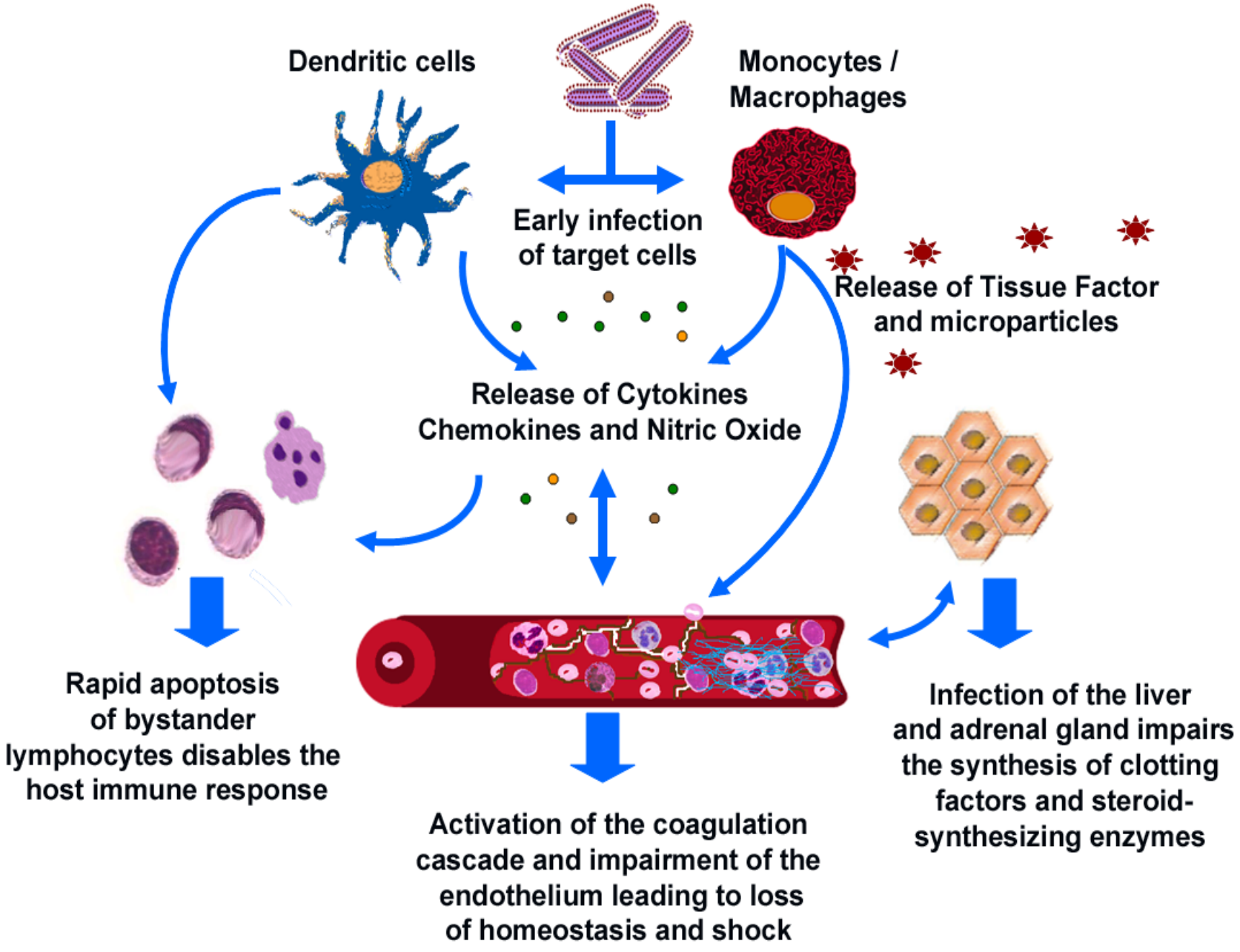
Pathogenesis of VHF

- Complex, incompletely understood, varies with specific viruses
- 3 key features of pathogenesis: coagulopathy, tissue necrosis, immune suppression
- Activation of complement / cytokine cascades
- Activation of coagulation cascades
- **Shock and multiorgan failure**
- Necrosis of liver, spleen, and kidney with some agents
- Damage to vascular endothelium with some agents

Pathogenesis of VHF

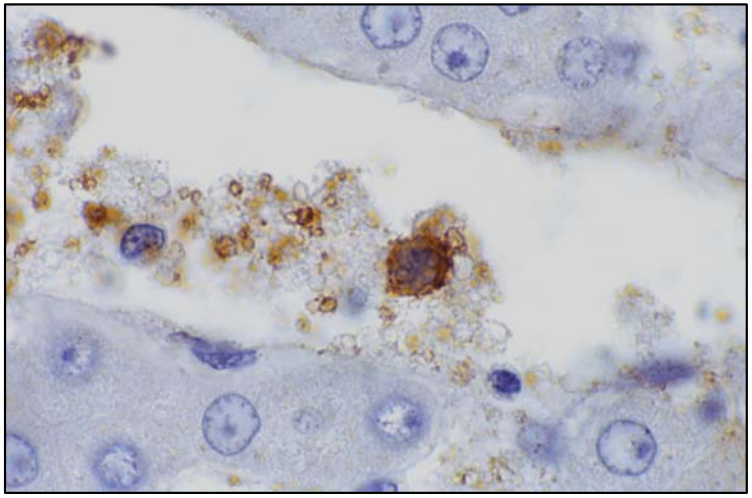


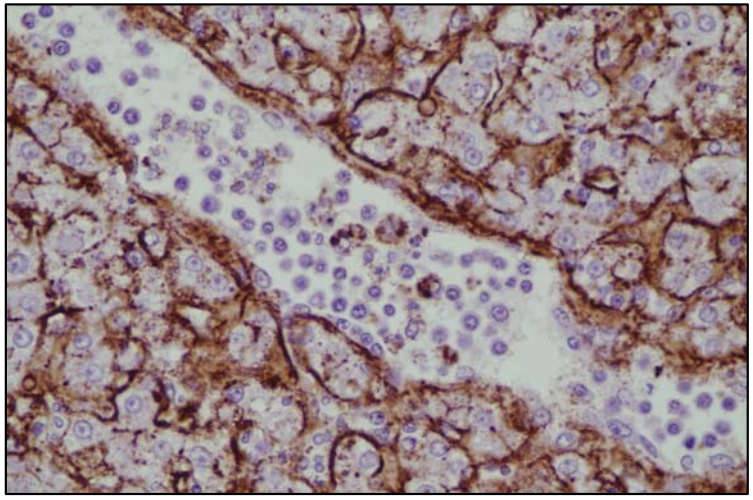
Model of Filoviral Pathogenesis in Primates



Infection of Macrophages

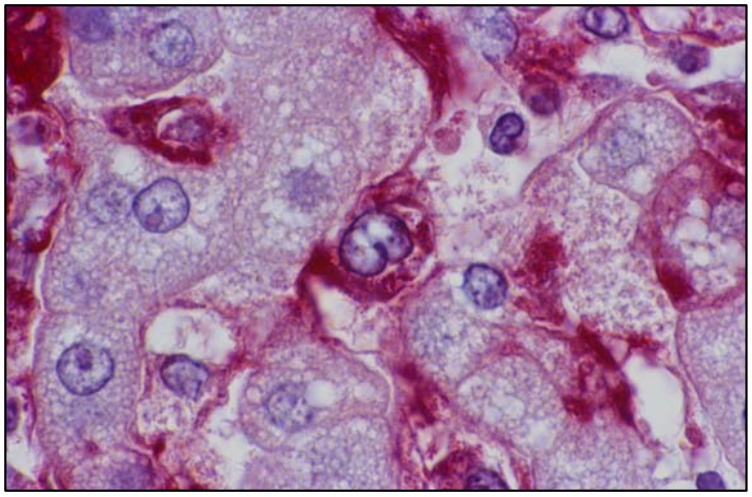
Lassa

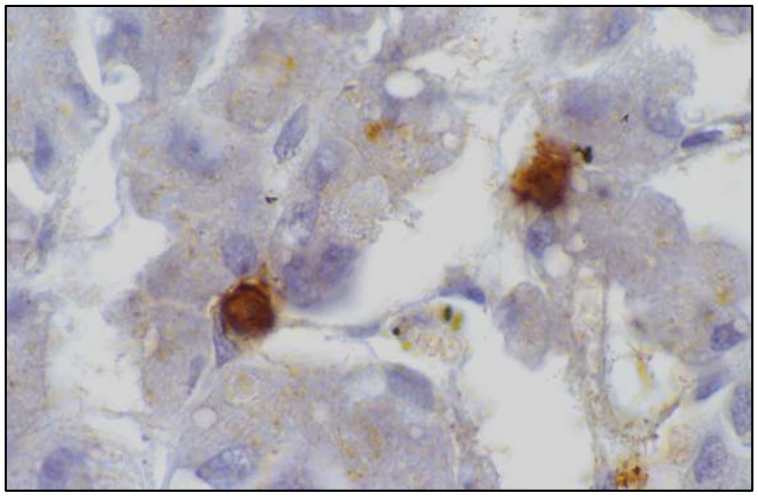




Marburg

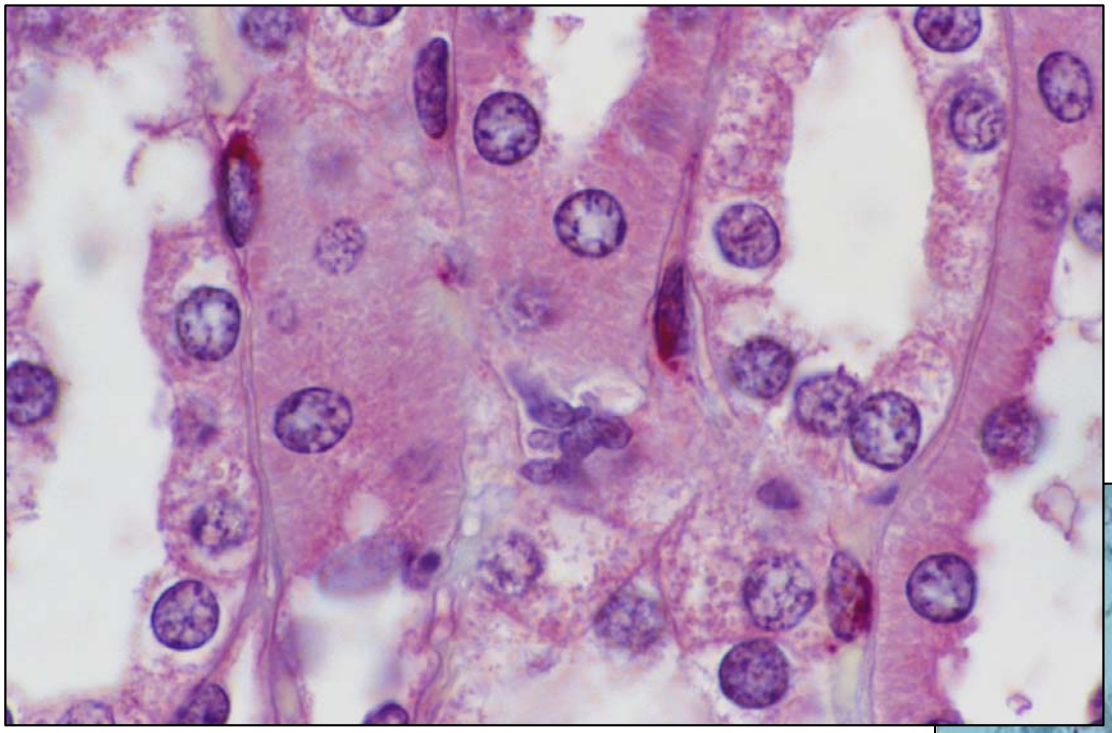
Ebola



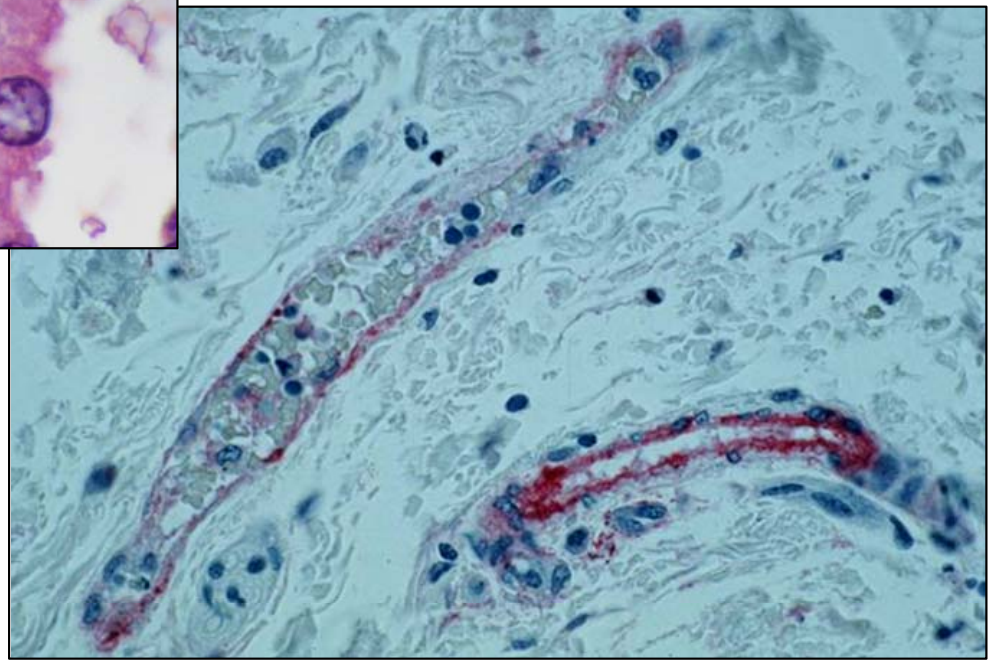


Dengue

Endothelial infection with some VHFs



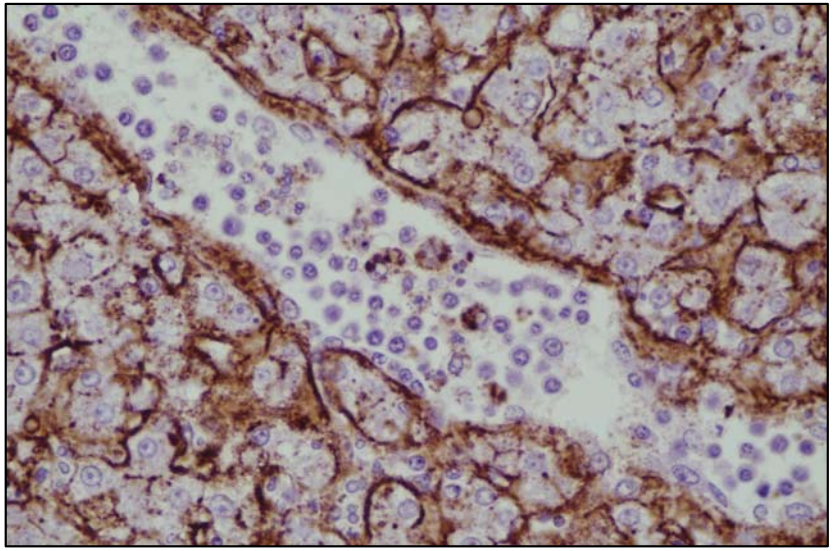
Hantaan



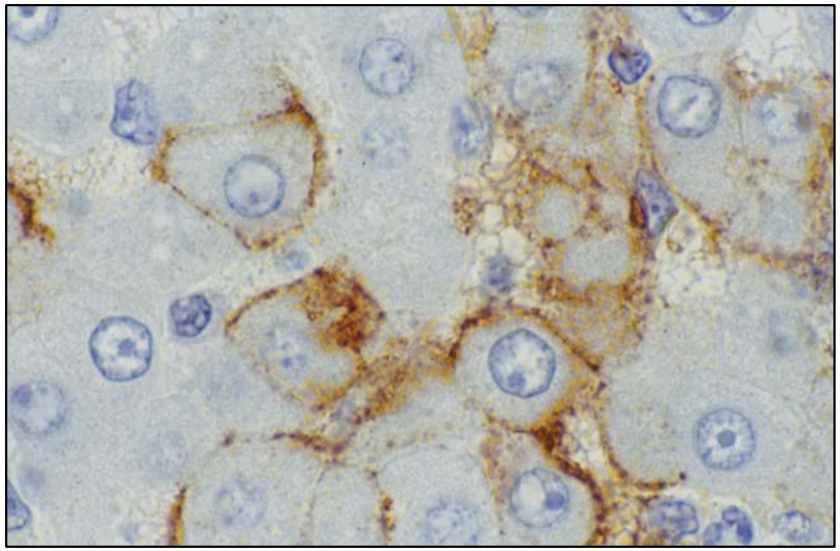
Lassa - Photo courtesy of Dr. Sherif Zaki, CDC

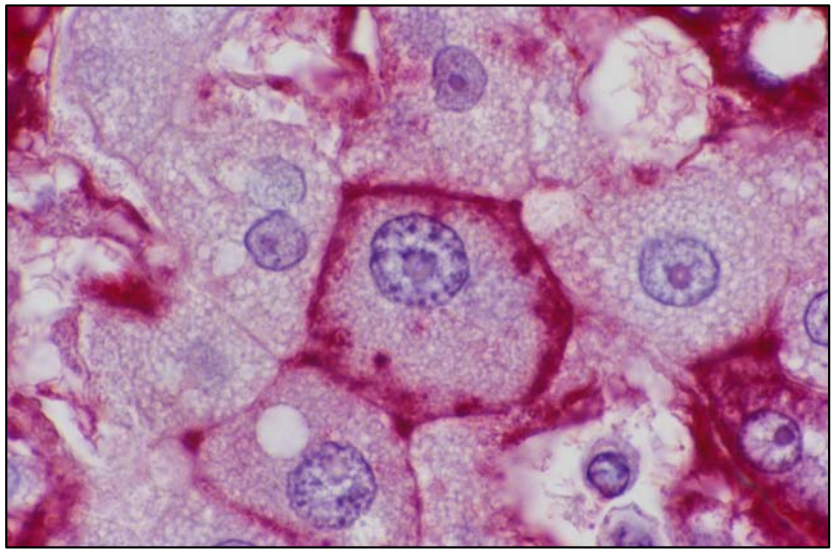
Infection of Liver

Marburg

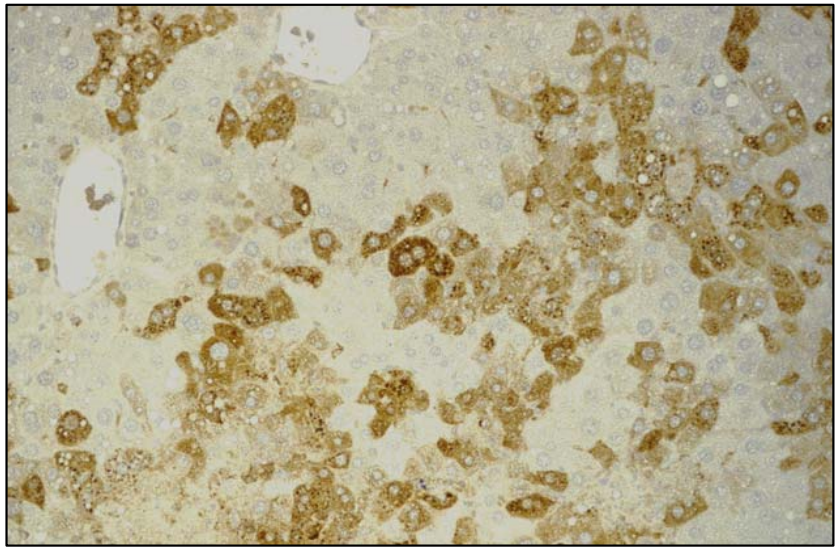


Lassa



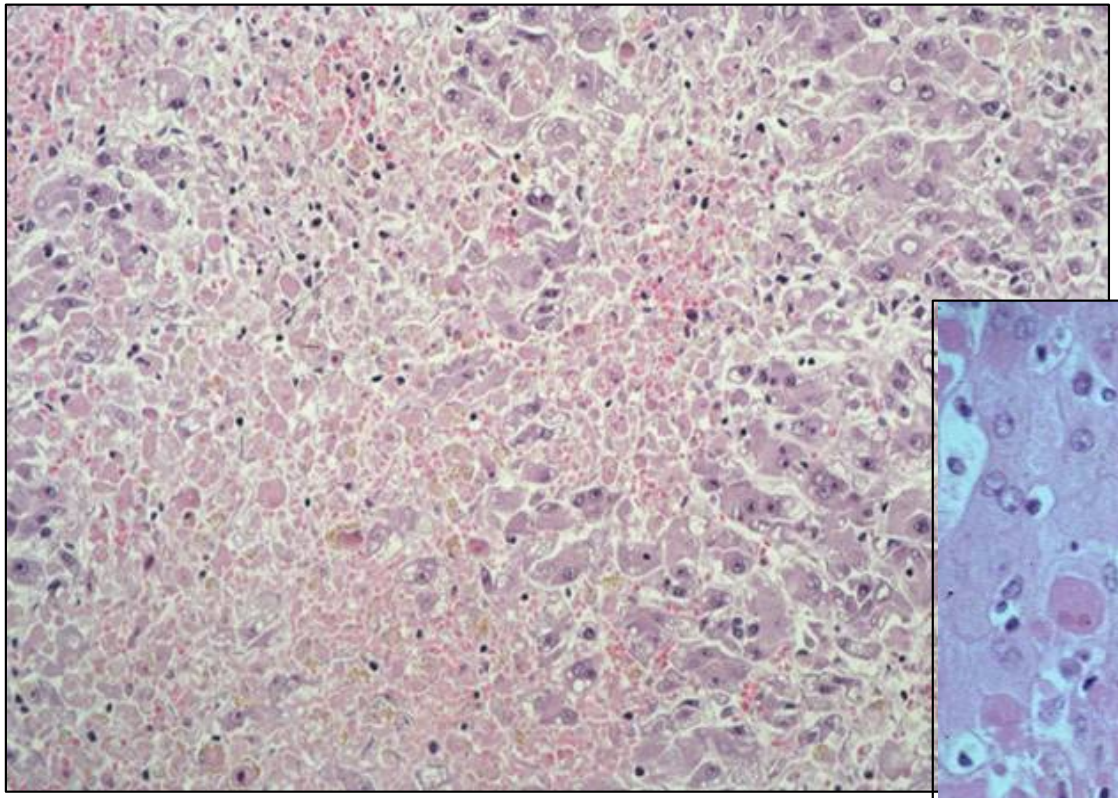


Ebola

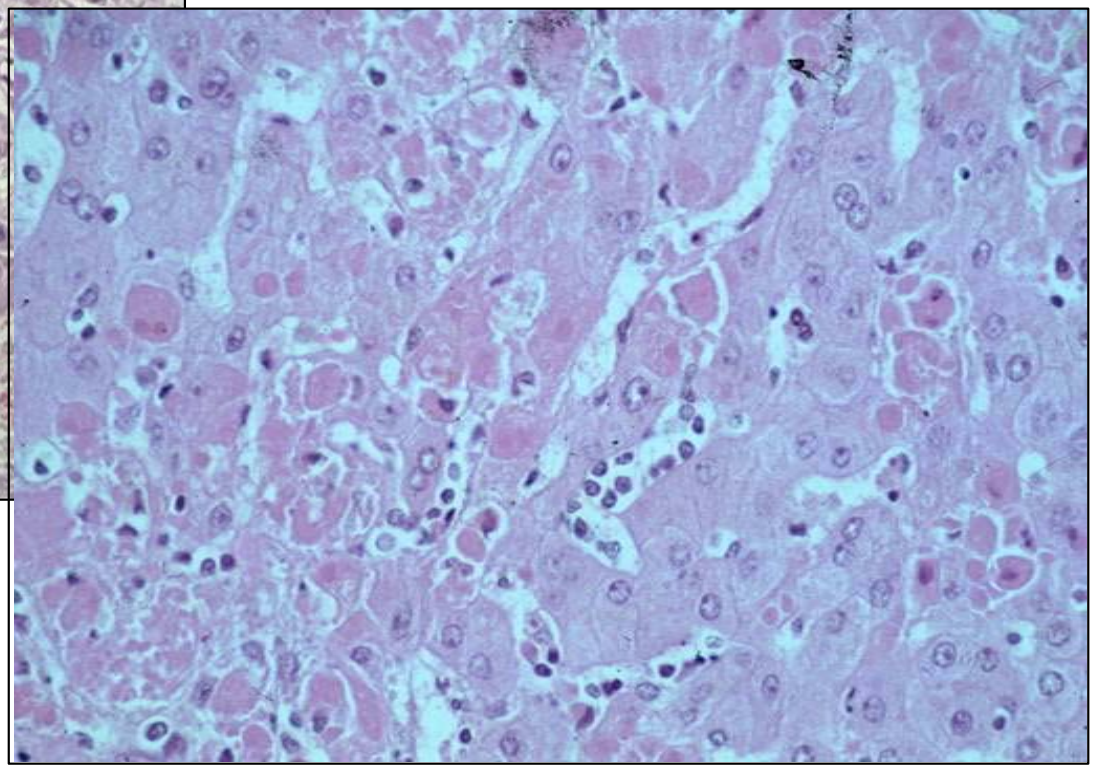


CCHF

Hepatic Necrosis



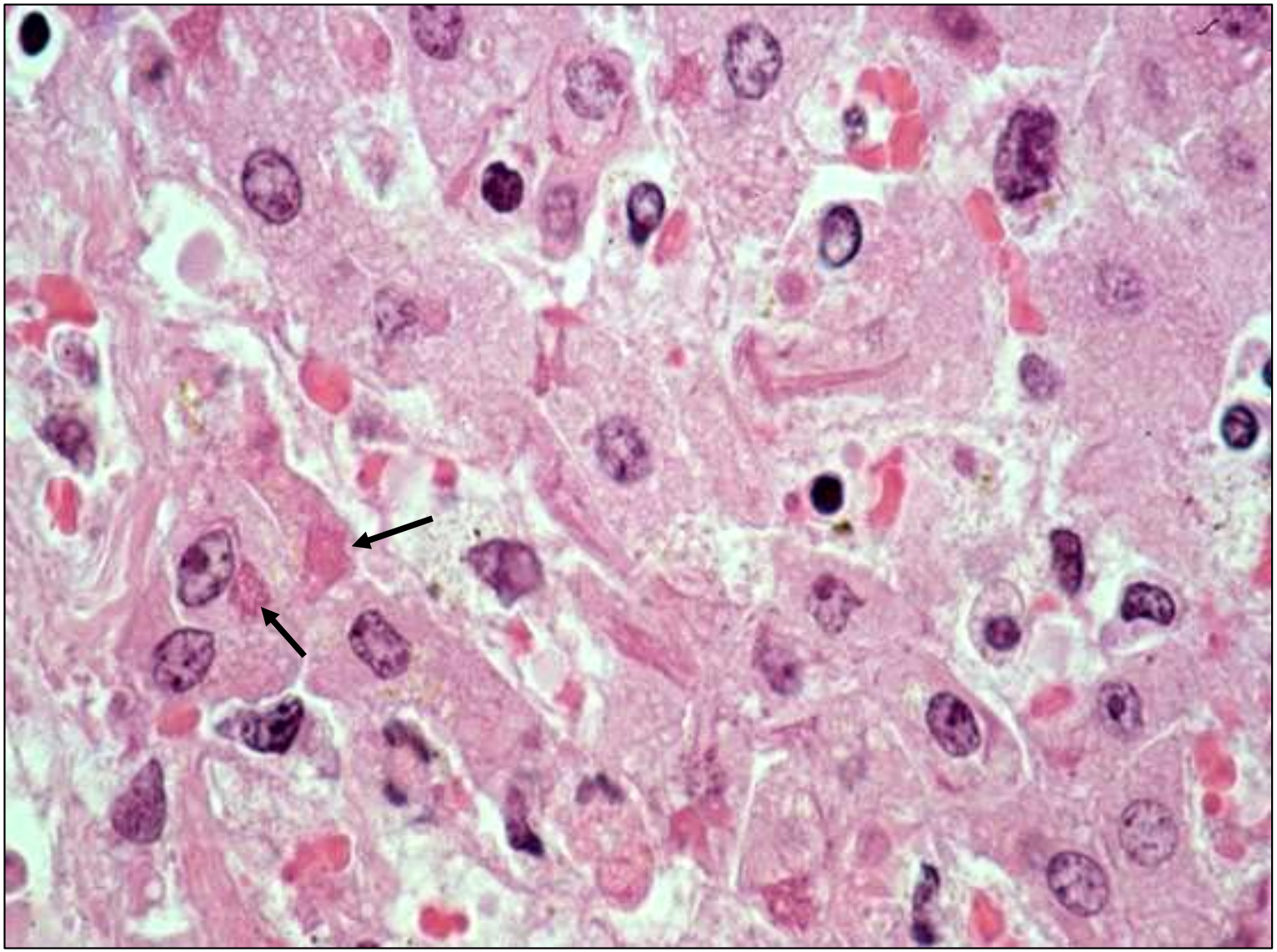
Rift Valley Fever



Lassa Fever

Photos courtesy of Dr. Sherif Zaki
Pathology, CDC

Liver - Ebola virus



Necrosis with viral inclusions (arrows)

Photo courtesy Dr. Sherif Zaki
Pathology, CDC

Clinical Features - VHF

- Early Nonspecific Symptoms
  - Fever, headache, malaise, dizziness
  - Myalgias
  - Nausea / vomiting / diarrhea (some VHFs)

- Initial Signs of Hemorrhage
  - Flushing, conjunctival injection
  - Petechiae, maculopapular rash
  - Positive tourniquet test (capillary fragility test)

- Progressive Signs
  - Hypotension
  - Bleeding manifestations
  - Shock and death

Clinical Features - VHF

DISEASE	Hemorrhage	Thrombocyto-penia	Leucocyte count	Rash	Icterus	Renal Disease	Pulmonary Disease	Tremor, Dysarthria	Encephalo-pathy	Deafness	Eye Lesions
ARENAVIRIDAE											
South American HF	+++	+++	⇩⇩⇩	0	0	0	+	+++	++	0	0
Lassa fever	+/S	+	0	++	0	0	+	+	+/S	++	0
BUNYAVIRIDAE											
Rift Valley fever	+++	+++		0	++	+		0	E	0	Retina
Crimean Congo HF	+++	+++	⇩⇩/⇧	0	++	0	+	0	+	0	0
HFRS	+++	+++	⇧⇧⇧	0	0	+++	+	0	+	0	0
HPS	+	++	⇧⇧	0	0	+	+++	0	+	0	0
FILOVIRIDAE											
Marburg and Ebola HF	++	+++		+++	++	0	+	0	++	+	Uveitis Retina?
FLAVIVIRIDAE											
Yellow fever	+++	++	0/⇩⇩	0	+++	++	+	0	++	0	0
DHF/DSS	++	+++	⇧⇧	+++	+	0	+	0	+	0	0
KFD/OHF	++	++	⇩⇩	0	0	0	++	0	E	0	Retina

Courtesy of Drs. Zaki & Peters

\+ occasional or mild
++ commonly seen, may be severe
+++ characteristic and usually marked

S characteristic, seen in severe cases

⇧ occasionally or mildly increased
⇧⇧ commonly increased, may be marked
⇧⇧⇧ characteristically increased and usually marked

E Develop true encephalitis but either after HF (KFD, Omsk) or in other patients (RVF)

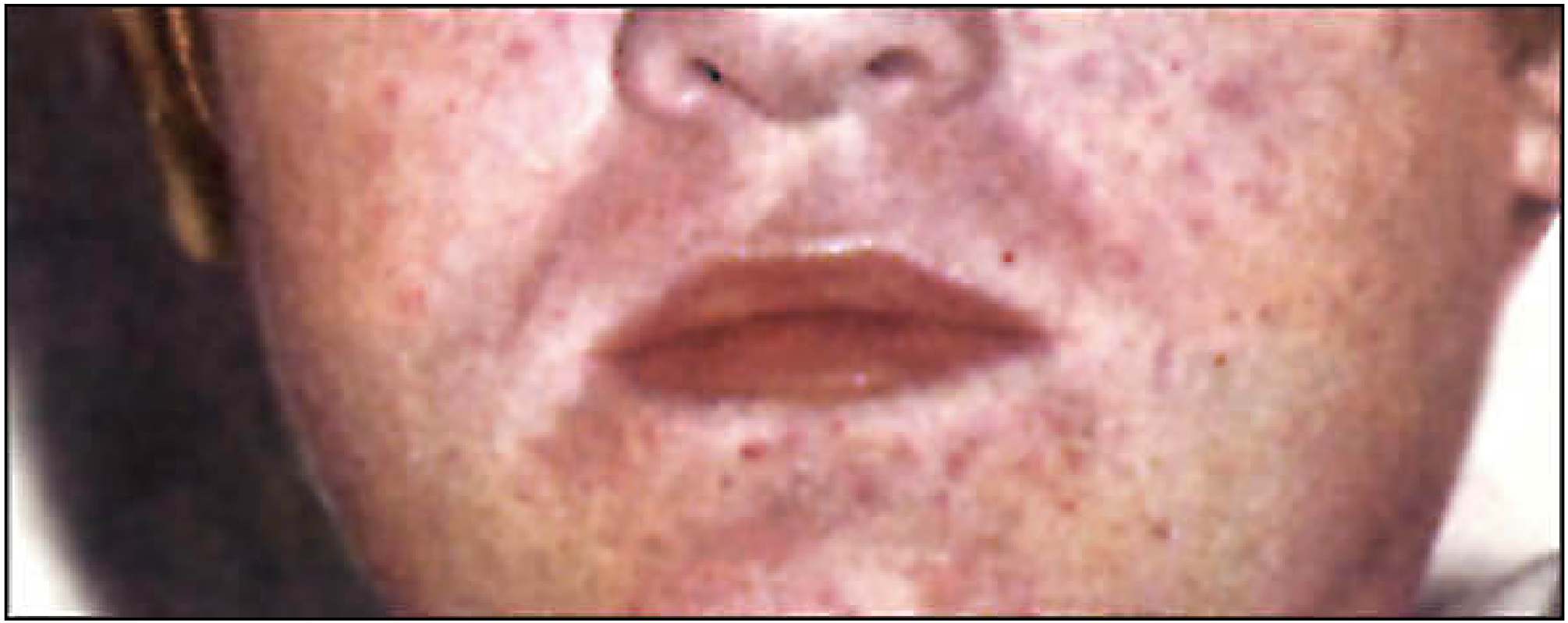
Clinical Features - Sequelae

- Prolonged Convalescence
- Hair Loss, Furrowed Nails
- Deafness (Lassa, EBOV)
- Retinitis (RVF, KFD)
- Uveitis (RVF, MBGV)
- Encephalitis (AHF, BHF, RVF, KFD, OHF)
- Pericarditis (Lassa)
- Renal insufficiency (HFRS)

Clinical Features - Filovirus

- Incubation time: 2 - 21days for EBOV & 2 - 14 days for MBGV
- High fever, headache, prostration, and myalgia
- Pharyngitis, diarrhea, **nonpruritic maculopapular rash**
- Disseminated intravascular coagulation (DIC)
- Hemorrhage (petechiae & ecchymoses)
- Terminal shock with multi-organ failure
- CBC: Severe thrombocytopenia & lymphopenia
- Clin Chemistry: Increased liver AST and ALT (reflects hepatic necrosis)

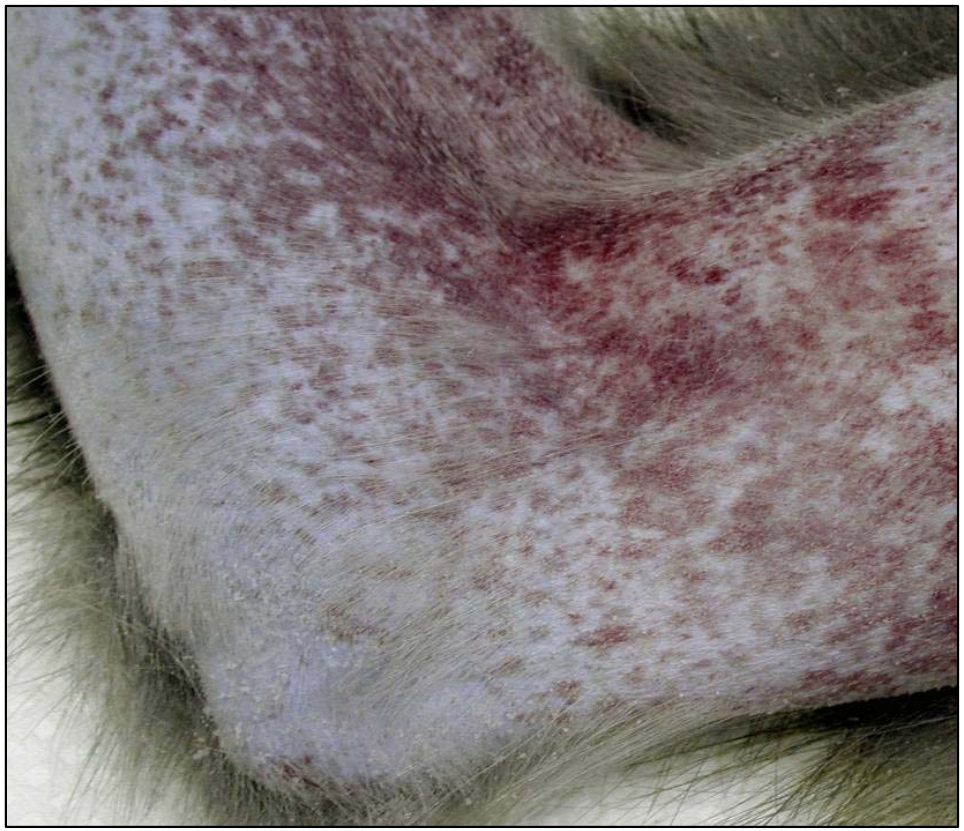
Marburg Infection Human



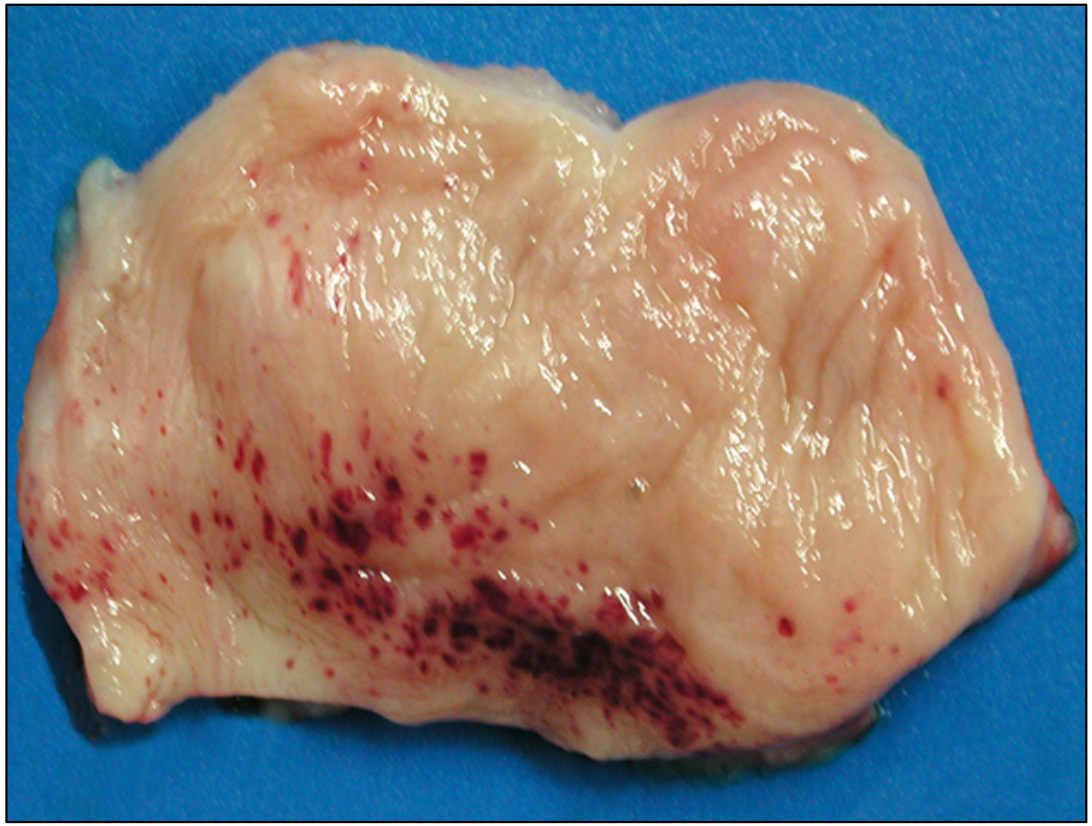
Maculopapular rash

Photo credit: Martini GA, Knauff HG, Schmidt HA, et. al. *Ger Med Mon*. 1968:13:457-470.

Ebolavirus Infection Primate



Maculopapular rash



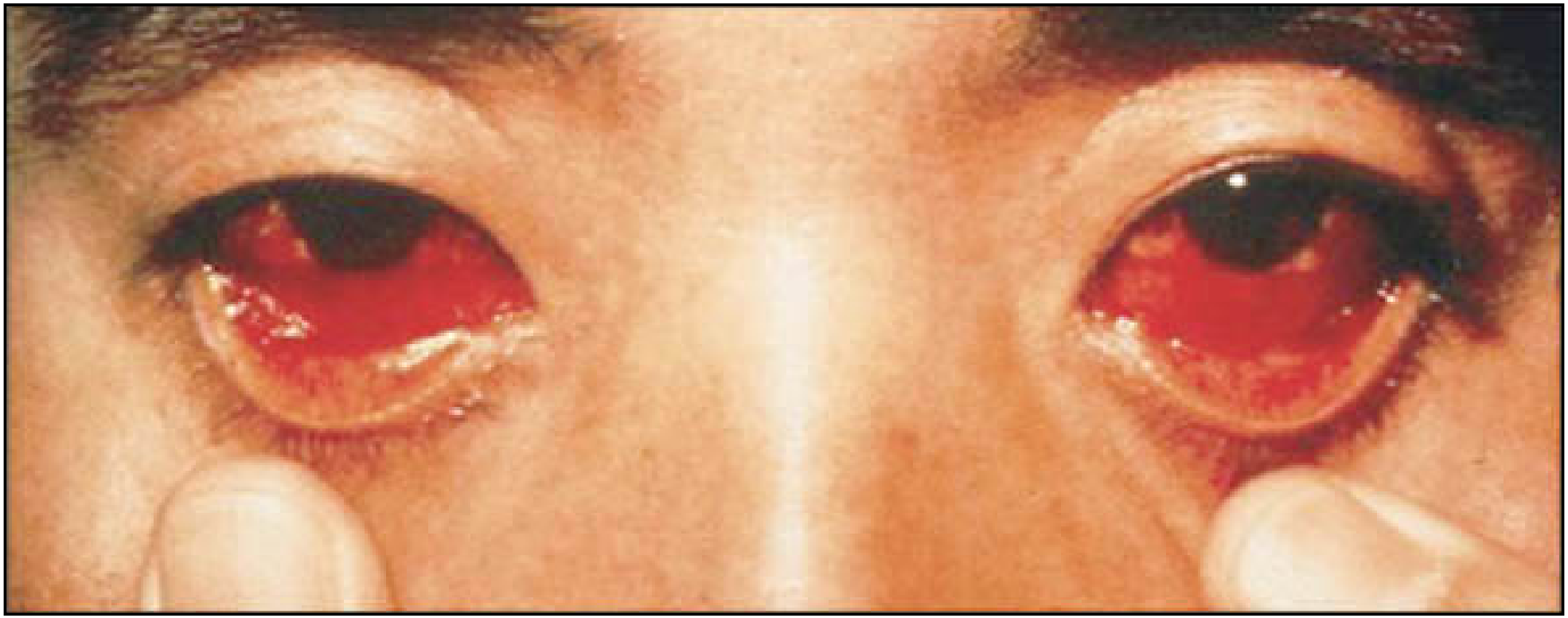
Urinary bladder hemorrhage

Photo credit: Larsen TL

Clinical Features - Arenavirus

- Incubation time is 7 - 16 days depending on virus
- Gradual onset of malaise, headache, retro-orbital pain
- **Conjunctival injection and hemorrhage**, fever, sweats, prostration
- Sore throat (described as severe w/ Lassa - exudative pharyngitis), nausea, vomiting, **lymphadenopathy**
- Petechia / ecchymoses may be present, facial erythema
- Can lead to shock, hemorrhage, pleural effusion, **encephalopathy** (tremors, generalized seizures)
- Fetal loss in greater than 80% of pregnant females
- **Hair loss and loss of coordination** may occur in convalescence

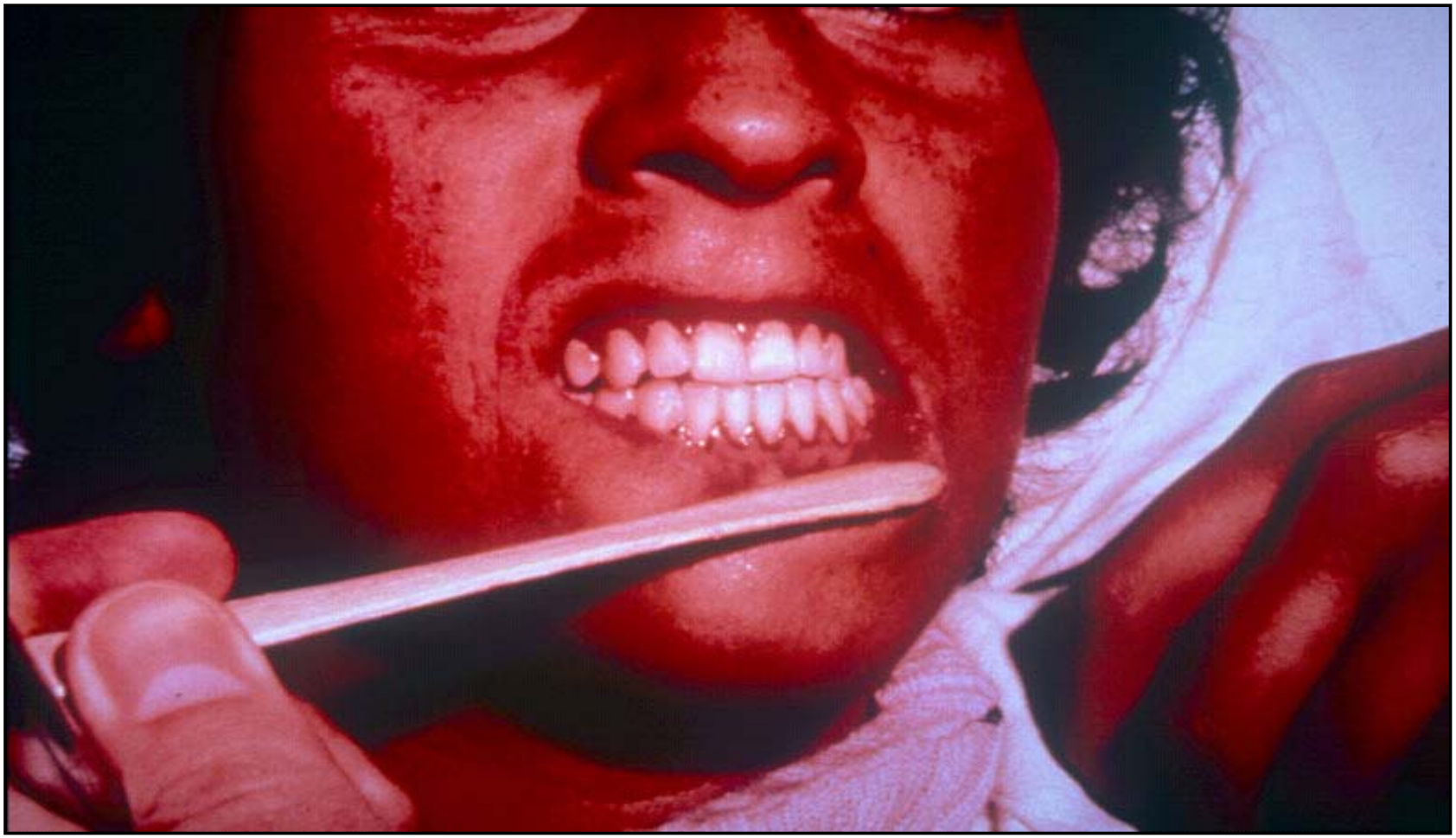
Bolivian Hemorrhagic Fever (Machupo virus – New World Arenavirus)



Conjunctival injection & subconjunctival hemorrhage

Ref: Current Science/Current Medicine (Peters CJ, Zaki SR, Rollin PE). Viral hemorrhagic fevers. In: Fekety R, vol ed. Atlas of Infectious Diseases, p10.1-10.26, Volume VIII, 1997.

Argentine Hemorrhagic Fever (Junin virus – New World Arenavirus)



Gingival hemorrhage

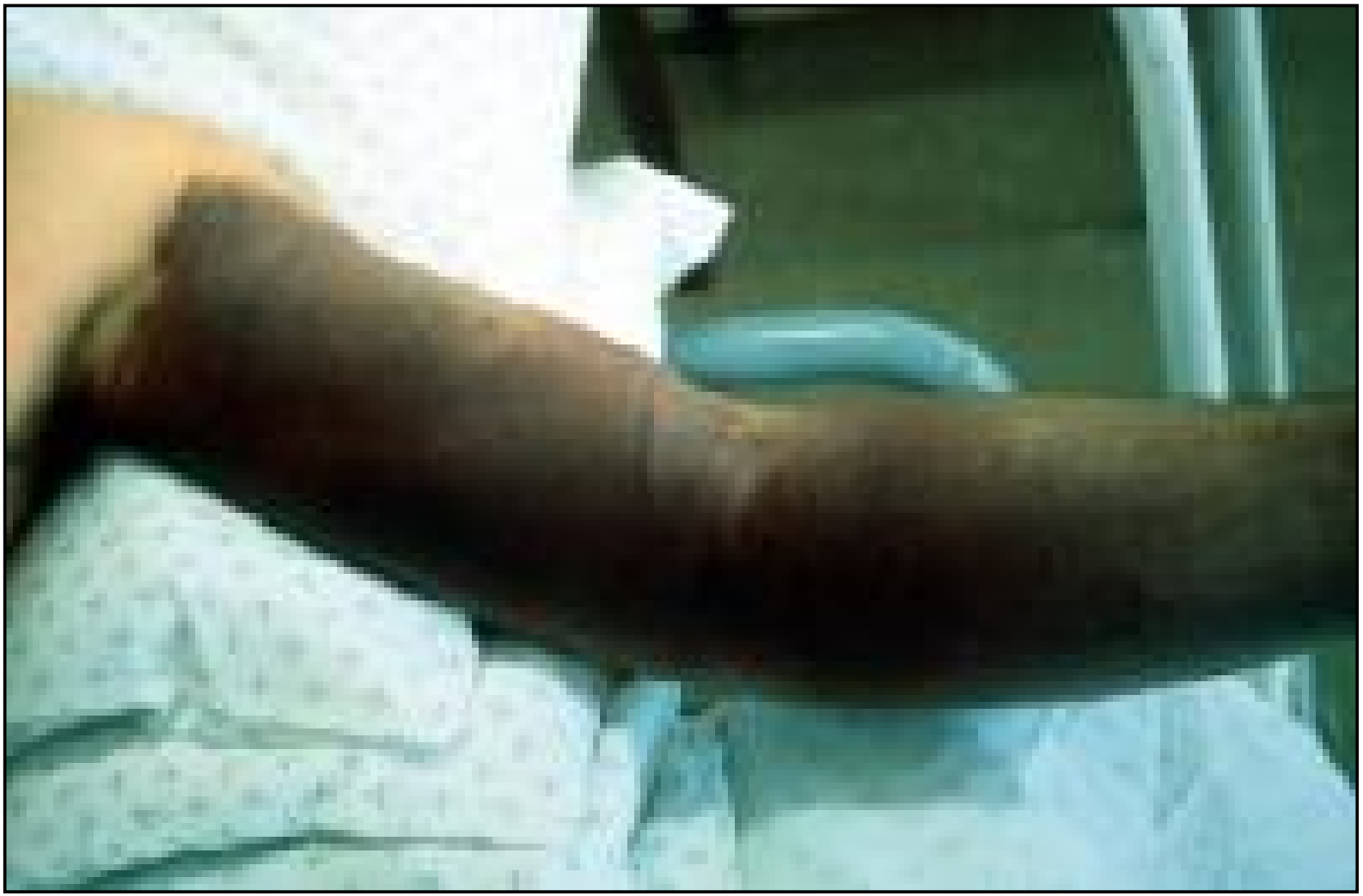
Clinical Features
Rift Valley Fever

- 2 to 6 day incubation period
- Mild Cases
  - Most human cases present as mild flu-like illness characterized by sudden onset of fever, myalgia, headache, backache, and photophobia with retro-orbital pain.
- Severe Cases
  - Eye disease (0.5-2.0%)
    - Retinal lesions with blindness if the macula is affected
    - Death is uncommon
  - Meningoencephalitis (1%)
    - Death is uncommon
  - Hemorrhagic fever syndrome (1%)
    - Liver disease, jaundice, vomiting blood, blood in feces, rash, bleeding from the gums
    - **Case fatality rate is 50%**

Clinical Features
Crimean Congo HF

- 3 - 12 day incubation period
- Sudden onset of fever, myalgia, stiffness, neck pain, dizziness, sore eyes, photophobia. May be diarrhea, nausea, vomiting, & generalized abdominal pain
- Restlessness, confusion, mood swings
- Detectable hepatomegaly occurs
- Petechia often giving way to ecchymoses, epistaxis, melena, hematuria, and gingival bleeding
- Hepatic and renal failure often ensue
- Mortality rate is around 30%.

CCHF



Left arm. Ecchymosis, diffuse, severe.
(1 week after clinical onset)

Photo credit: Robert Swaneopoel, PhD, DTVM, MRCVS, National Institute of Virology, Sandringham, South Africa.

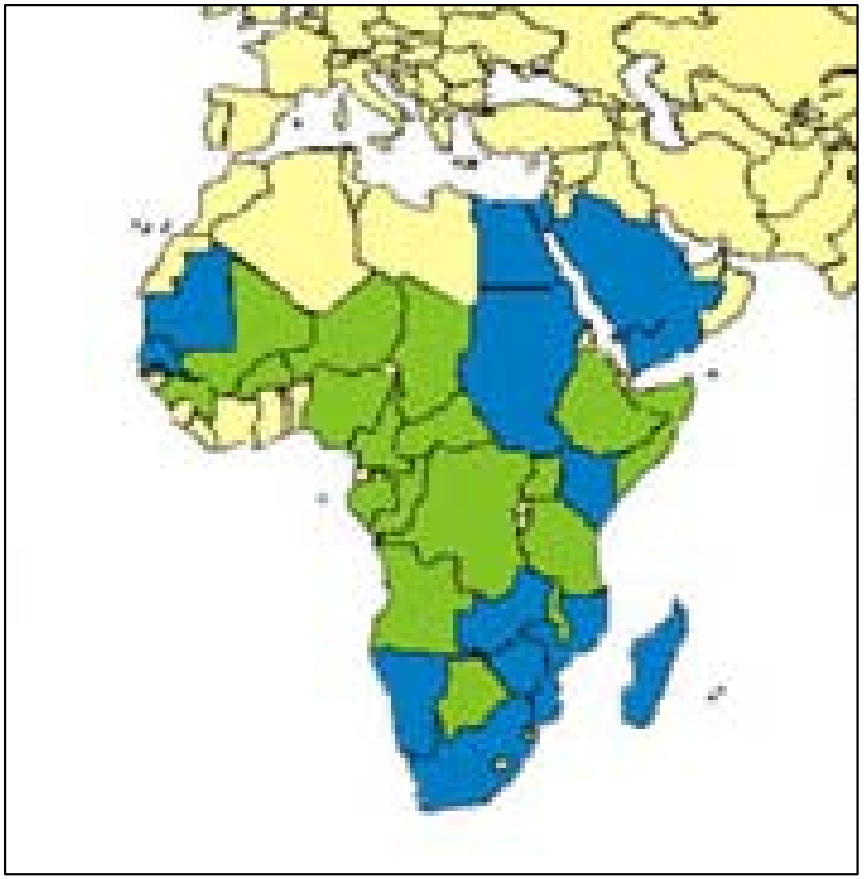
Differential Diagnosis of VHF

Clinical presentation: Flu-like illness, febrile, hemorrhage, thrombocytopenia, CNS signs, elevated liver enzymes (ALT, AST), leukopenia, DIC, multisystemic / multi-organ failure

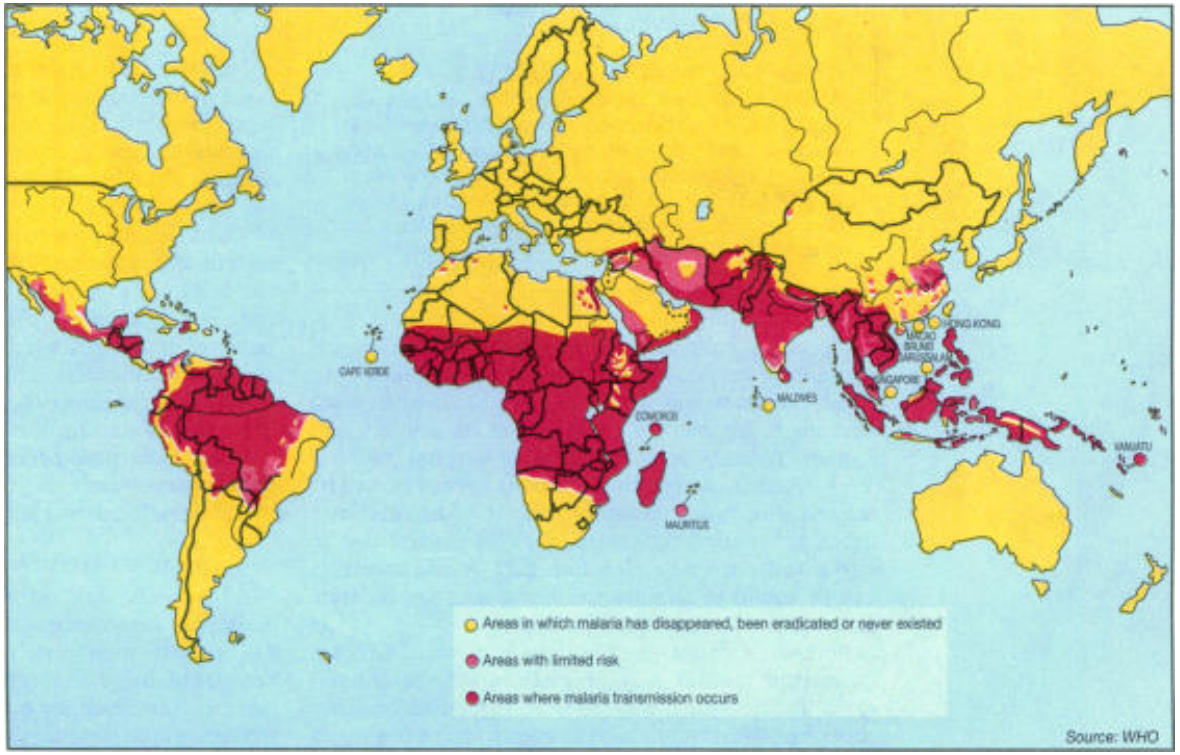
- **Protozoal**
  - Malaria
- **Bacterial**
  - Typhoid fever (*Salmonella typhi*)
  - Rocky Mountain Spotted Fever (*Rickettsia rickettsii*) & other rickettsioses
  - Leptospirosis
  - Meningococci
  - Q fever (*Coxiella burnetti*)
  - Plague
- **Viral**
  - Influenza
  - Viral meningitis / encephalitis (e.g. henipaviruses)
  - HIV / co-infection
  - Hemorrhagic form of smallpox in NHP model looks similar to VHFs.
- **Other**
  - Vasculitis, thrombotic thrombocytopenic purpura (TTP), hemolytic-uremic syndrome (HUS), heat stroke

Differential Diagnosis

Distribution of Rift Valley Fever



Distribution of Malaria



Diagnosis - Clinical Pathology

- **Thrombocytopenia or abnormal platelet function**
- Leukopenia (exception is Lassa, which has a leukocytosis)
- Some patients have anemia
- Most have elevated liver enzymes (ALT / AST)
- Bilirubin is elevated in RVF and YF
- **Prothrombin time, activated partial thromboplastin time (APTT) and bleeding time are prolonged**
- Some have disseminated intravascular coagulation (DIC); those that have DIC have elevated d-dimers (FDP's) and decreased fibrinogen

Diagnosis of VHFs

- **History (incl. travel, animal illness / death,…)**
- **Epidemiology: Geography, season, occupation, exposure to vectors**
- **Clinical signs and symptoms**
- **Clinical pathology**
- **Laboratory confirmation required**

Diagnosis
Laboratory Confirmation

- Rapid ELISA techniques most easily employed
  - Antigen capture detection
  - IgM (test of choice for Hantaviridae, yellow fever, & Dengue) or IgG antibody capture
- Serology on paired sera
- Immunohistochemistry (IHC) & in situ hybridization (ISH) of infected tissues
  - Formalin-fixed tissue
  - CDC has developed a skin biopsy procedure for detection of EBOV using IHC

Diagnosis
Laboratory Confirmation (cont.)

- **Virus isolation from blood, serum or tissue biopsy is Gold Standard**
- **Electron microscopy can provide definitive evidence**
- **Reverse transcription - polymerase chain reaction (RT-PCR)**
  - **Increasingly important tool**

Processing Clinical Specimens

- Whole blood w/ anticoagulant (however, be careful as some anti-coagulants may be inhibitory in PCR assays)
- Urine, throat swab or wash
  - In sealed plastic tube w/10% FBS or 1% HSA final conc.
- Label each specimen
- Swab exterior of each container with disinfectant
- Double-bag, swab exterior with disinfectant before removal from patient's room

Send to a Level 4 Biosafety Lab

Clinical Laboratory Procedures

- Strict barrier precautions
  - Gloves, gown, mask, shoe covers, protective eye and faceshield
  - Consider respirator with HEPA filter
  - Handle specimens in biosafety cabinet when possible
- Spills/splashes
  - Immediately cover with disinfectant, allow to soak for 30 minutes
  - Wipe with absorbent towel soaked in disinfectant
- Waste disposal
  - Same as for patient isolation practices

Medical Management

The foundation of treatment is supportive care

- Hemodynamic resuscitation & monitoring
- **Careful management of fluid and electrolytes, blood pressure, and circulatory volume**
  - Use of colloid: Usually fluid of choice
  - Hemodialysis or hemofiltration as needed
    - Esp. HFRS patients
- Vasopressors and cardiotonic drugs (some cases do not respond to i.v. fluids)
- Cautious sedation and analgesia

Medical Management

- DIC may be important in some VHFs (RVF, CCHF, filoviruses)
- Coagulation studies and clinical judgment as guide
  - Replacement of coagulation factors / cofactors
  - Platelet transfusions
- No aspirin, NSAIDs, anticoagulant therapies, or IM injections

Medical Management
Antiviral Therapy

- Ribavirin
  - Investigational drug, compassionate use
  - most effective against Lassa fever and Hantaviruses
  - Arenaviridae (Lassa, AHF, BHF)
  - Bunyaviridae (HFRS, RVF, CCHF)
  - No utility for Filoviridae or Flaviviridae

- Immune (convalescent) plasma
  - Arenaviridae (AHF & BHF; +/- Lassa): TOC
  - Passive immunoprophylaxis post-exposure?
  - Experimental studies in animals have not proven efficacy against filovirus infection

Medical Management For Arenavirus & Bunyavirus

- Ribavirin Treatment
  - 30 mg/kg IV single loading dose
  - 16 mg/kg IV q 6 hr for 4 days
  - 8 mg/kg IV q 8hr for 6 days

- Prophylaxis
  - 500 mg PO q 6 hr for 7 days

Note: Parenteral and oral Ribavirin are investigational and available only through human use protocols

Borio L, *et al. JAMA* 287(18):2391-2405, 2002
McCormick JB *et. al. N Eng J Med* 314(1):20-26, 1986
Jahrling PB *et al. J Infect Dis* 141:580-589, 1980

Medical Management
HFRS (bunyavirus) Therapy

- Intravenous Ribavirin treatment regimen:
  - 33 mg/kg (2.0 gm/60kg) single loading dose
  - 16 mg/kg (1.0 gm/60kg) q 6h for 4 days
  - 8 mg/kg (0.5 gm/60kg) q 8h for 3 days

- Note: parenteral Ribavirin is investigational and available thru human use protocols only

Huggins et. al. ***J Infect Dis*** **164:1119-27, 1991.**

Medical Management of Hemorrhagic Syndrome
Potential of Activated Protein C (Xigris®)

- **VHF's are grouped by the syndrome they produce – not by agent: Activated protein C (rhAPC / Xigris®) targets the syndrome.**
- rhAPC labeled for use in syndrome, not as a specific antiviral chemotherapeutic. rhAPC has no anti-EBOV activity *in vitro*.
- Serves as an exogenous source of activated protein C. Has **anti-thrombotic, pro-fibrinolytic, and anti-inflammatory effects**.
- DIC a common manifestation in several VHFs, especially filoviridae; rapid and significant depletion of endogenous protein C during disease.
- Significant declines in protein C levels also reported in patients with Argentine hemorrhagic fever.
- Recent study at RIID: rhAPC (Xigris®) had beneficial effects in most NHPs (including survival in 2) challenged w/ lethal dose of ZEBOV.

Hensley et. al.: In publication 2007.

Medical Management of Hemorrhagic Syndrome Potential of Xigris®

- Approved Xigris dose in humans for severe sepsis is 24 μg/kg/hr for 96 hrs. Highest NOAEL* (No Observed Adverse Event Level) from toxicology studies in monkeys and in phase 1 studies is 48 μg/kg/hr.

- Disadvantages of Xigris: Administered by continuous I.V. infusion, short high-life, expensive, and potential for development of immune antibodies against recombinant product (patient becomes refractory to drug treatment after prolonged therapy).

- **Not the "magic bullet",** but one possible component in combination therapy protocols for various VHFs.

Hensley LE, et. al.: In publication 2007.

Infection Control

Source of Contaminant

Patient fluids, tissues

Local Environment (fomites)

Aerosol

Host / Vector

Agents of Concern

Arenaviruses

Filoviruses

KFV, RVFV

Route of Entrance

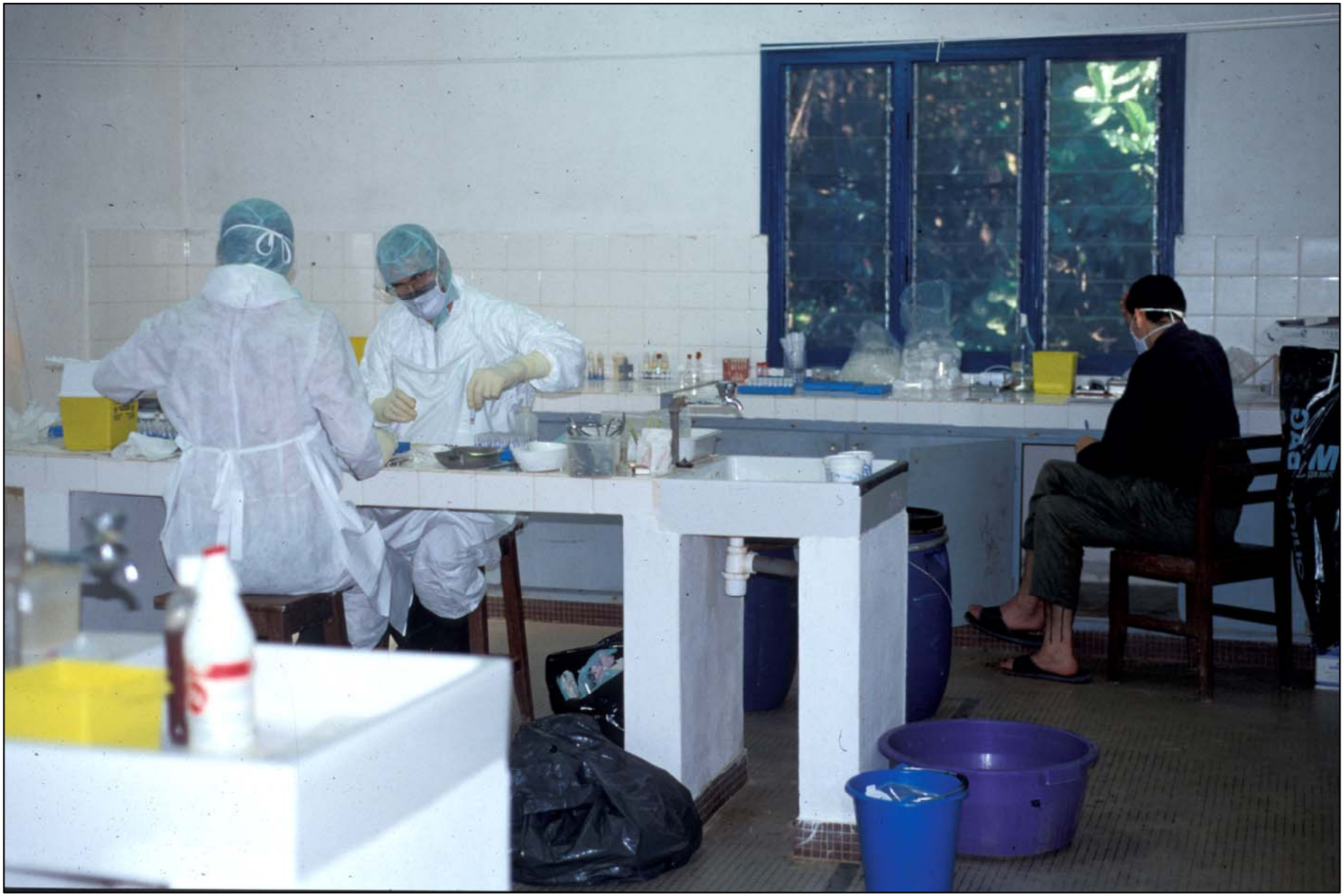
Inhaled

Mucous Membranes, skin

Parenteral inoculation

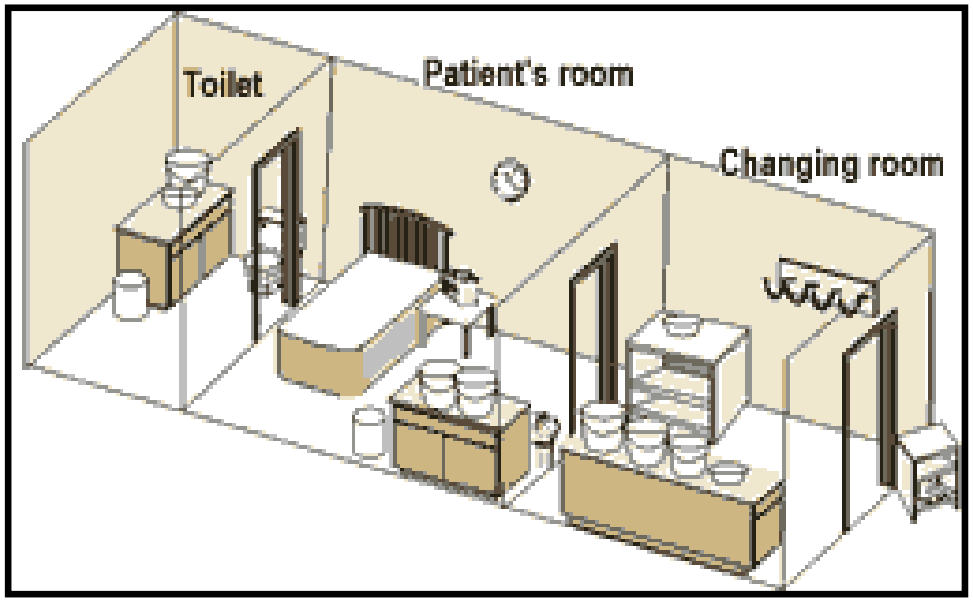
Effective Prevention Methods ?

Effective Prevention Methods ?



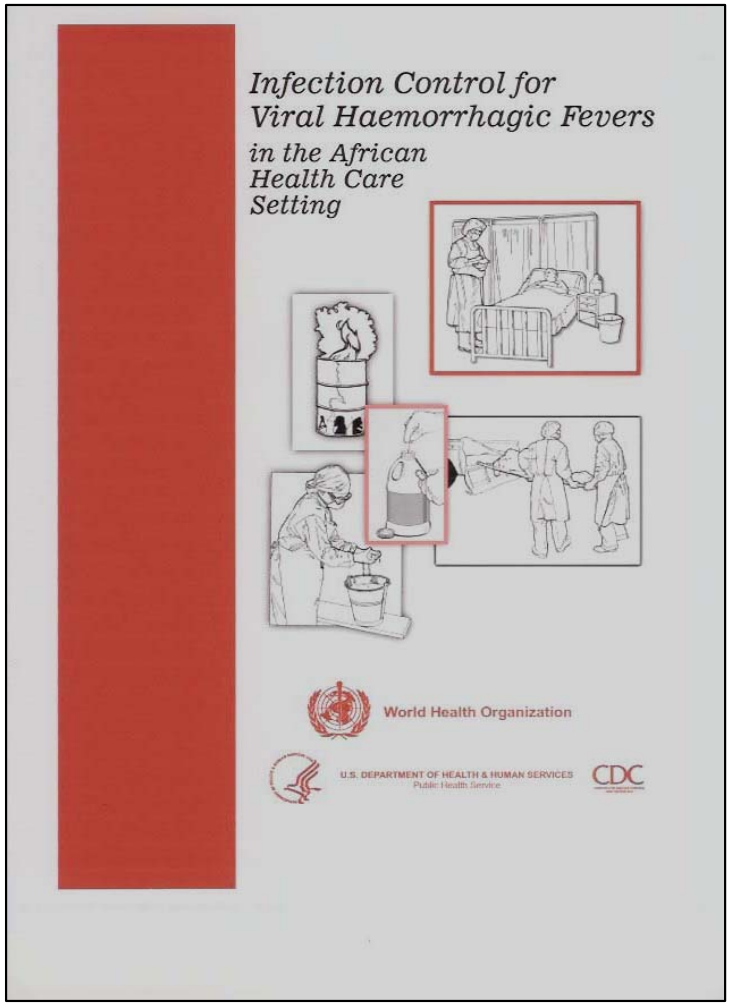
Infection Control

- **Single room with adjoining anteroom as only entrance**
  - Changing area/protective equipment
  - Disinfection solutions
    - 0.5% sodium hypochlorite, 2% glutaraldehyde, phenolic disinfectants (0.5%-3.0%), soaps and detergents
  - Hand washing stations
  - Chemical toilets



Infection Control

- **Negative air pressure; air not recirculated**
  - ✓ If prominent hemorrhage, cough, vomiting, diarrhea present
  - ✓ Not required early in the disease, but if available use to prevent having to transfer patient later



http://www.cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual.htm

Infection Control (cont.)

- Strict barrier precautions
  - Double gloves, gown, mask, shoe covers, protective eyewear / faceshield
- Personal Protective Equipment
  - Powered air-purifying respirators (PAPR) vs. N-100 disposable mask
  - Prominent hemorrhage, cough, vomiting, diarrhea
- Limit patient care to minimal # of caregivers
  - Reliable and competent individuals
  - Minimize exposure risk
- Education
  - Demonstrated decrease in disease incidence

Medical Management
First Aid for Exposures

- Wash / irrigate wound or site immediately
  - within 5 minutes of exposure
- Mucous membrane (eye, mouth, nose)
  - continuous irrigation with rapidly flowing water or sterile saline for > 15 minutes
- Skin
  - scrub for at least 15 minutes while copiously soaking the wound with soap or detergent solution
    - fresh Dakin's solution (0.5% hypochlorite):
      - 1 part standard laundry bleach (5% hypochlorite - note that bleach is sold in different concentrations)
      - 9 parts tap water

Management of Patient Contacts

- Casual contacts
  - Remote contact with index patient (e.g., same airplane)
  - No known risk
- Close contacts
  - Same household, physical contact, nursing care, handling lab specimen
  - Report as soon as VHF considered likely in the index patient; place under surveillance
  - Record temp b.i.d. for 3 weeks post-exposure
  - Therapy: fever ($T^{o} > 101^{o}$ F) or other systemic symptoms within 3 weeks post-exposure

Management of Patient Contacts (cont.)

- High-risk contacts
  - Mucous membrane contact with infected person (e.g., kissing, sexual intercourse).
  - Needlestick or other penetrating injury involving exposure to patient's secretions, excretions, blood, tissues, or other body fluids.
  - Any patient in this category that develops a Tº of 101ºF or higher or other symptoms consistent with VHF should be treated as a VHF patient to include quarantine and post-exposure prophylaxis measures if available.

Remember: Some of the HFVs can be excreted for many weeks / months in semen as demonstrated with MARV and in urine with Lassa.

Prevention / Control

- RIFT VALLEY FEVER Vaccines
  - Formalin-inactivated
    - safe but requires 3 shots, intermittent booster
    - limited supply
  - Live, attenuated MP-12
    - Phase II testing

- Ebola Vaccines
  - All experimental in primates; success with adenovirus- & VSV-vector based platforms in nonhuman primates

- Marburg Vaccines
  - Recent NHP study at RIID: 100% survival following challenge w/ lethal dose of MBGV and then post-exposure treatment w/ recombinant VSV-GP Marburg vaccine

Daddario et. al.: *Lancet* 367:1399-1404, May 2006

Prevention / Control

- YELLOW FEVER
  - Licensed 17D vaccine, highly efficacious
  - Recent reports of vaccine associated deaths
  - Cannot be used in persons with egg allergy

- ARGENTINE HEMORRHAGIC FEVER
  - Live, attenuated vaccine
  - Safe and efficacious
  - Protects monkeys against Bolivian HF

Prevention / Control

http://www.who.int/csr/resources/publications/ebola/whoemcesr982sec1-4.pdf

Questions?

USAMRIID
United States Army
Medical Research Institute
of Infectious Diseases

Biodefense solutions to protect our nation

The Alphaviruses

Presented by:
Pamela Glass, Ph.D.
Microbiologist, Virology Division
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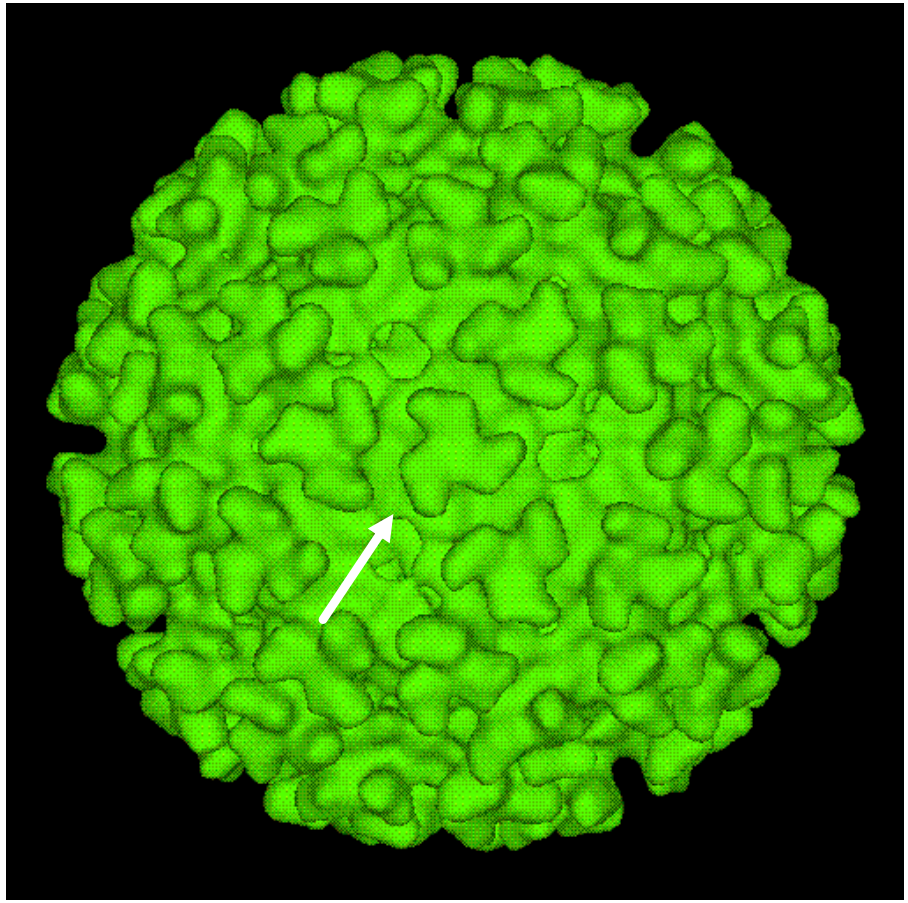
Learning Objectives

- **Students should be able to:**
  - **Name the important *Alphavirus* threat agents**
  - **Discuss natural and aerosol transmission of alphaviruses**
  - **Identify assays for diagnosis in medical and field settings**
  - **Describe clinical illness associated with VEE**
  - **Describe management of infection**
  - **Describe countermeasures against infection**

What is an *Alphavirus*?

- **Family *Togaviridae***
  - **Genus *Alphavirus***
  - **25 virus species**
- **50 – 60 nm, icosahedral, enveloped**
- **Genome = 11-12 kB of single-stranded, positive-sense RNA**



USAMRIID
United States Army Medical Research Institute of Infectious Diseases
Biodefense solutions to protect our nation

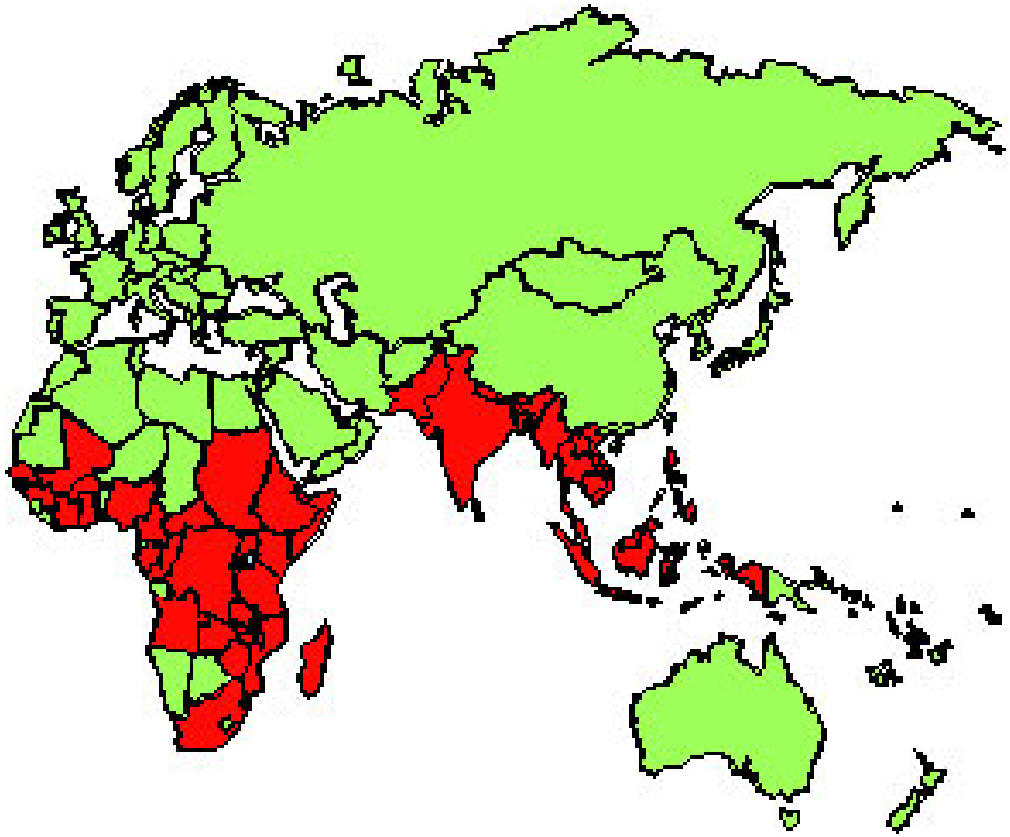
Alphaviruses

- Most cycle between mosquito and birds/small mammals

- Ten viruses infect humans
  - Old World – Polyarthritis group
    - Examples:
      - Ross River, O'nyong nyong, Chikungunya
  - New World – Encephalitis group
    - Examples:
      - Venezuelan equine encephalitis virus (VEE)
      - Eastern equine encephalitis virus (EEE)
      - Western equine encephalitis virus (WEE)

Chikungunya Outbreak 2004-2006

- **Islands of the Indian Ocean ~300,000 suspected cases**
  - 264,000 suspected on Reunion alone
  - islands of Comoros, Lamu, Madagascar, Mauritius, Mayotte, and the Seychelles
- **India**
  - >180,000 suspected cases
  - “173 people have died of chikungunya in the state of Kerala this year” - may be due to co-infection or underlying causes
  - Outbreak ongoing



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History

- **Epidemic (epizootic) encephalitis in horses**
  - **Eastern US from 18th – 19th century**
- **Viruses isolated from brains of ill horses**
  - **WEEV - CA, 1930**
  - **EEEV – NJ/VA, 1933**
  - **VEEV – Colombia, 1938**

- **Classified in 1954 as Group A arboviruses**
  - **Later re-classified as genus w/in *Togaviridae***

History

- **Human disease evident by 1938**
  - **Outbreak (30 cases) of human encephalitis in New England from EEEV**
  - **WEEV recovered from brain of child with fatal encephalitis**

- **Evidence of VEE in humans**
  - **Laboratory acquired infections in early 1940's**
  - **Epidemics in Colombia, Venezuela, Panama 1950's – 1960's: 100,000's of cases**

Why Should I Pay Attention?

- Encephalitic alphaviruses are CDC Category B agents
- VEEV is on NATO BW threat list
- New World alphaviruses (VEEV, EEEV, WEEV) are most significant threats
  - Particularly VEEV

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Optimal Characteristics of BW Agents

- Easily produced with minimal infrastructure requirements
- Adequate stability during storage and delivery
- High infectivity rates / short incubation period
- Consistent induction of desired disease
- Amenable to vaccination

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Alphaviruses As BW Threats

- Highly infectious by aerosol
- Incapacitating
- Readily produced in large quantities
- Chemically stable
- Multiple serotypes
- Large effective coverage (1g/10,000 Km2)

Alphaviruses as Weapons

- **US offensive program**
  - **Weaponized VEEV**
    - **Aerosol delivery**
  - **Experimented with EEEV, WEEV**

- **Laboratory acquired VEE at Ft. Detrick**

- **Soviet BW program**
  - **Weaponized VEEV**

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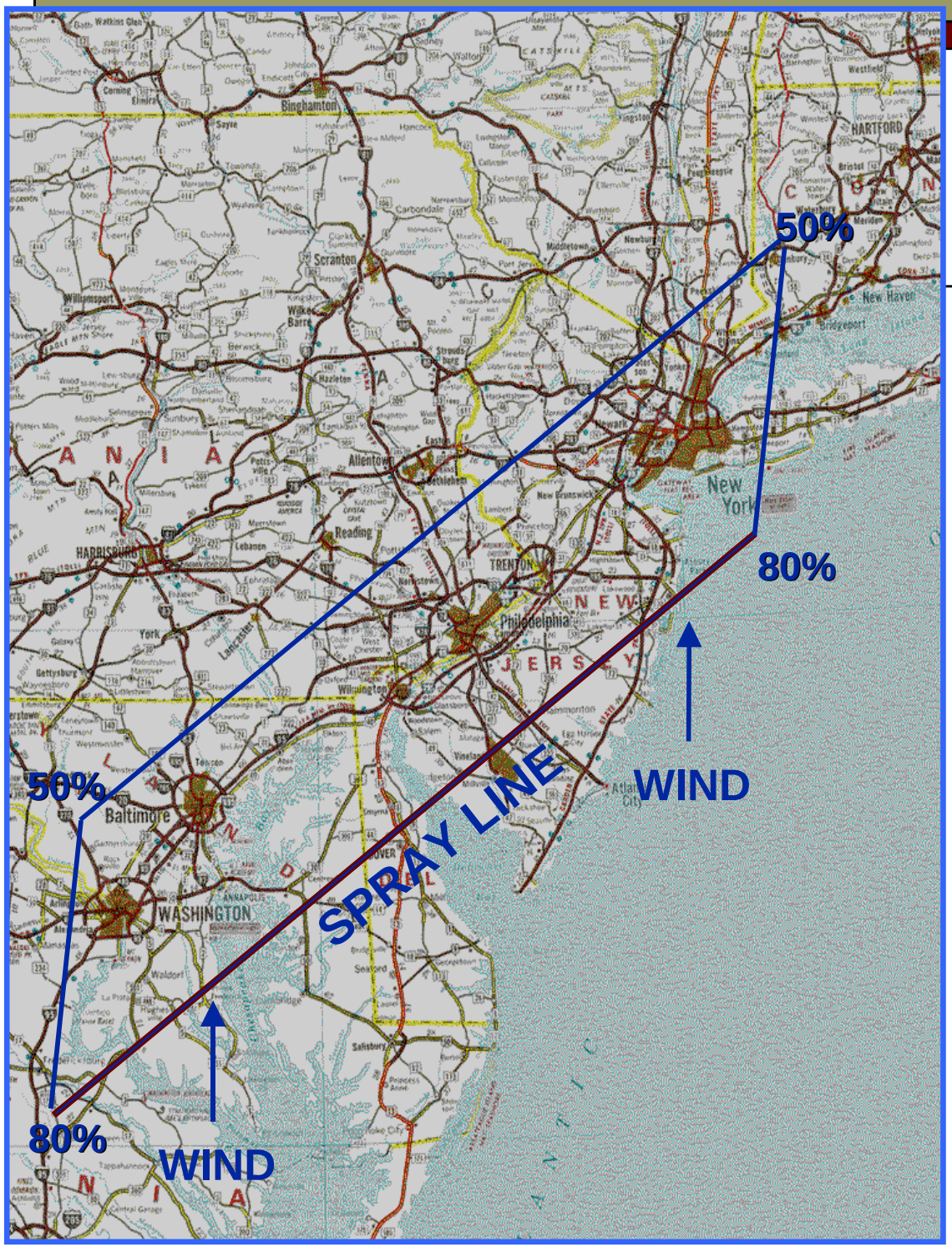
Superiority of VEEV as Weapon

- **Infection/disease ratio:**
  - **VEE 1:1**
  - **EEE 23:1**
  - **WEE 1150:1**
- **Clinical disease of VEE distinct**
  - **VEE is really an acute, febrile, self-limited but incapacitating illness that is only rarely associated with encephalitis**



VEE Virus
Predicted Casualty
Percentage Footprint

Meterological Assumptions

- Time: 1900 hrs
- Wind: 15 km/hr; S
- Temperature: 27
- Humidity: 50% RH
- Atmospheric Stability: Neutral
- Spray Line: 200 Miles

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VEEV Complex

- VEEV constitutes a whole clade of viruses
- Previously classified as six main subtypes of VEE (I-VI) with certain subtypes further divided
- Recent taxonomy changes (*species*)
  - *Venezuelan equine encephalitis virus*
    - Subdivided into IA/B, IC, ID, IE, IF viruses
    - Most epizootic and human cases associated with subtypes I-AB (Trinidad donkey strain) and I-C (P676 strain)
  - *Everglades virus* (subtype II)
  - *Mucambo virus* (subtype IIIA)
  - *Tonate virus* (subtype IIIB)
  - *Pixuna virus* (subtype IV)
  - *Cabassou virus* (subtype V)
  - *Rio Negro virus* (subtype VI)

Transmission Cycle - VEEV

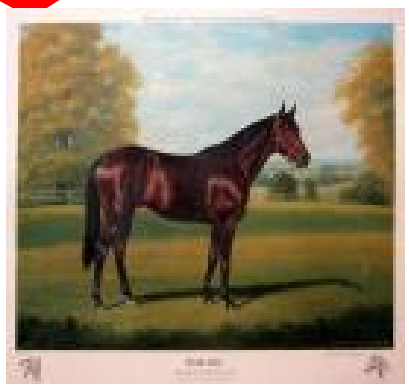
Normal (Endemic)

Virus mutation

+

Bridge vector

Outbreak (Epizootic/Epidemic*)



*epizootics usually precede epidemics by 1-2 weeks

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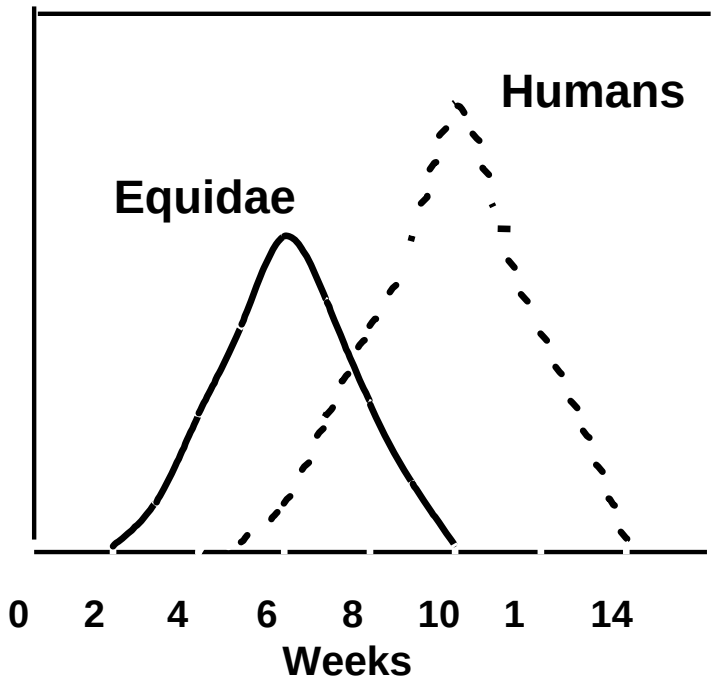
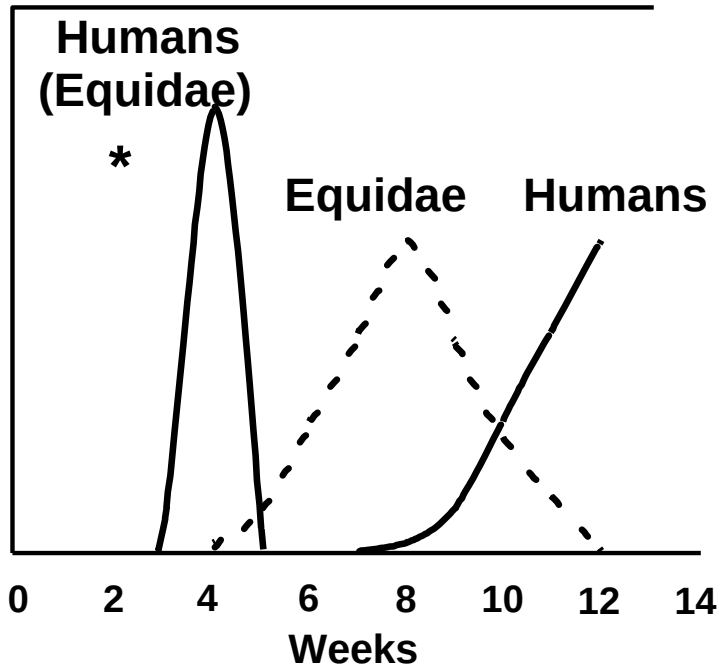
Epidemic Curve: Natural vs. BW Attack

Natural Epidemic



Aerosol with Secondary Spread



* Mean Incubation Period + 2 SD

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VEE Disease

Equids

- Fever, encephalitis, leukopenia occ. pulmonary symptoms,
- Morbidity = 40-50%
- Inapparent:apparent infection = 2:1
- Mortality = 20-40%

Humans

- Morbidity = 10-50%
- Inapparent:apparent infection = 1:1
- Neurologic illness
  - 0.4% adults
  - 4.0% Children
- Case fatality
  - <0.5% all cases
  - 20% neurologic cases

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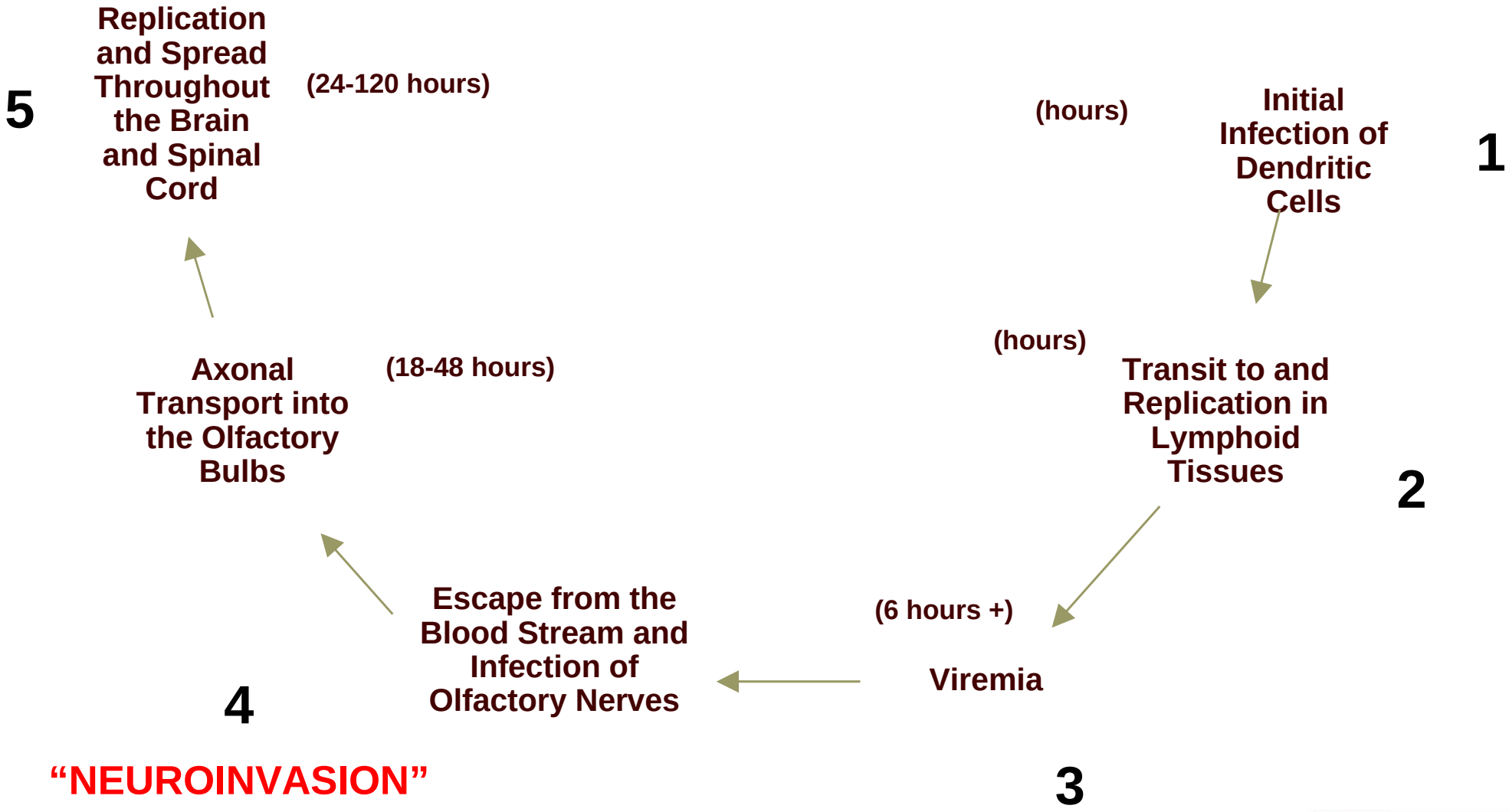
VEE: Pathogenesis

- **Primary Site of Replication/Injury:**
  - **lymphoid tissue, bone marrow**
- **Secondary Hematogenous Invasion**
  - **brain, spleen, muscle, liver, lungs, placenta, fetus.**

VEE Virus Kinetics Following Peripheral Infection of Mice



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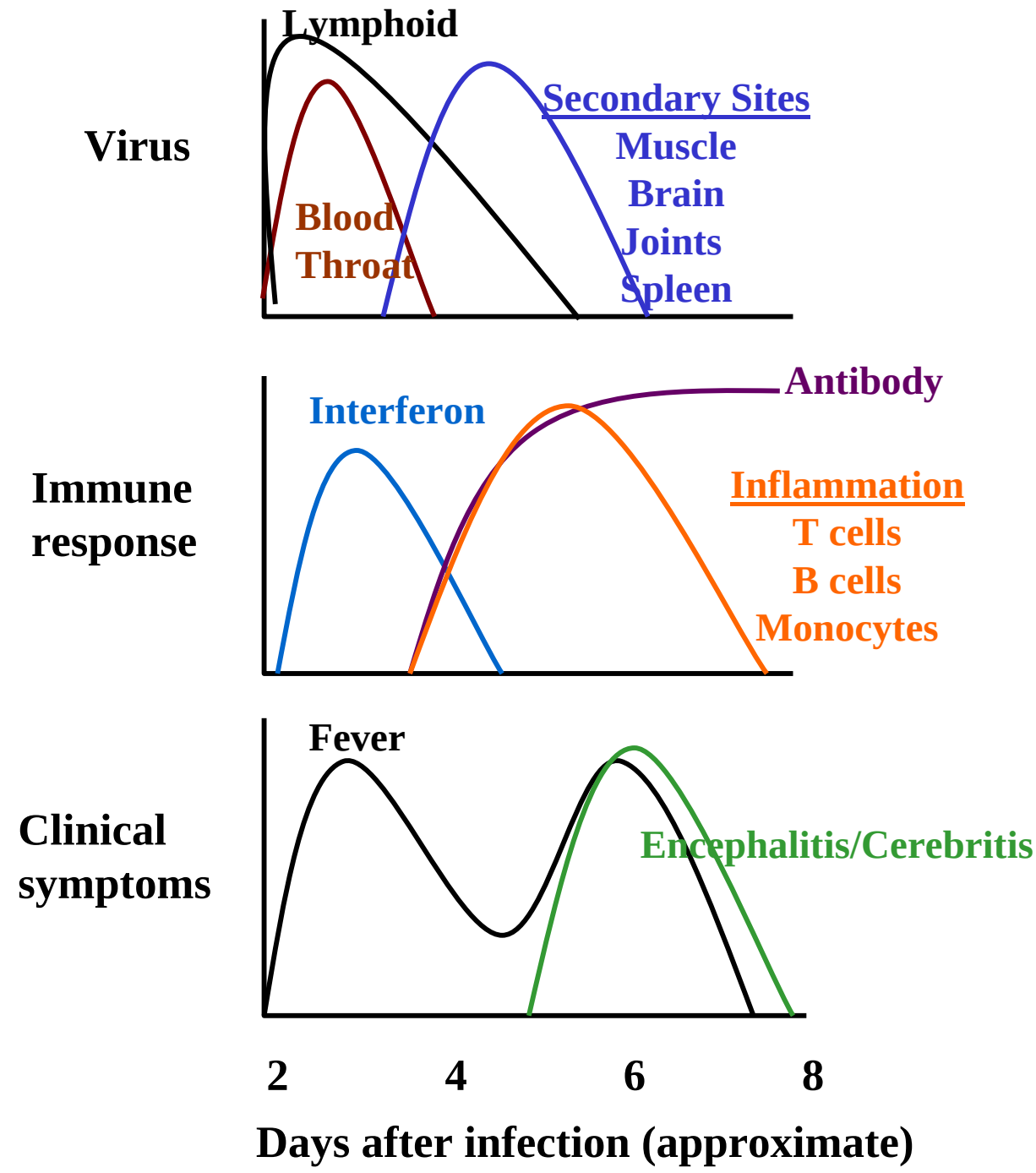
Pathogenesis of VEE-Induced Disease in Humans



Modified from Fields Virology

VEE Clinical Course

- **Incubation period: 1-6 days**
- **Acute febrile phase**
  - **Lasts few days to a week**
  - **Often have brief defervescense on first day**
- **Encephalitis phase**
  - **In 4% of children and <1% of adults**
  - **Usually occurs 4-10 days into illness**

Symptoms in VEE Infection

Sudden Onset Fever	**100%**
Headache	**100%**
Myalgia	**72%**
Vomiting	**50%**
Drowsiness	**40%**
Chills	**20%**
Sore Throat	**20%**
Diarrhea	**20%**

Abortions also reported during large outbreaks

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Clinical Case: Laboratory Aerosol Infection with VEEV

- 35 yr. old male physician
- Day 1: general malaise and headache
- Day 2-4: "almost unbearable" frontal headache, severe back and muscle aches, "every movement demanded considerable effort," 103 F. fever, felt cold, weak, nauseated, no appetite, slept continually for 22 hours.
- Day 5-7: some improvement, then relapsed on day 8
- Day 14: resumed work, easily fatigued
- Sequelae: insomnia, minor tremors (>4 mos)

Lennette and Koprowski, J.A.M.A. (1943) 123:1088.

Encephalitis in VEE Infection

- **Onset several days into febrile illness**
  - **Headache, N/V, nuchal rigidity**
  - **Ataxia**
  - **Altered mental status**
  - **Focal paresis or paralysis**
  - **Seizures**
  - **Coma**
  - **Long term sequelae possible**

VEEV Laboratory Abnormalities

- **Frequent leukopenia/neutropenia**
- **Mild thrombocytosis**
- **Elevated liver-associated enzymes (ALT, LDH)**
- **CSF usually with:**
  - **Lymphocytic pleocytosis (100 – 500 cell/uL)**
  - **Elevated protein**
  - **Relatively normal glucose**

Key Features of EEE and WEE

EEE

- incubation 5 to 15 days
- febrile prodrome (age dependent)
- vomiting, stiff neck, drowsiness
- generalized, facial, or periorbital edema
- paresis, disturbances of autonomic function -- impaired respiratory regulation or excess salivation
- 30% - 70% of survivors have long-term neurological sequelae -- seizures, spastic paralysis, and cranial neuropathies; cognitive impairment --- minimal brain dysfunction to severe dementia

WEE

- incubation 5 to 10 days
- malaise, headache, fever, nausea and vomiting
- nuchal rigidity, impaired sensorium, and upper motor neuron deficits
- severity of neurological involvement is inversely related to age, with over 90% of children younger than 1 year exhibiting focal or generalized seizures.
- Most patients recover over months; sequelae in some patients include motor weakness, cognitive deficits, or a seizure disorder

As with VEE, young patients w/ EEE or WEE have faster onset, more severe CNS impairment and higher mortality

Differential Diagnosis

- **Biowarfare Agents** that imitate a meningoencephalitic syndrome include *Brucella sp.*, *Yersinia pestis*, *Salmonella typhi*, *Coxiella burnetii*, *and Clostridium botulinum*

- **Sporadic viral agents** include *WNV*, *SLEV*, *JEV*, *DV*, *TBEV*, *RVFV*, *Henipaviruses*, *Machupo virus*, *Junin virus*, *herpes viruses*, *rabies virus*, *etc.*

- **Noninfectious causes** - *vascular*, *autoimmune*, *and neoplastic diseases*

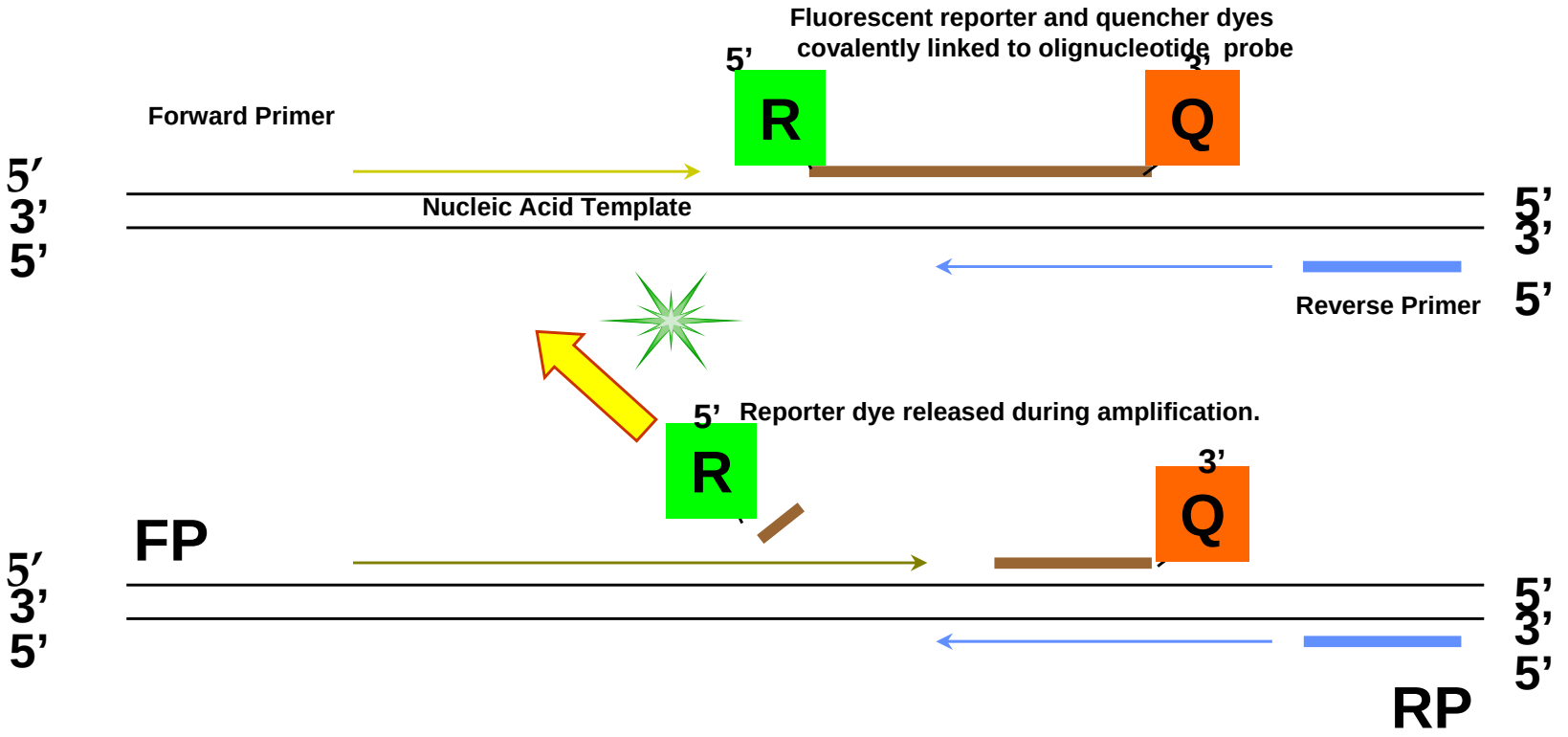
Diagnosis of Alphaviruses in Medical Settings

- **Suspected on clinical and epidemiologic grounds, confirmed by:**
  - **Virus Isolation**
    - Low level viremia (24-72 hrs) or from pharynx
  - **Serology**
    - IgM Capture
    - Rising antibody titer in paired samples
  - **PCR**

Virus Isolation

- Virus grown in cell culture, examining for cytopathology.
- Virus characterized by RT-PCR, sequencing, immunofluorescence assay (IFA) and/or ELISA methods.

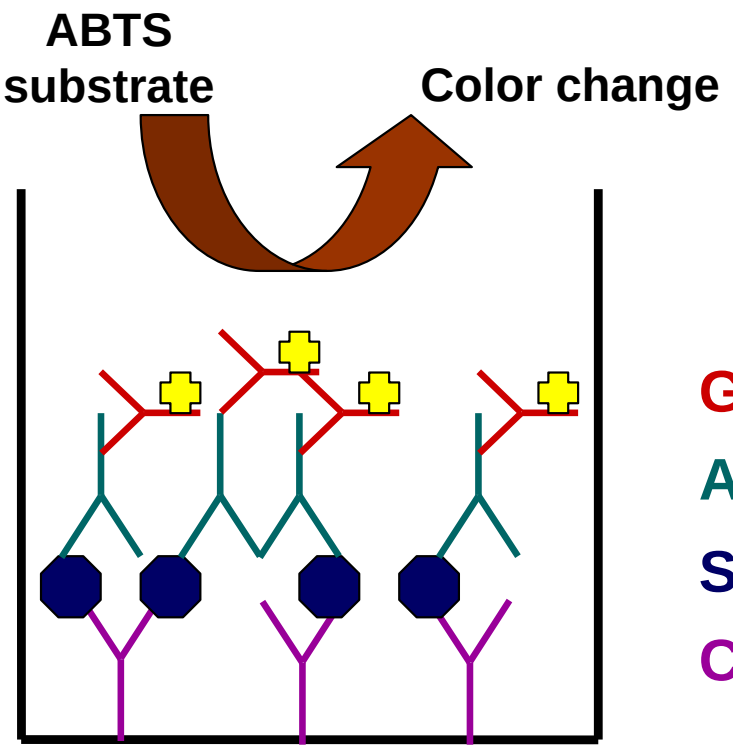
RT-PCR Detection of Virus



- Common gene amplification chemistry
- Assays for over 26 biological agents
- COTS technology
- Over 50 assays developed

Slide courtesy of Dr. Schoepp, DSD Division

ELISA Assay for Virus Detection



Goat anti-horse HRP

Ab anti-virus (horse)

Sample/Antigen (virus)

Capture Ab (mouse)

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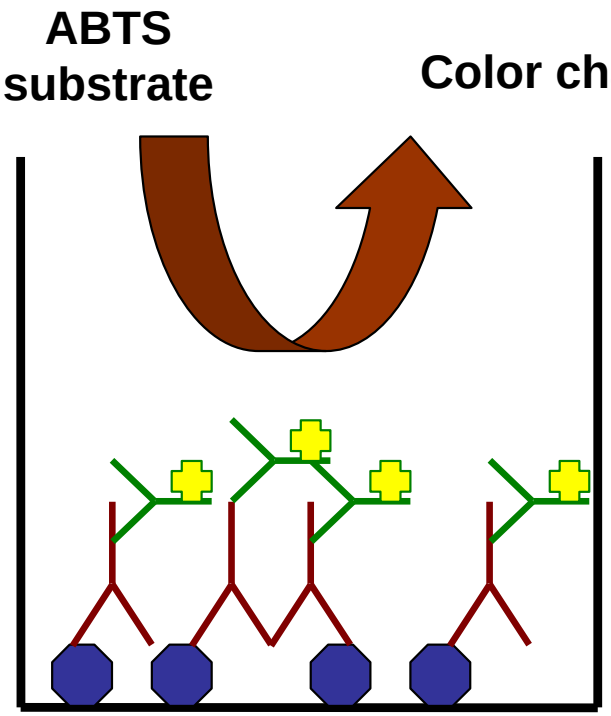
ELISA Assay for the Measurement of the Immune Response



Goat anti-human IgM/G HRP

Sample (anti-virus Ab)

Antigen

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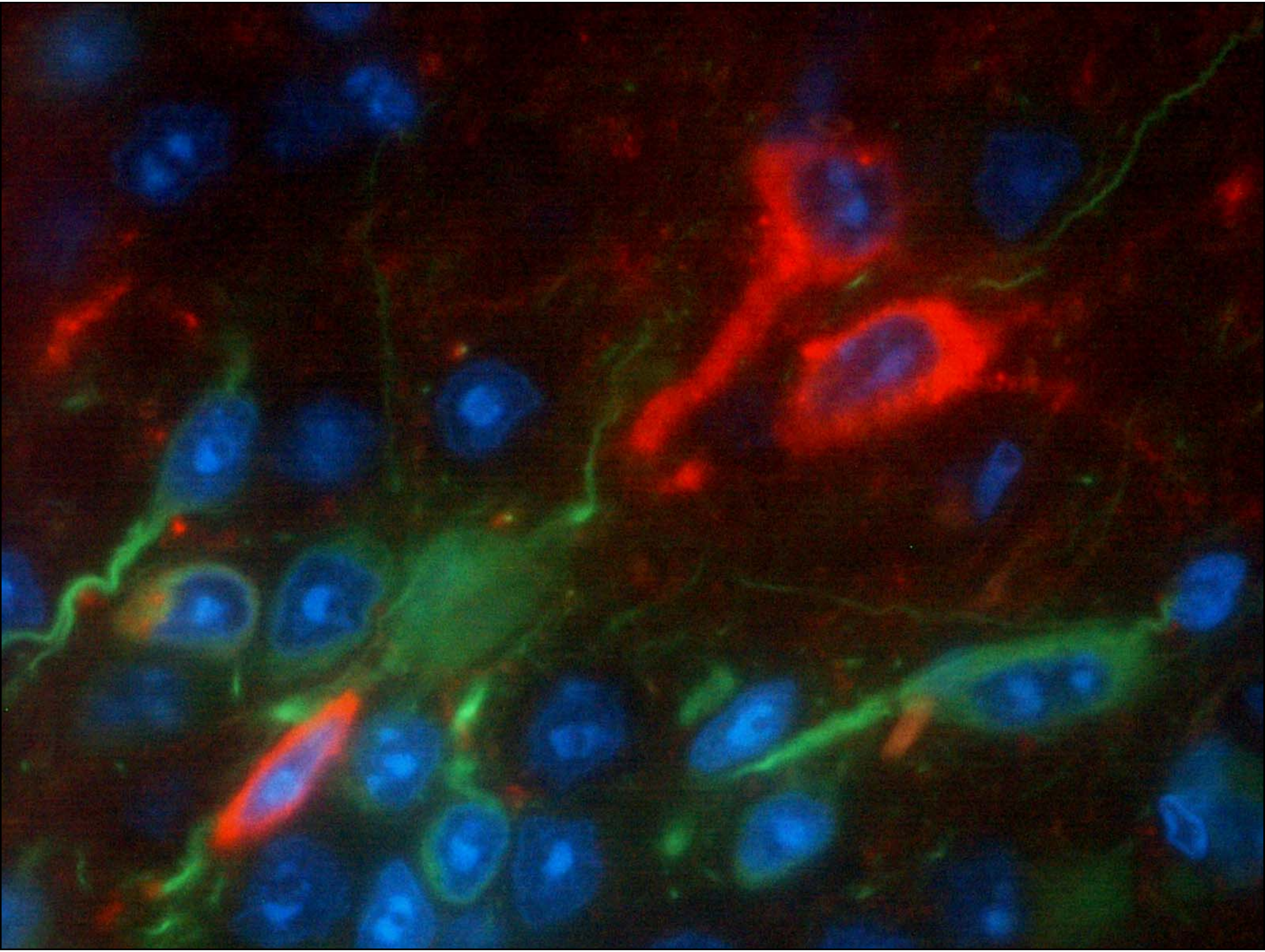


Assays used in field settings

- **Real Time PCR: RAPID/Light cycler**
- **Electrochemiluminescence (ECL) ORIGEN®**

Real-time PCR Instrument

RAPID/LightCycler™

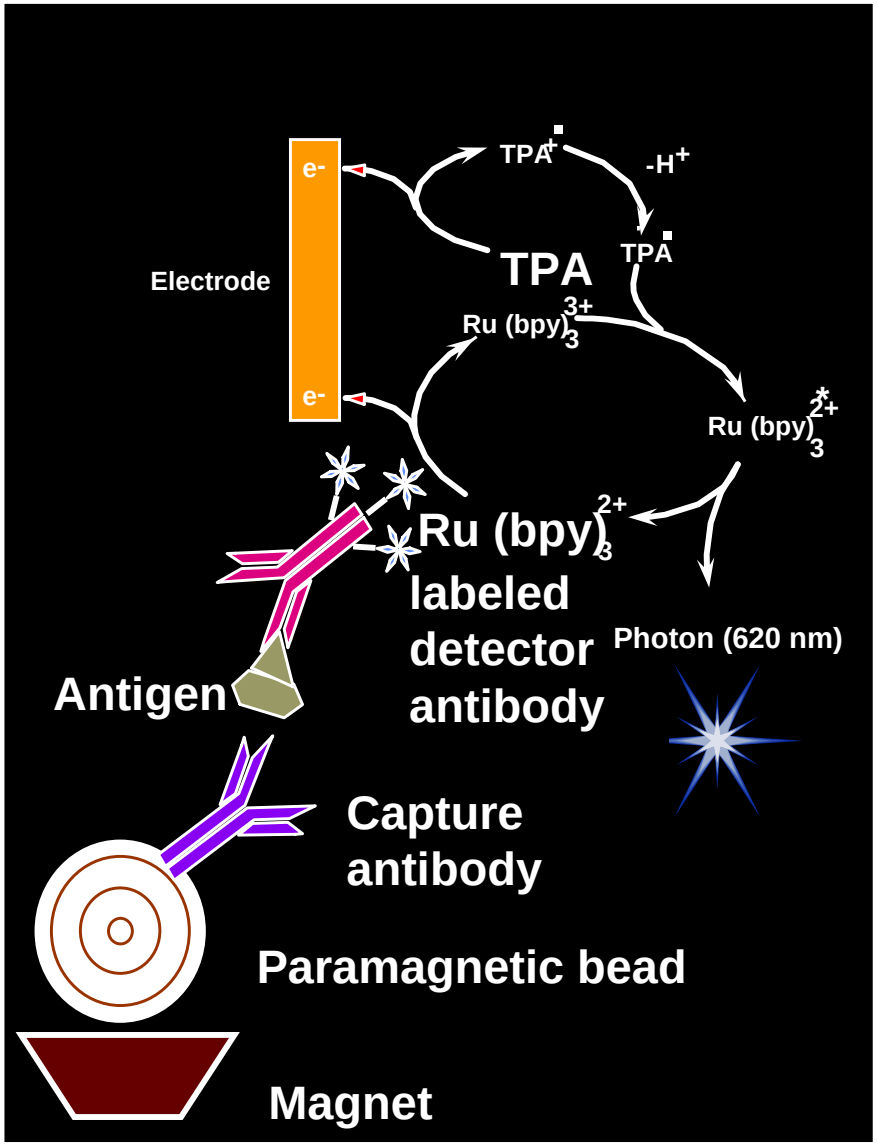
- Rugged and portable
- Rapid (25 to 40 mins after specimen processing)
- Sensitive
- Common fluorescent probe chemistry

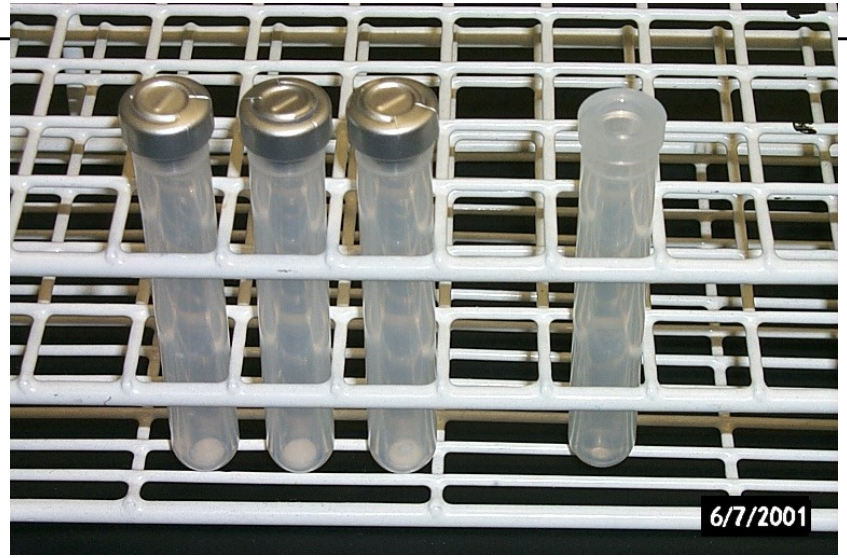
Slide courtesy of Dr. Schoepp, DSD Division

Electrochemiluminescence (ECL) ORIGEN®

Slide courtesy of Dr. Schoepp, DSD Division

ECL Immunoassay





- High sensitivity
- Wide dynamic range
- Single tube, 15 min assay
- Stable reagents

Slide courtesy of Dr. Schoepp, DSD Division

ECL Assays

- SEB Toxin
- Ricin Toxin
- Bot A/B/E Neurotoxin
- Y. pestis F1 Antigen
- B. anthracis PA/Spore/Cap
- Alphavirus Group
- VEE virus
- Flavivirus Group
- Orthopox
- Tularemia
- Brucella sp.
- Coxiella burnetti

- **Fielding**
  - TAML
  - CENTCOM
  - PACOM
  - JPO/DoA
- **Future**
  - Miniaturization and optimization of next generation device

Slide courtesy of Dr. Schoepp, DSD Division

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Treatment of Alphaviruses

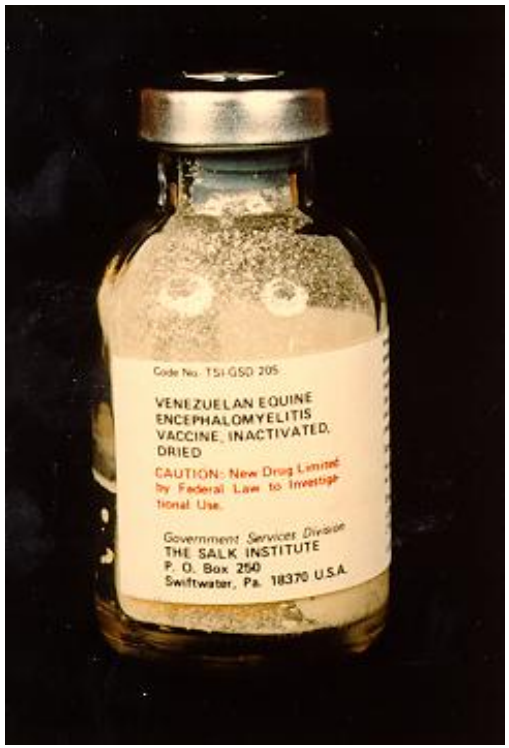
- No specific therapy
- Supportive therapy – esp. with encephalitis
  - ICU monitoring to manage ICP
- Analgesics/antipyretics
- Fluids

TC-83 VEE Vaccine

- Experimental, Live-attenuated, IND vaccine
- Heterogeneous virus populations
- High reactogenicity (approximately 20%)
- Vaccinees shed rodent virulent virus
- 20% nonresponder rate
- Fetal infection and wastage in rodents
- Viremia in equines sufficient to infect vectors
- Incomplete protection vs. subtypes 1D, 1E, III
- Induces heterologous vaccine interference

C-84 Inactivated VEE Vaccine

- Formalin-inactivated, TC-83 passage
- Experimental, used under IND
- Requires multiple injections and periodic boosters
- Expensive
- Does not protect rodents against aerosol challenge
- Used to boost TC-83 nonresponders



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VEE Laboratory Infections in TC-83 Recipients

Case #	Yrs Post TC-83	PRNT (TrD) PRE	PRNT (TrD) POST	PRNT (IE) PRE	PRNT (IE) POST	Probable Source
1	2	80	320	10	2560	Centrifuge
2	0.5	10	640	<10	80	Centrifuge
3	3	40	640	<10	2560	Seed Prep
4	0.5	10	640	<10	160	HA Prep
5	2	320	>2560	80	>10240	Centrifuge

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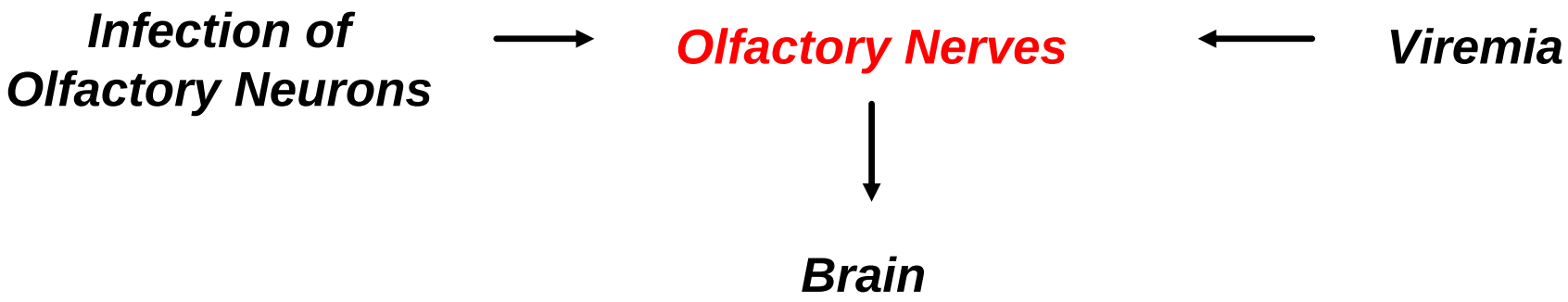
VEE – Immunity for Peripheral & Aerosol Infection

- **Peripheral Infection >> serum neutralizing IgG antibodies>> prevents viremia**
- **Aerosol Infection >> mucosal neutralizing IgA antibodies>> prevents infection of olfactory bipolar neurons**



Inactivated EEE and WEE Vaccines

- Only immunogens available
- Require multiple inoculations
- Require periodic boosters
- Poorly Immunogenic
  - EEE 58%
  - WEE 50%
- Short duration
- Interfere with TC-83 vaccination
- Aerosol protection?

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Approaches to Improved Vaccine Candidates

- **Infectious clones**
  - Incorporation of attenuating mutations
  - Incorporation of mutations that increase immunogenicity by targeting to the lymphatic system
- **Methods of Inactivation**
  - Irradiation, chemical inactivating agents (INA, BEI)
- **DNA vaccines**
  - Selective expression of targets of immune response
- **VEE Replicon Strategy**
  - Multiple plasmid delivery system for production of “virus-like particles”

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Questions?

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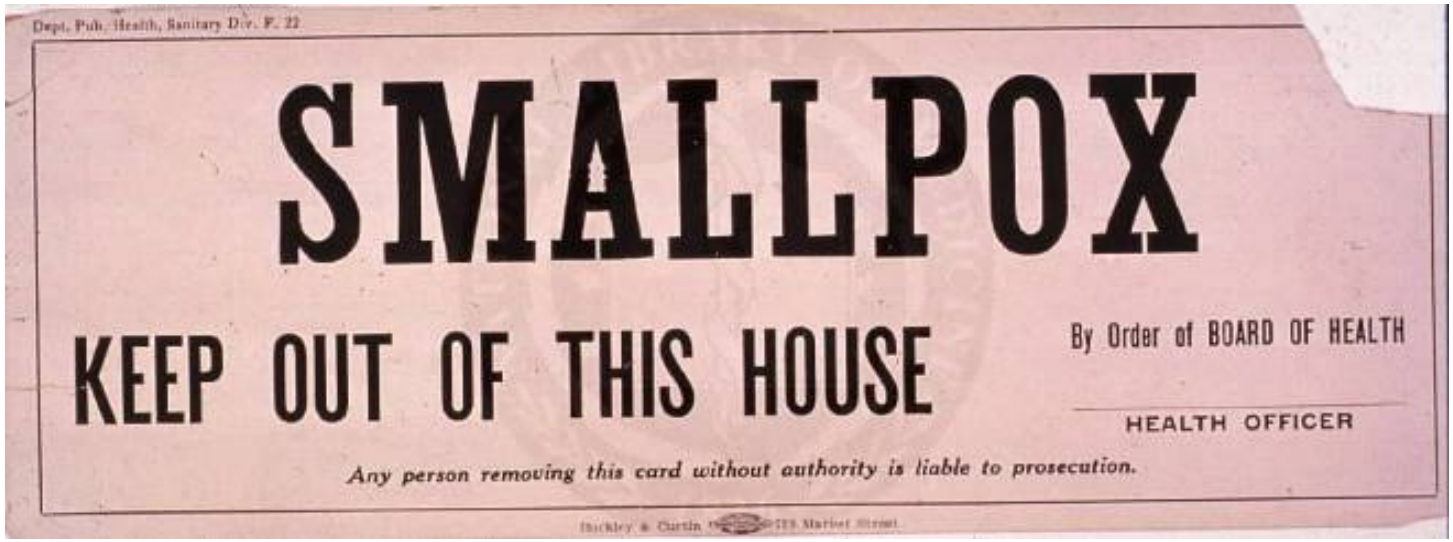

Presented by
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Learning Objectives

- **Students should be able to:**
  - **Discuss the threat of smallpox re-emergence through biological warfare or terrorism**
  - **Explain dynamics of smallpox transmission both person to person and in a community**
  - **Recognize clinical presentation of smallpox**
  - **Discuss treatment, control and prevention of smallpox**

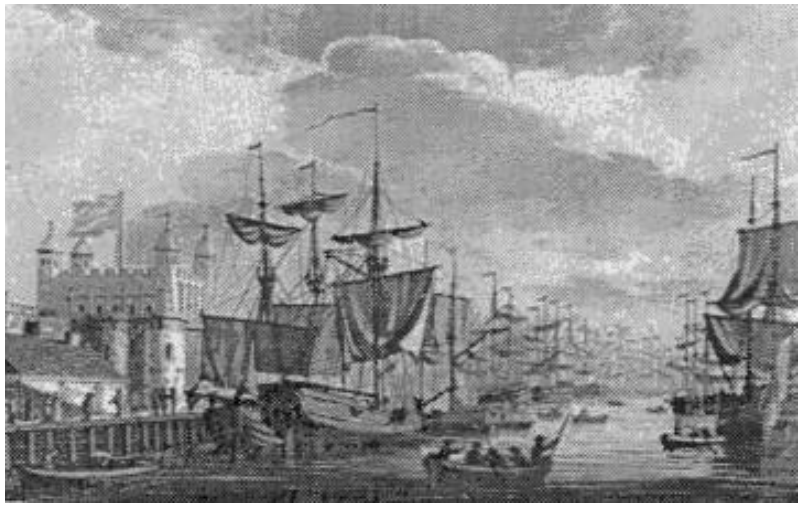
Smallpox History

- **Ancient historical evidence**
  - **18th Egyptian Dynasty (1580 – 1350 B.C.)**
    - **Ramses V**
  - **Chinese writings 1122 B.C.**
    - **“Tai-tou”**
  - **References in ancient Indian writings**
    - **Brahmin mythology god of smallpox - *Kakurani***

Pockmarks on mummy of Ramses V

History

- Smallpox peaks in 18th century Europe
  - 400,000 deaths/yr
  - 1/10 deaths in London



London, 1700

- Deaths in royal lineages 1695 - 1775
  - Mary II (England)
  - Joseph I (Austria)
  - Luis I (Spain)
  - Peter II (Russia)
  - Ulrika Eleonora (Sweden)
  - Louis XV (France)

Smallpox Importation into New World

Cortez

Pizarro

- **Aztec**
  - Aztec population suffers 3.5 million deaths in 2 years
  - Death of Cuitlahuatzin (successor to Montezuma)
- **Incas**
  - Smallpox introduced 2 yrs before Pizarro arrives
  - 2 emperors dead – empire in turmoil

"Although many Spaniards die also, smallpox kills incomparably more Indians"

Missionary in northern Mexico

North American Natives

- **Effect on Native Americans in U.S.**
  - **Mortality quoted as > 50% in many eyewitness accounts of outbreaks**
  - **Mandan Indian village, 1837**
    - **Population falls from 2000 to < 40 in a few weeks**

Other Naïve Populations

- **Iceland epidemic 1707 – 1709**
  - **First known introduction of smallpox into Iceland**
  - **18,000 deaths among a population of 50,000**
- **Underlying factors –**
  - **Naïve population?**
  - **Increased genetic susceptibility to variola?**
  - **Comorbidities?**

Stearn and Stearn, 1945

History - Vaccination

- Ancient practice of inoculation
  - At least 1000 years old in India and China
- European “variolation”
  - English physicians in early 18th century
  - Lady Montague
- Observations of Edward Jenner
  - Milkmaids rarely had smallpox scars

Lady Montague

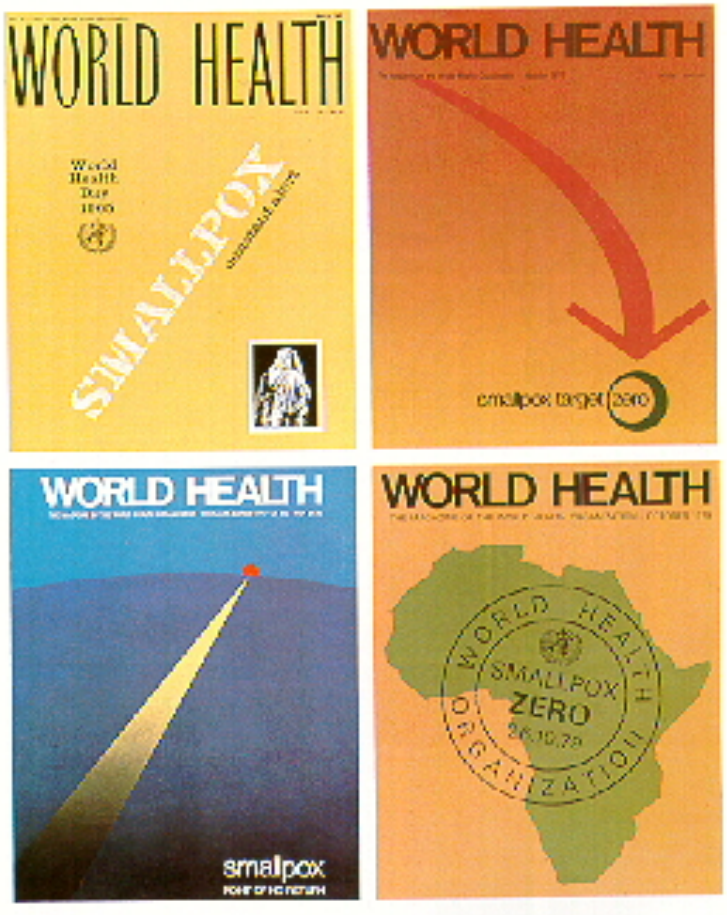
Jenner

History

- **First vaccination**
  - **Jenner inoculates boy with cowpox**
  - **Demonstrates smallpox immunity**
  - **Coins term “vaccinate” (from *vacca*, L. - cow)**

Smallpox : 20th Century

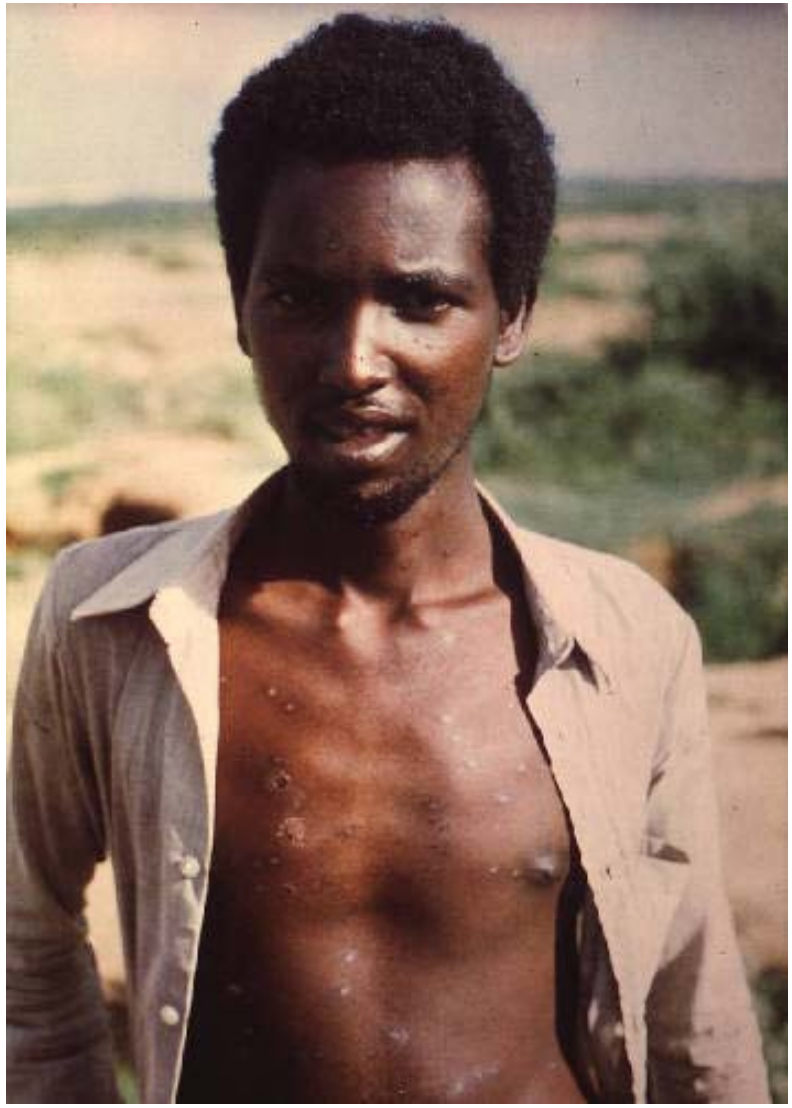
- Smallpox eliminated in Western countries by early 20th century
  - Multiple vaccines used
- WHO smallpox control efforts
  - Culminates in Intensified Smallpox Eradication Program (1968 -1979)
    - Standardized vaccines, lyophilization, understanding of epidemiology and excellent public-health groundwork



WHO

Global Eradication

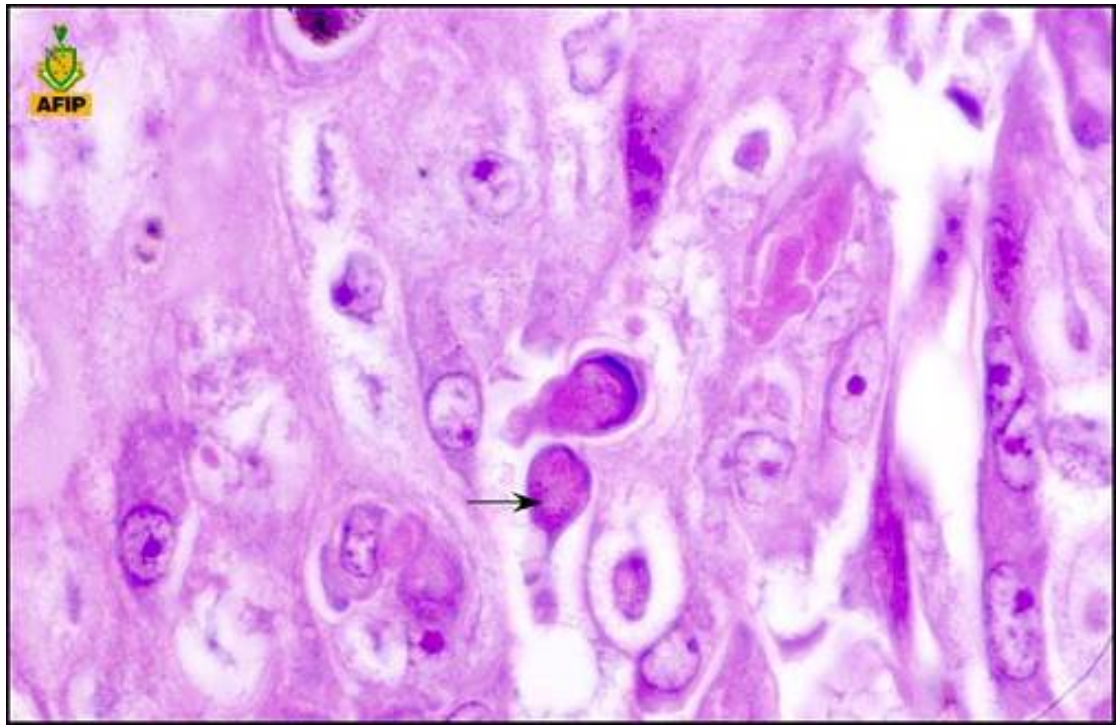
- Last naturally acquired case: Somalia 1977
- Last cases: laboratory acquired in England 1978
- Smallpox declared eradicated in 1980
- Subsequent designation of official repositories
  - CDC, Atlanta
  - Vektor Institute, Novosibirsk Region, Russian Federation



CDC

Poxviruses

- **Largest of all viruses (only viruses visible with a light microscope)**
- **Non-segmented double stranded DNA genome**
- **Virions are ovoid or brick-shaped, measuring 200-400 nm**



AFIP

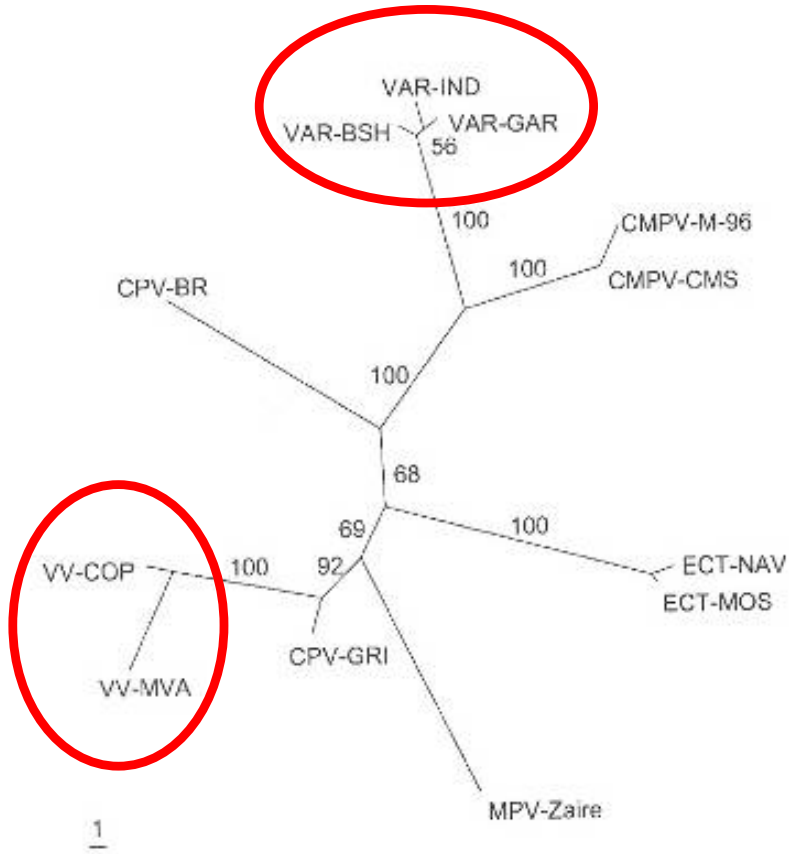
Taxonomy

Family Poxviridae, Subfamily Chordopoxvirinae

Genus	Examples
• Orthopoxvirus	• Variola, vaccinia, monkeypox, cowpox, camelpox viruses
• Avipoxvirus	• Canarypox virus
• Capripoxvirus	• Goatpox virus
• Parapoxvirus	• Orf virus
• Molluscipoxvirus	• Molluscum virus
• Yatapoxvirus	• Tanapox virus

Orthopoxviruses

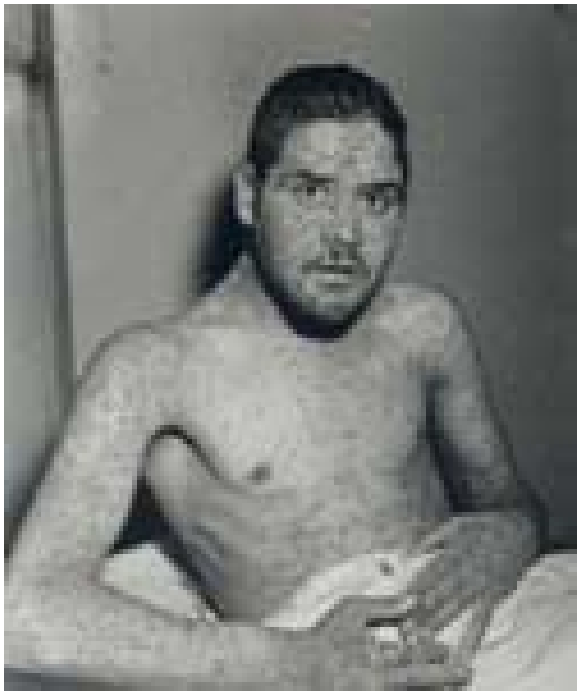
- **Genetically closely related**
- **Cross-react serologically and induce cross-reactive immunity in vivo**



Phylogenetic relationship of orthopoxviruses, from Guber et al 2004

Smallpox Disease

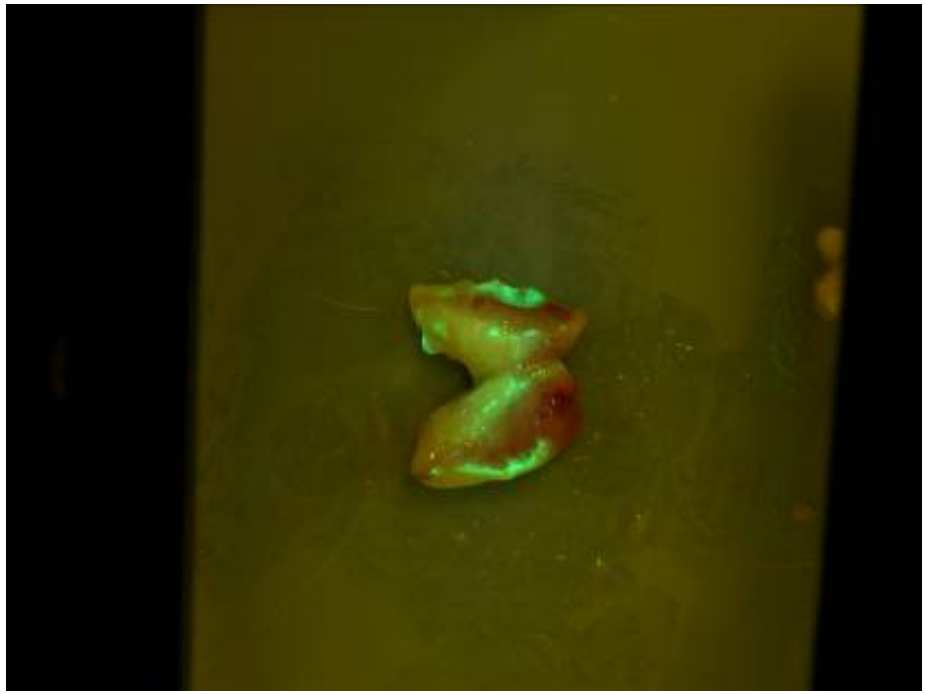
- **Human infection with variola virus**
  - **Variola major = classic smallpox**
    - **Mortality roughly 30%**
  - **Variola minor = alastrim**
    - **Distinct virus**
    - **Mortality 1%**
  - **Cannot distinguish on individual clinical presentation**
  - **Distinguished by epidemiologic clues or genetic analysis**



National Museum of Health and Medicine

Pathogenesis

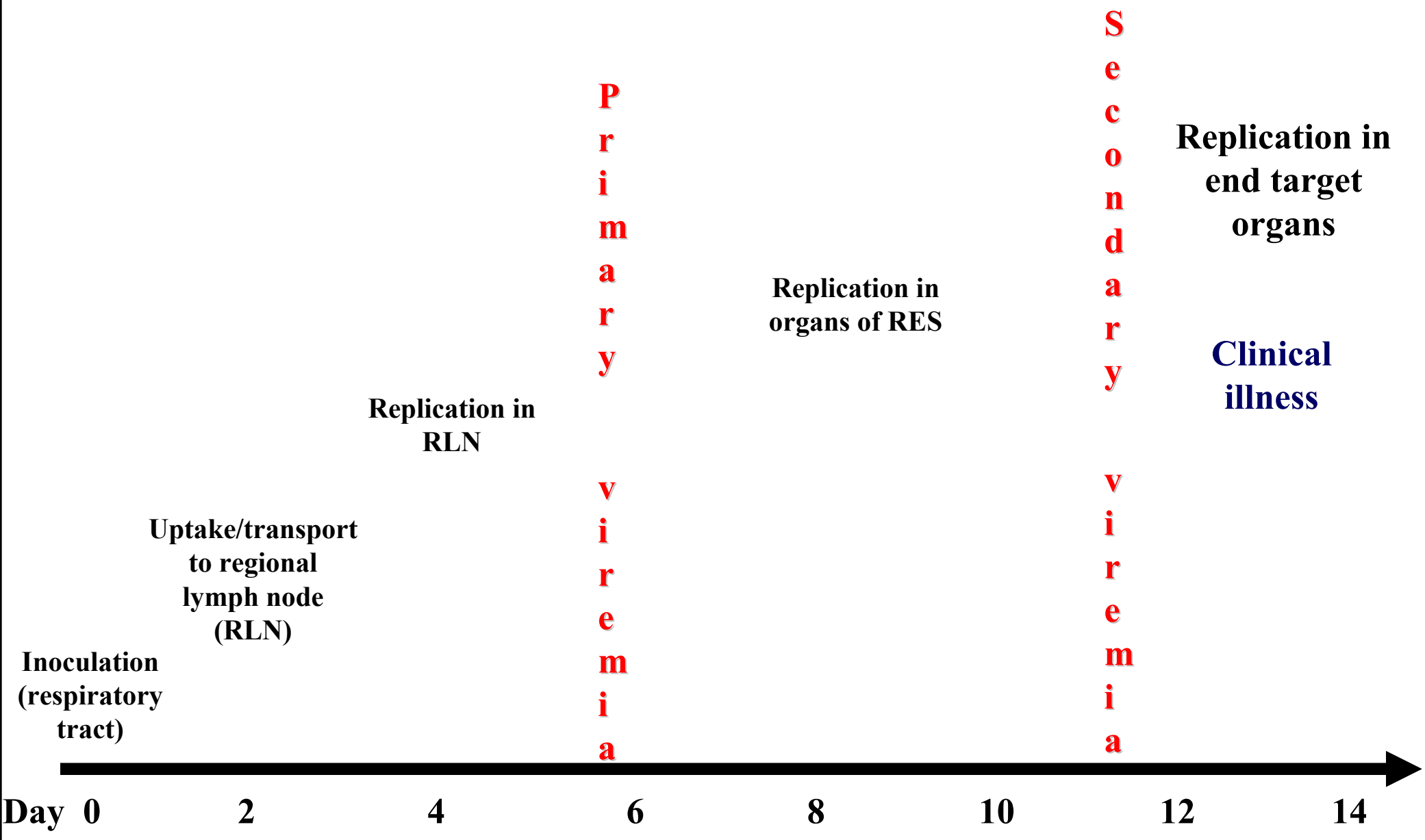
- Transmission primarily from oropharyngeal secretions
- Route of entry through respiratory tract
- Initially an infection of immune system tissues



GFP expression in lymph node of NHP infected with eGFP-MPV

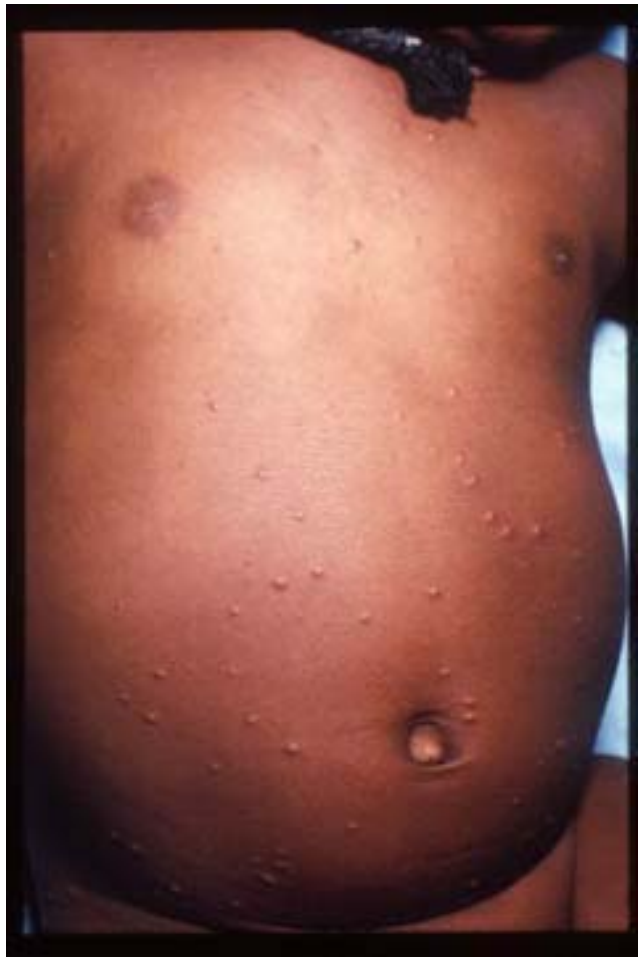
Courtesy of Jason Paragas, PhD

Pathogenesis



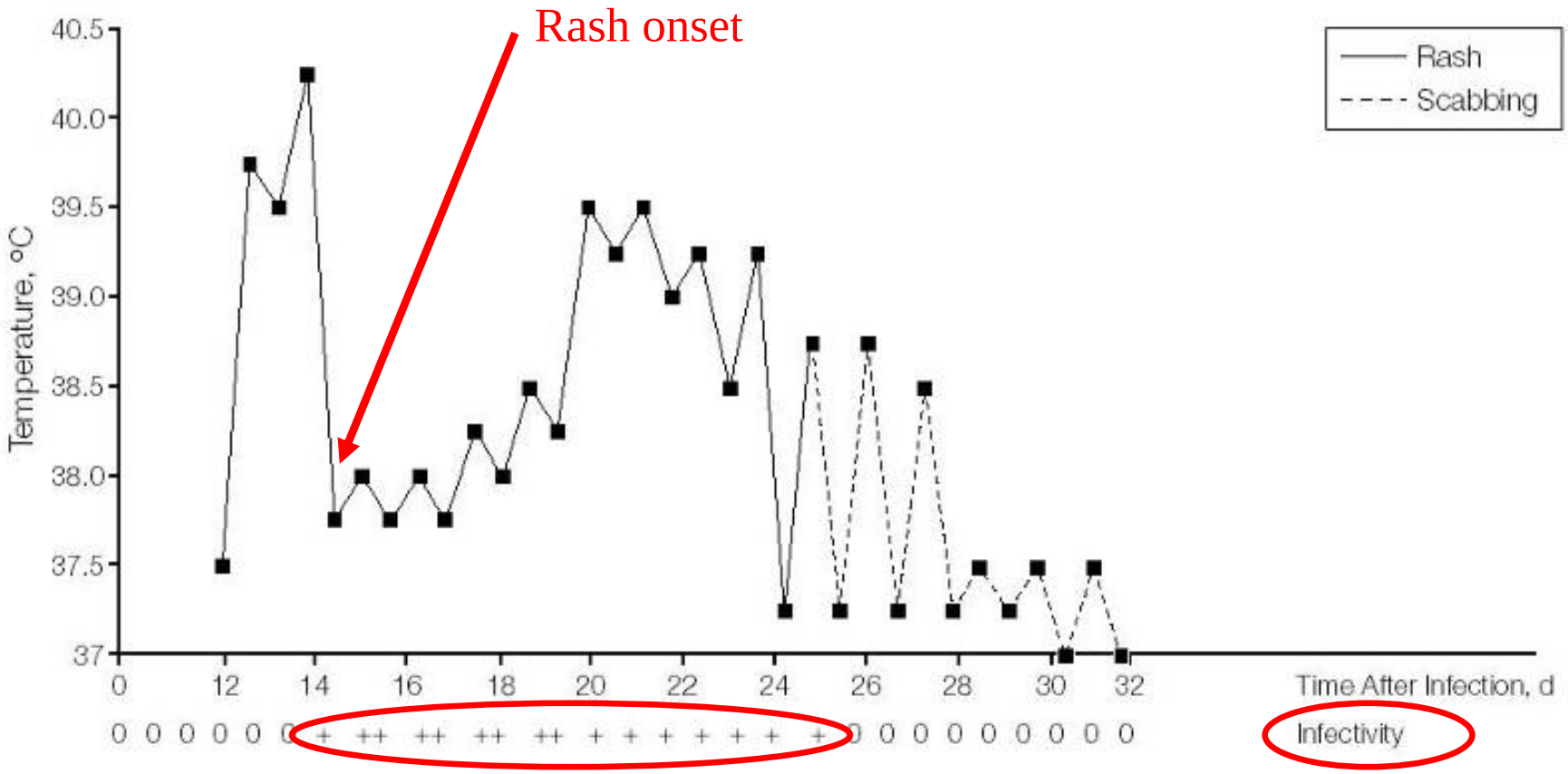
Clinical Course

- **Incubation averages 12 days**
- **Fever and constitutional sx's for 2-3 days**
  - **"Pre-eruptive phase"**
  - **VERY ill**
- **Rash and enanthem appear**
  - **Temp drops**
  - **Patient feels somewhat better**



Day 3 of rash WHO

Disease Course

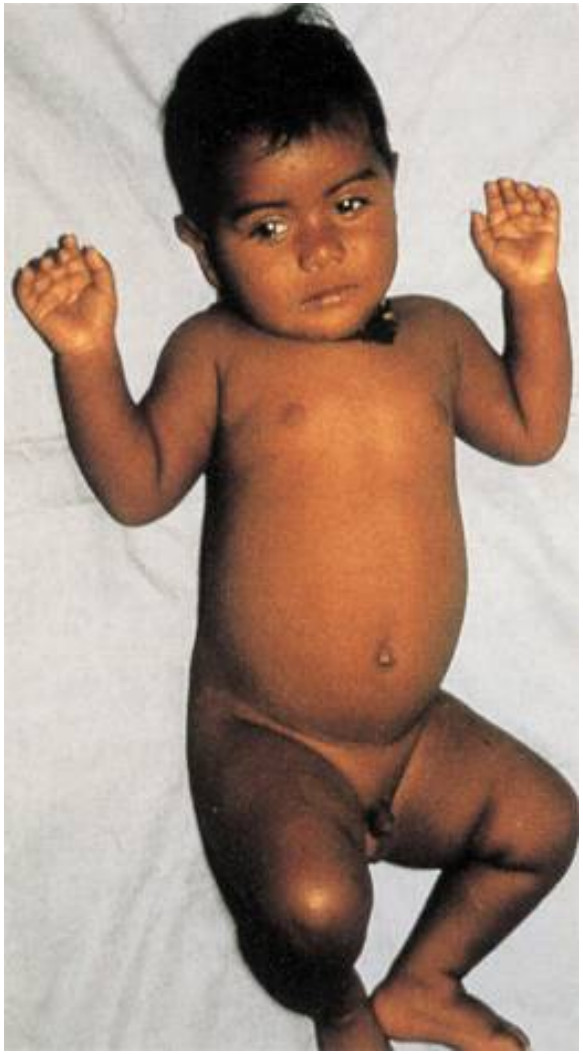


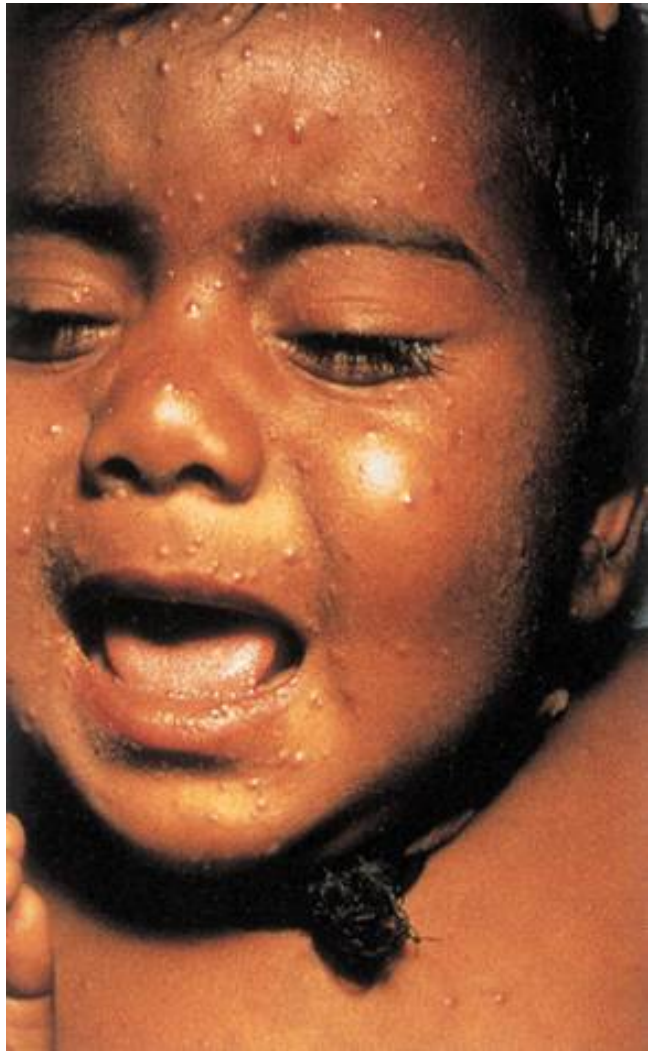
From Henderson et al. JAMA 1999

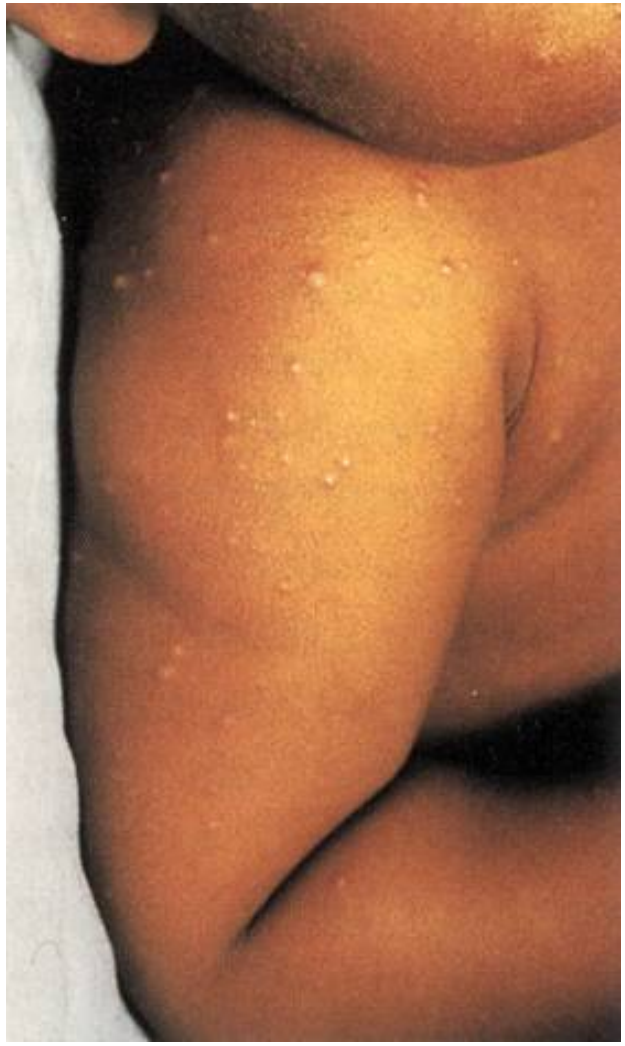
Clinical Features: Key Points

- Prodrome
- Centrifugal rash
- Palms and soles
- Slow and synchronous progression
- Deep, firm lesions

Day 3 of rash (day 6 of illness)



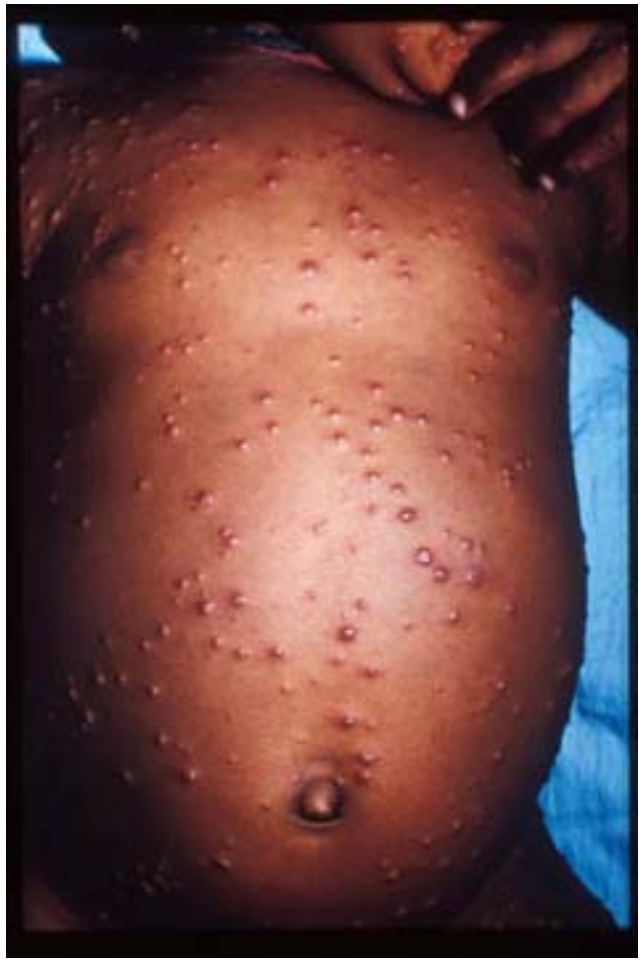




from: WHO

Clinical Course

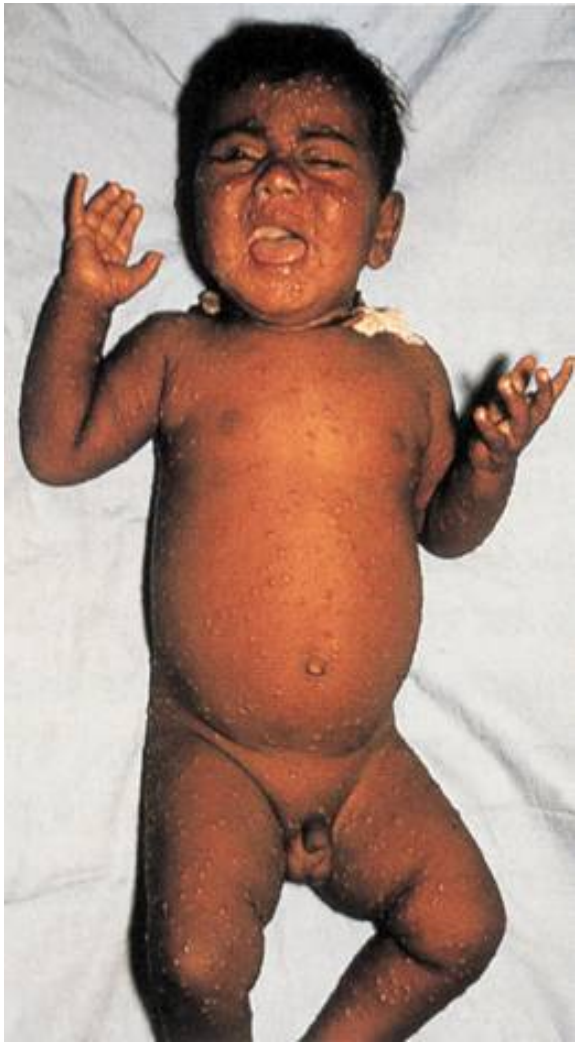
- Lesions start mostly on face and periphery
  - "Centrifugal" distribution
- Macules become papules

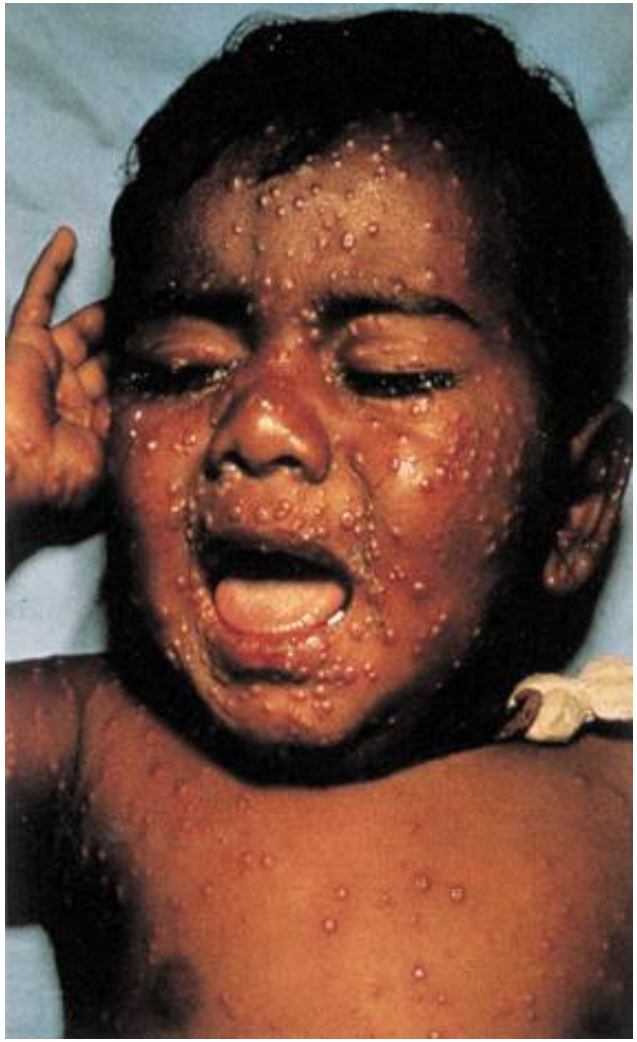


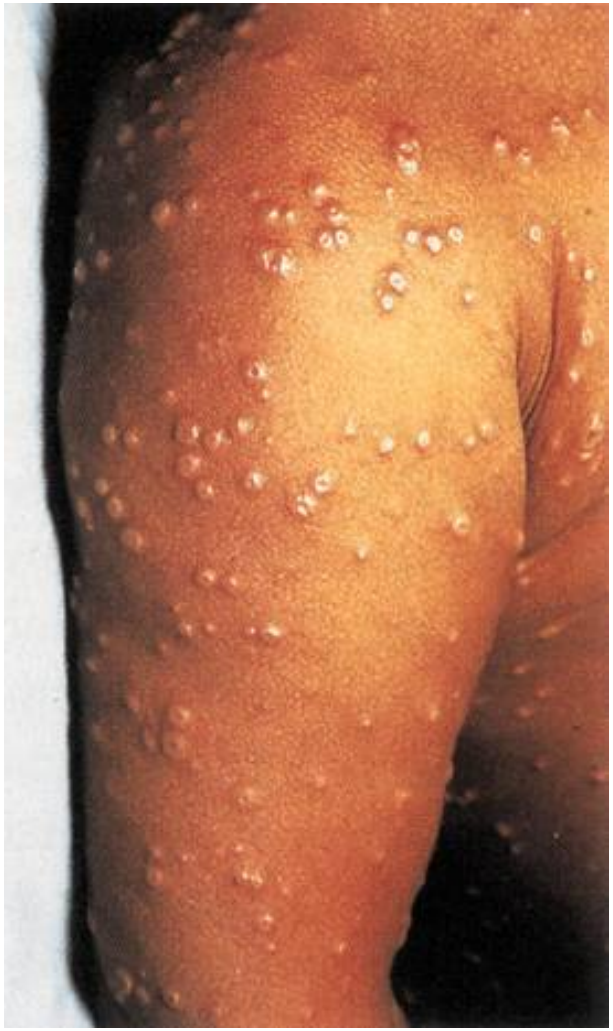
Day 5 of rash

from: WHO

Day 5 (8)



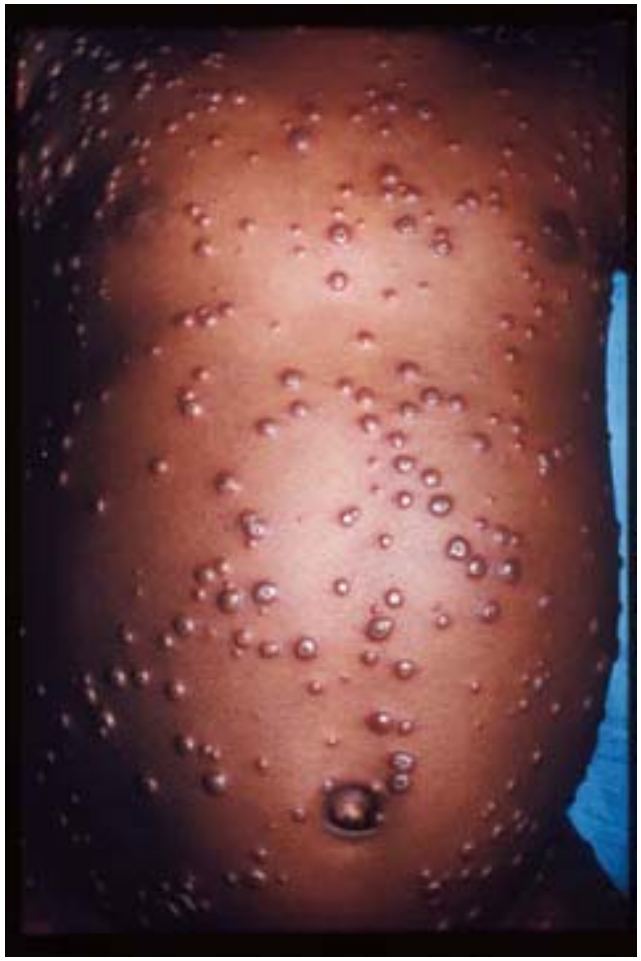




from: WHO

Clinical course

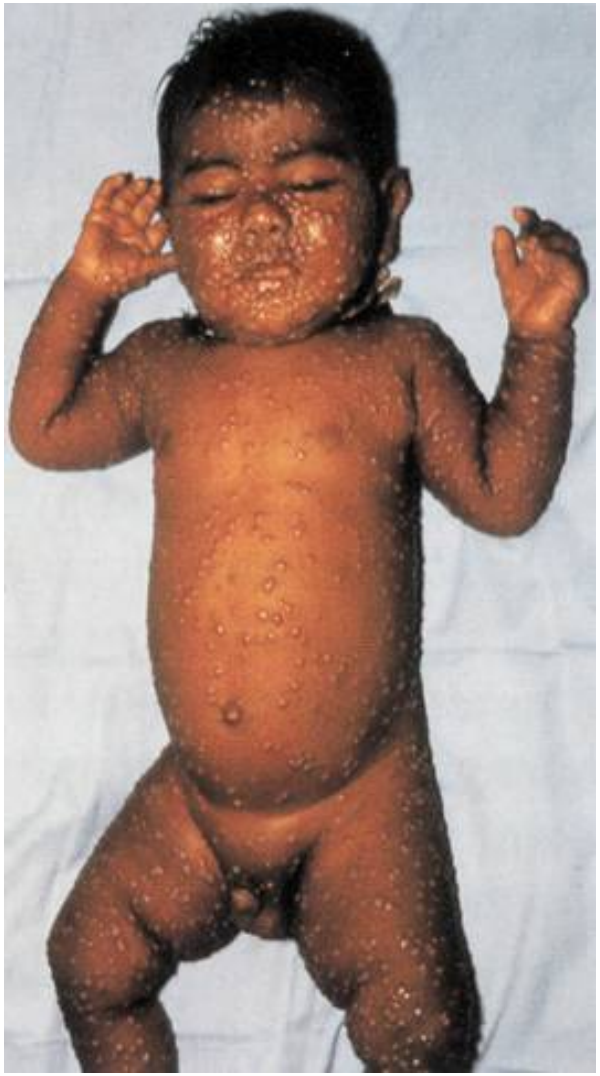
- Lesions spread centrally
- Papules become vesicles/pustules
- Patient much more ill
  - Fever has returned

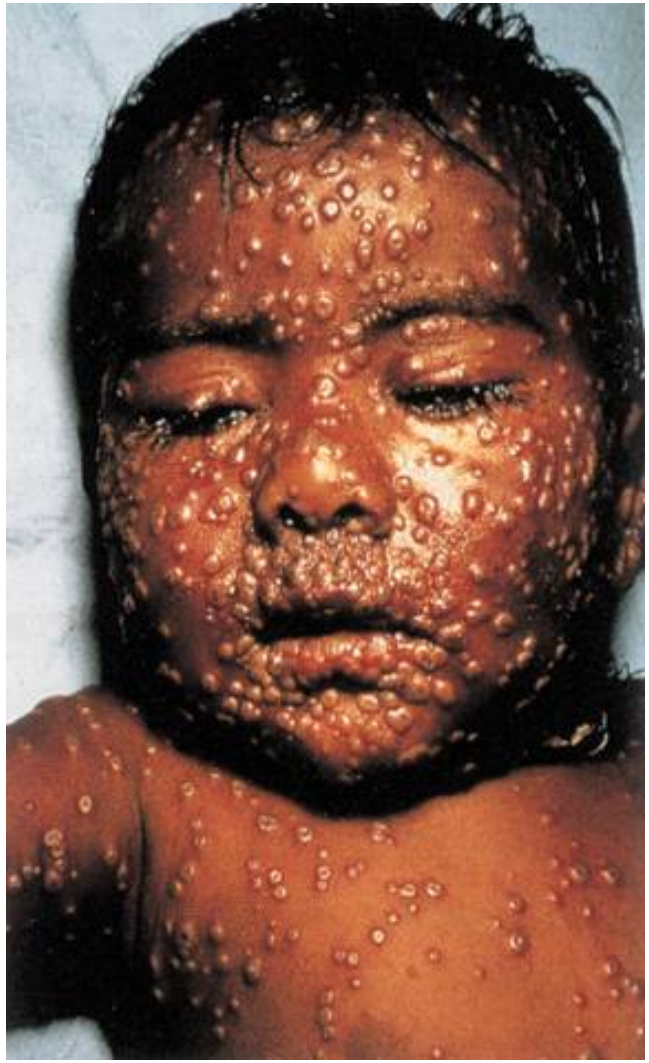


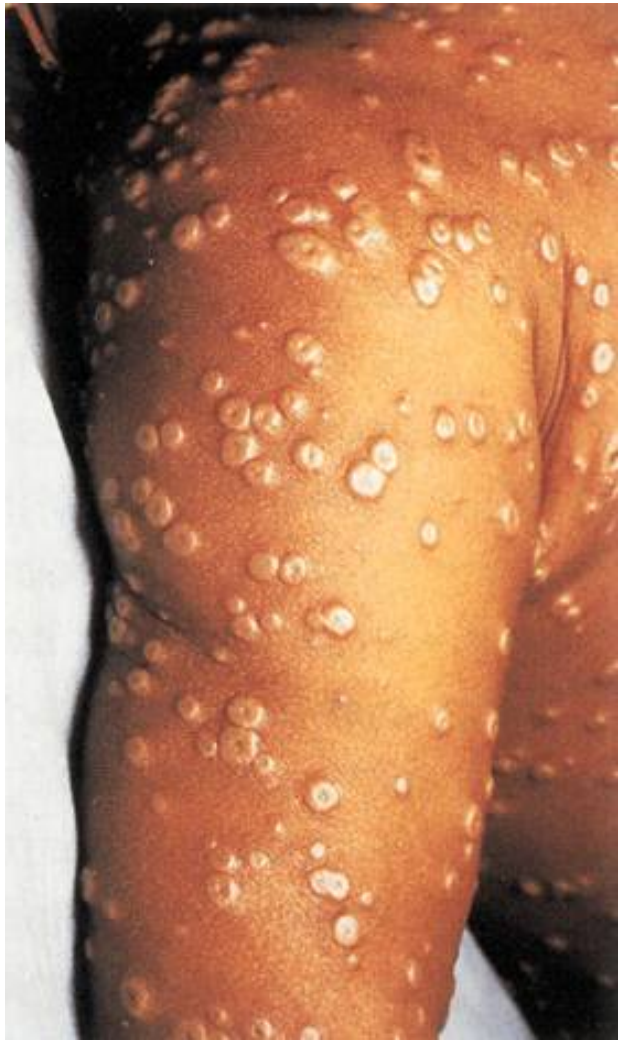
Day 7

from: WHO

Day 7



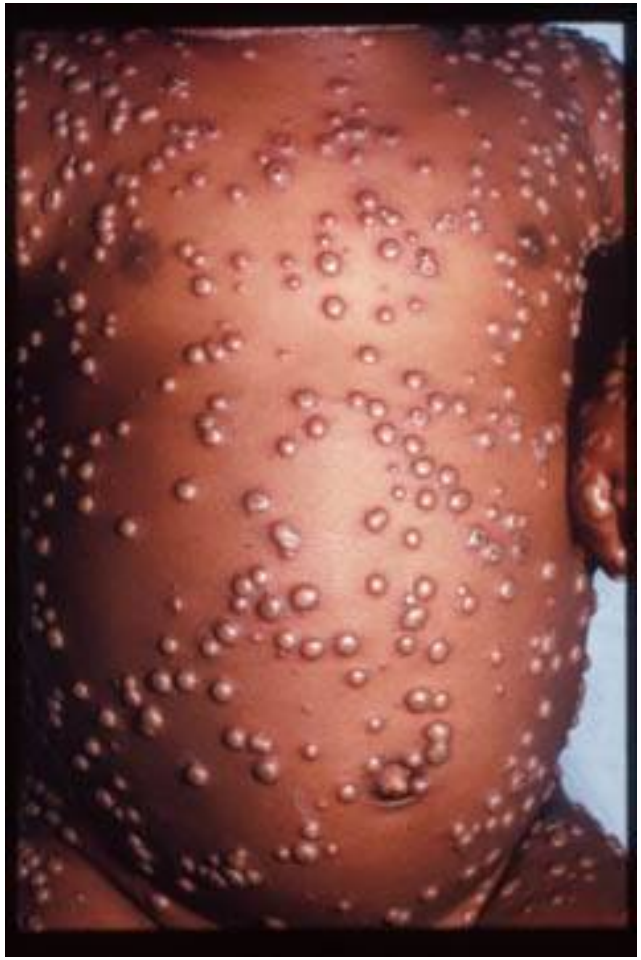




from: WHO

Clinical Course

- Synchronous progression of lesions in same region
- Lesions most abundant in face and extremities
- Death occurs day 10-16 of illness

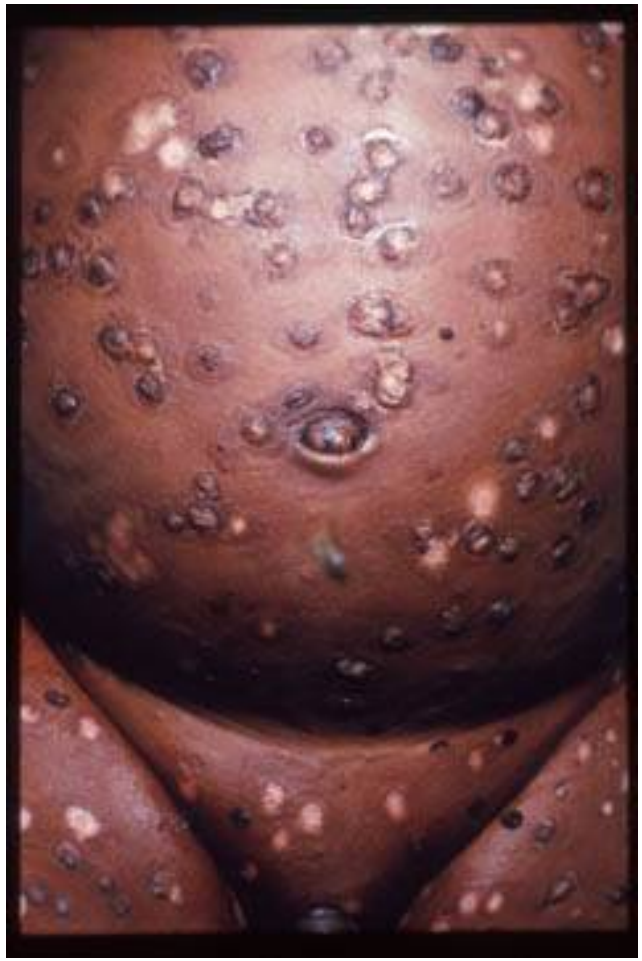


Day 9 (rash)

from: WHO

Clinical Course

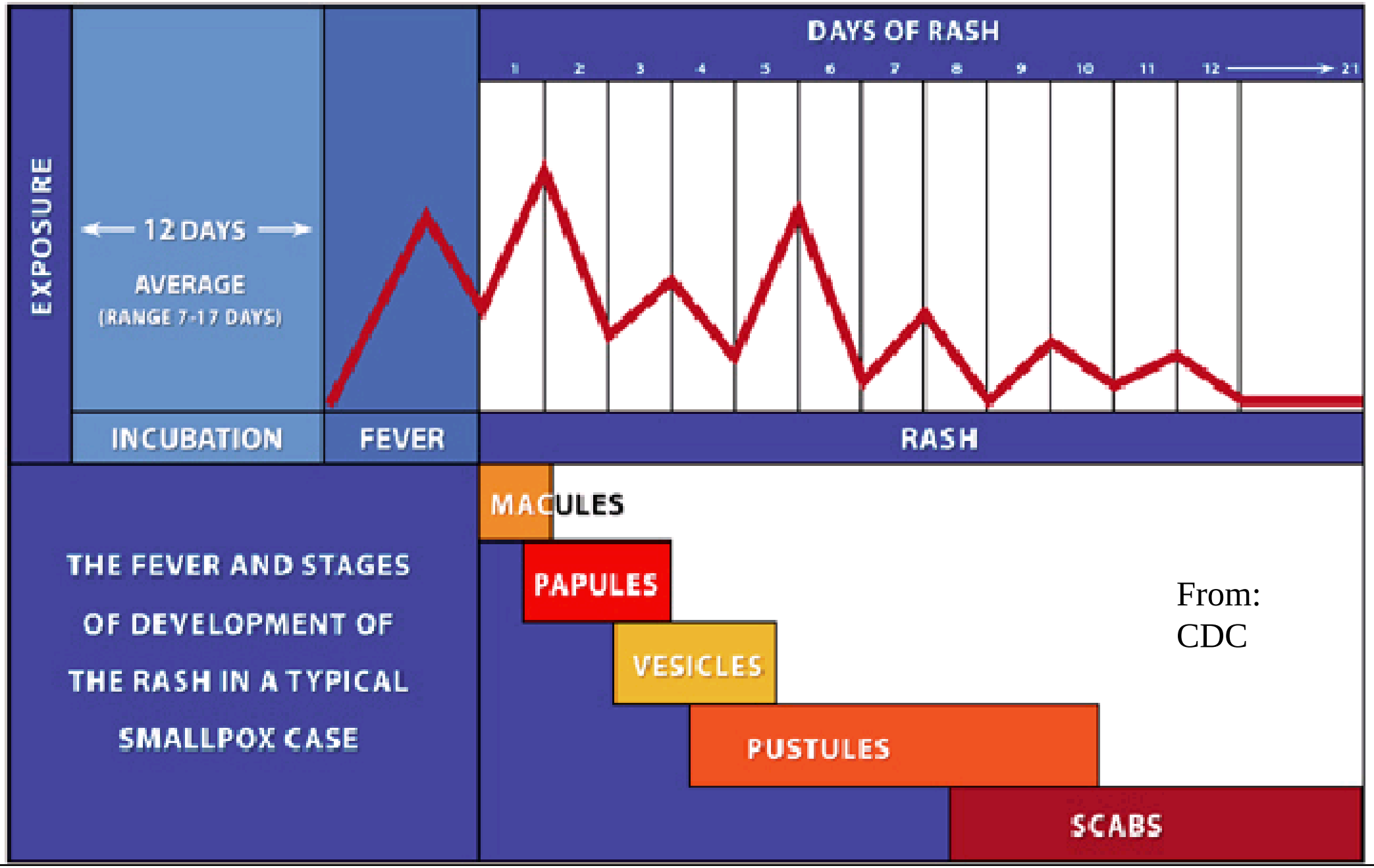
- Crusts form by day 14 of rash
  - First on face
- Scabs separate around day 22 – 27
  - Often leave depigmented scar



Day 14

from: WHO

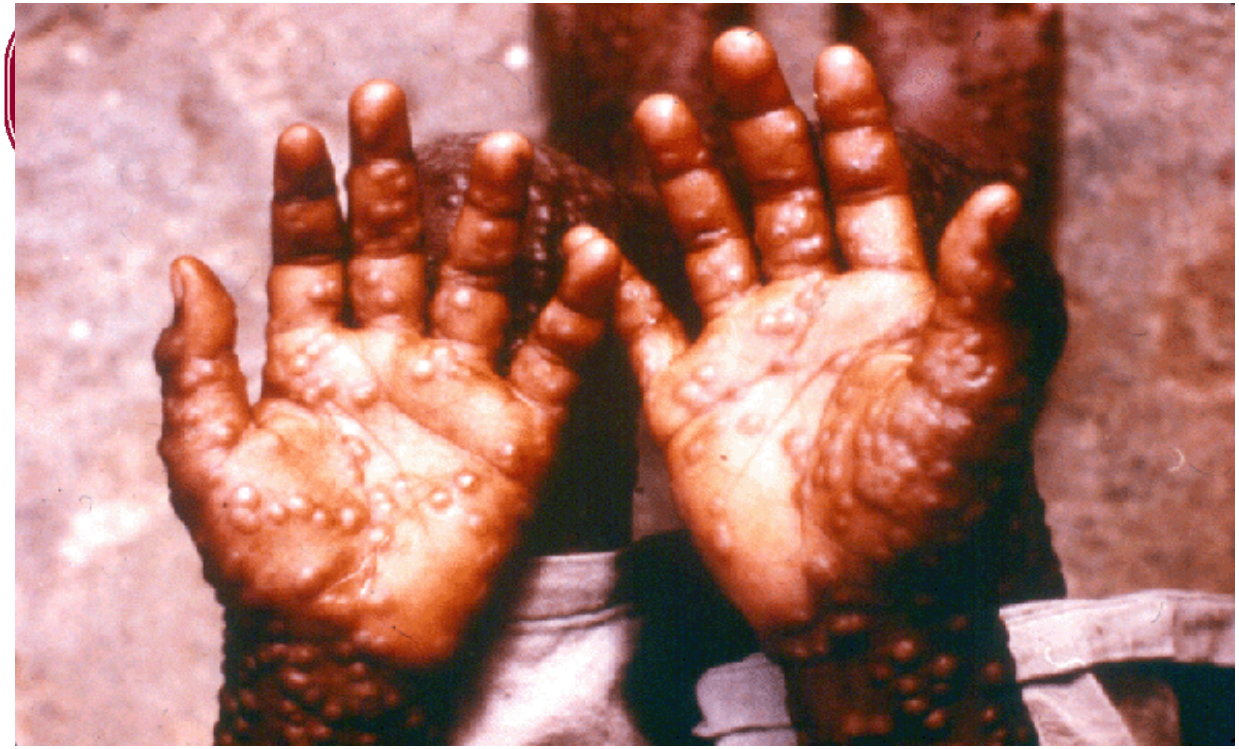
Clinical Progression



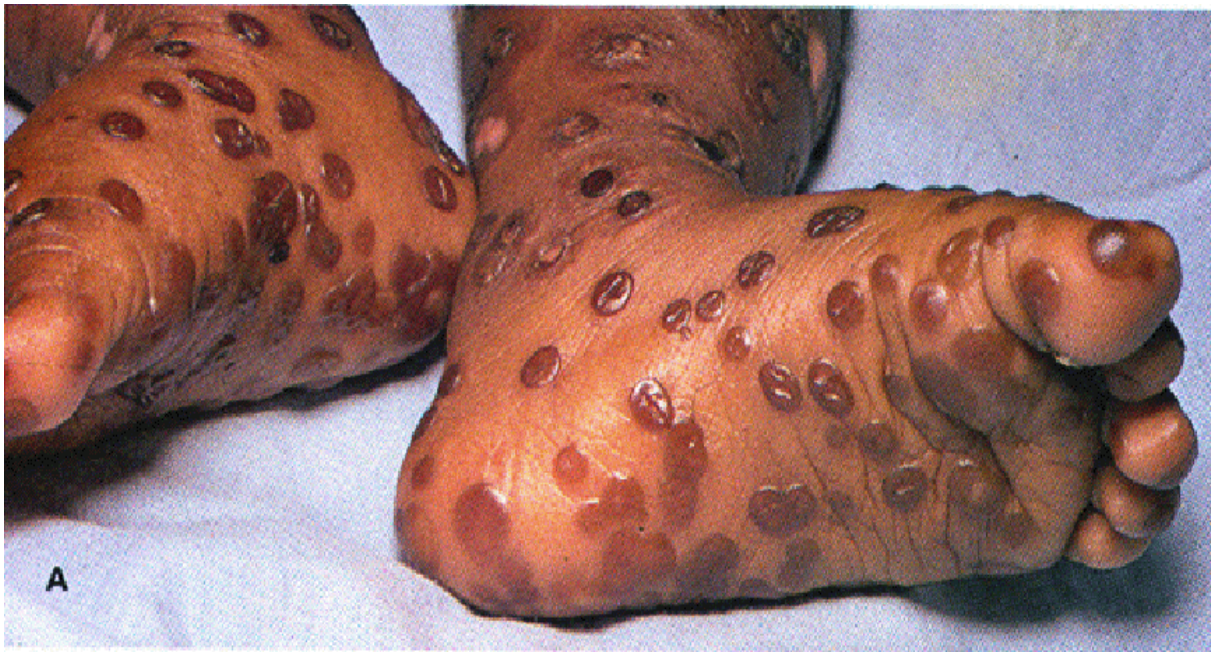
From:
CDC

Clinical Features: Key Points

- Prodrome
- Centrifugal rash
- Palms and soles
- Slow and synchronous progression
- Deep, firm lesions



Pustular lesions on palms



Flattened lesions on soles

CDC
Centers for Disease Control and Prevention

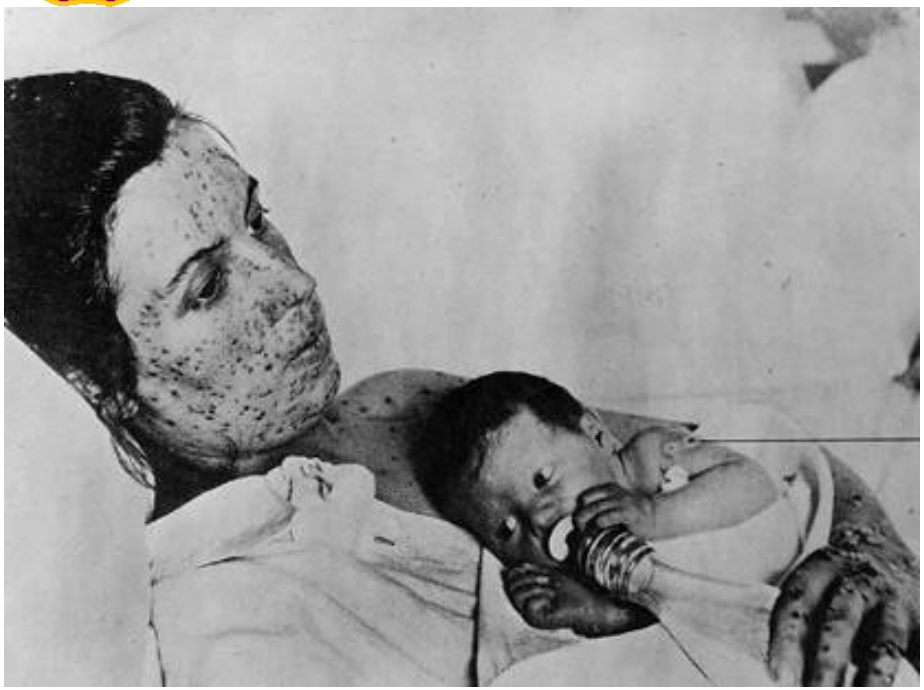
Clinical Types of Variola Major

Type	Frequency %	Mortality %
Ordinary Type (Classic)	88.8	30 (3)
– Discrete	- 42.1	- 9 (<1)
– Semiconfluent	- 23.9	- 37 (8)
– Confluent	- 22.8	- 62 (26)
Flat Type	6.7	97 (67)
Hemorrhagic Type	2.4	96 (94)
– Early	- 0.7	- 100 (100)
– Late	- 1.7	- 97 (90)
Modified Type	2.1%	0
Sine Eruptione	??	0

From Fenner et al 1988

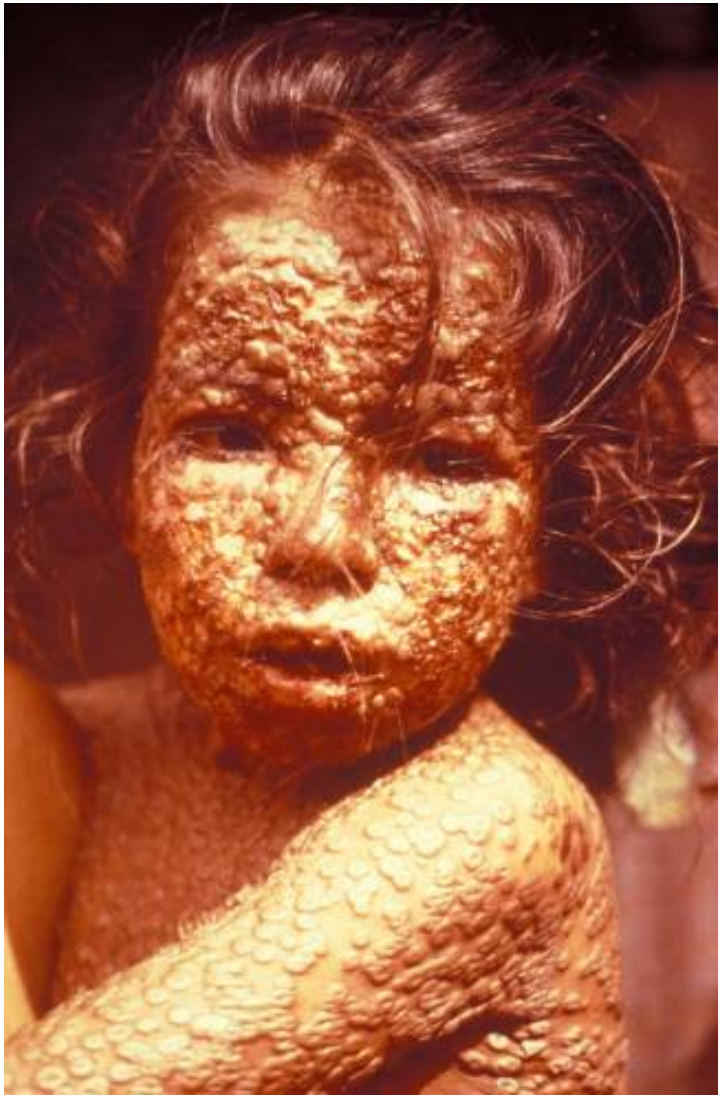
% in parentheses = for previously vaccinated individuals

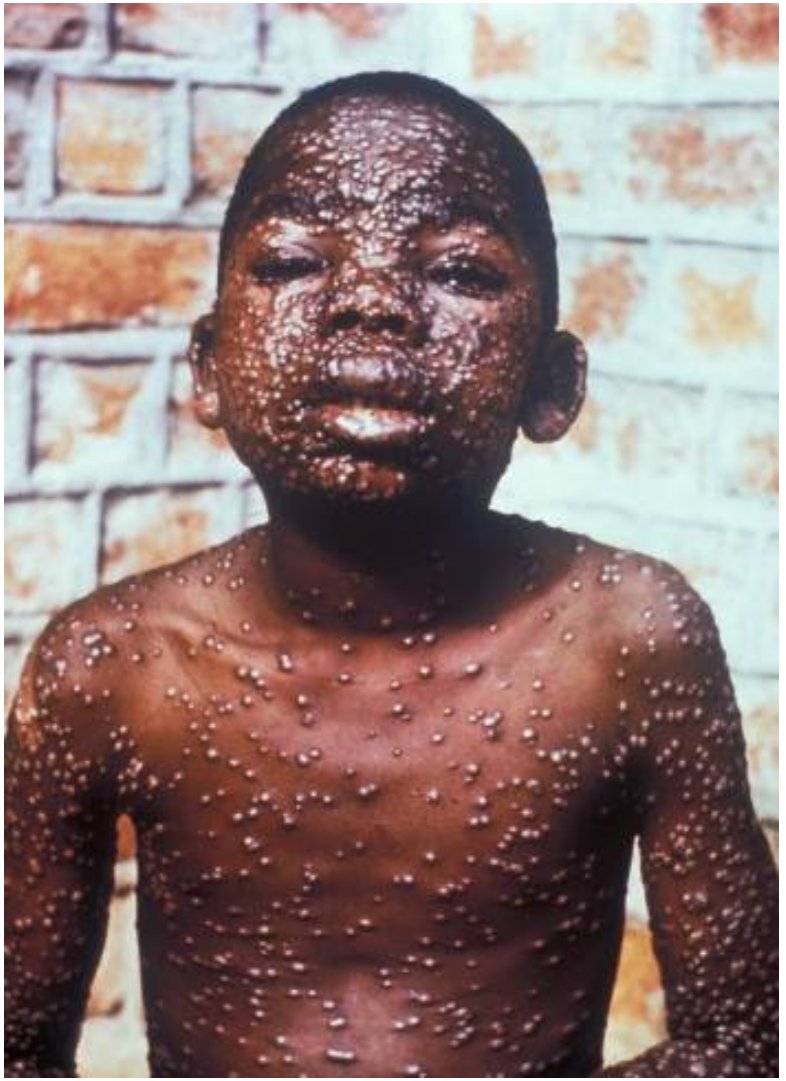
Ordinary Type Smallpox



Nat Museum Health and Med

Ordinary Type Smallpox





WHO

Flat Type and Hemorrhagic Smallpox

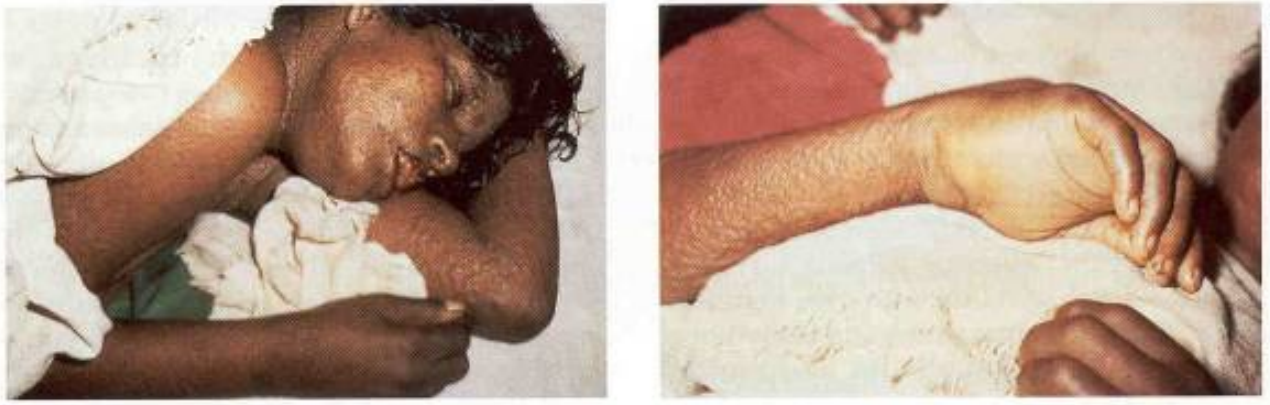


Fig. 27-3. Flat-type smallpox in an unvaccinated woman on the sixth day of rash. Extensive flat lesions (**a** and **b**) and systemic toxicity with fatal outcome were typical. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 33. Photographs by F. Dekking.

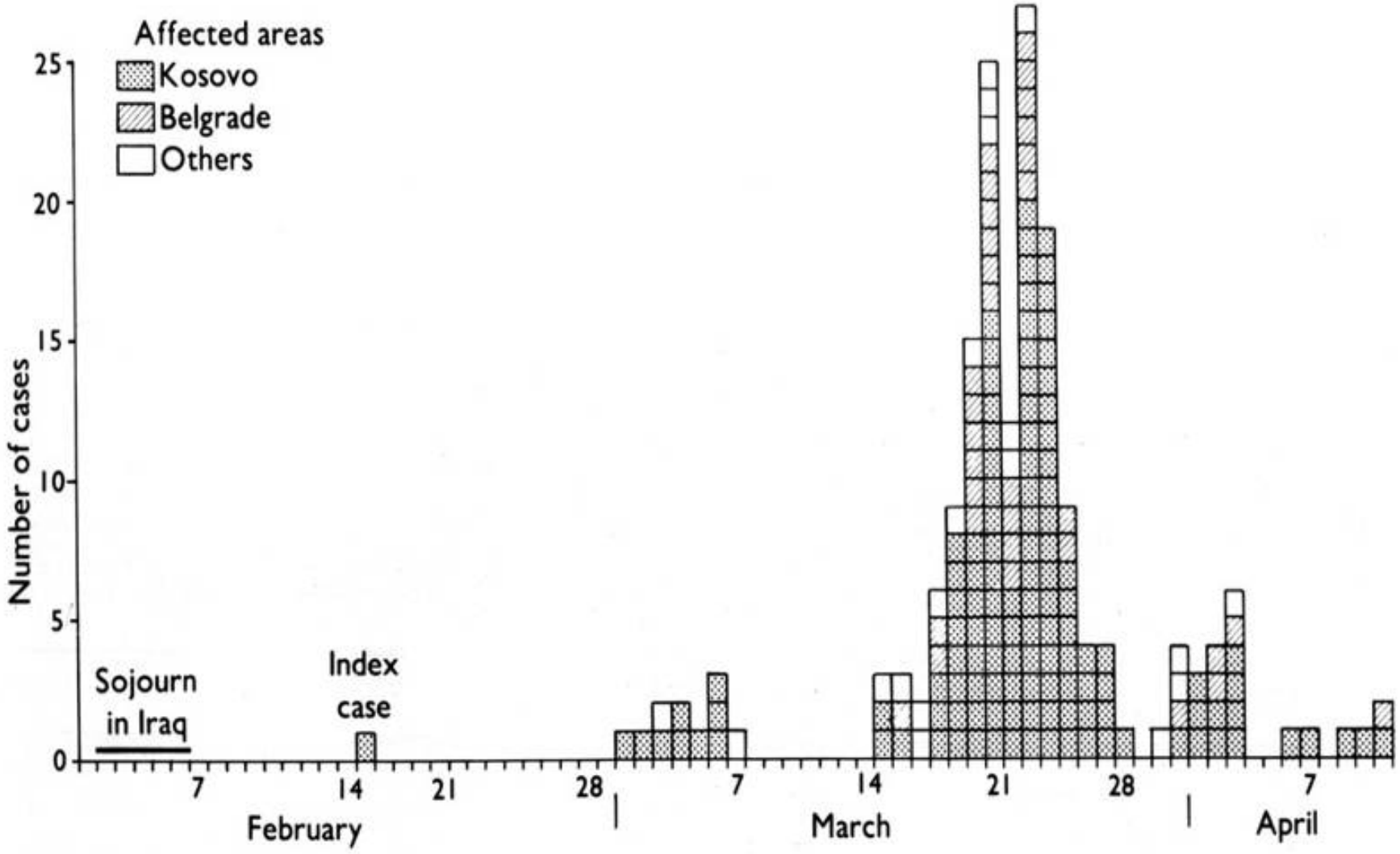
Fig. 27-4. Early hemorrhagic-type smallpox with cutaneous signs of hemorrhagic diathesis. Death usually intervened before the complete evolution of pox lesions. Reprinted with permission from Herrlich A, Mayr A, Munz E, Rodenwaldt E. *Die pocken; Erreger, Epidemiologie und klinisches Bild*. 2nd ed. Stuttgart, Germany: Thieme; 1967. In: Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 35.

Textbook of Military Medicine

Epidemiology

- Transmitted through respiratory secretions
  - Close, prolonged, face-to-face contact
- Rarely transmitted by other routes
  - Fomites (esp. clothing and linens)
  - Skin contact (oozing from lesions)
    - Theoretically from scabs
  - Small particle aerosol

Yugoslavia Outbreak, 1972

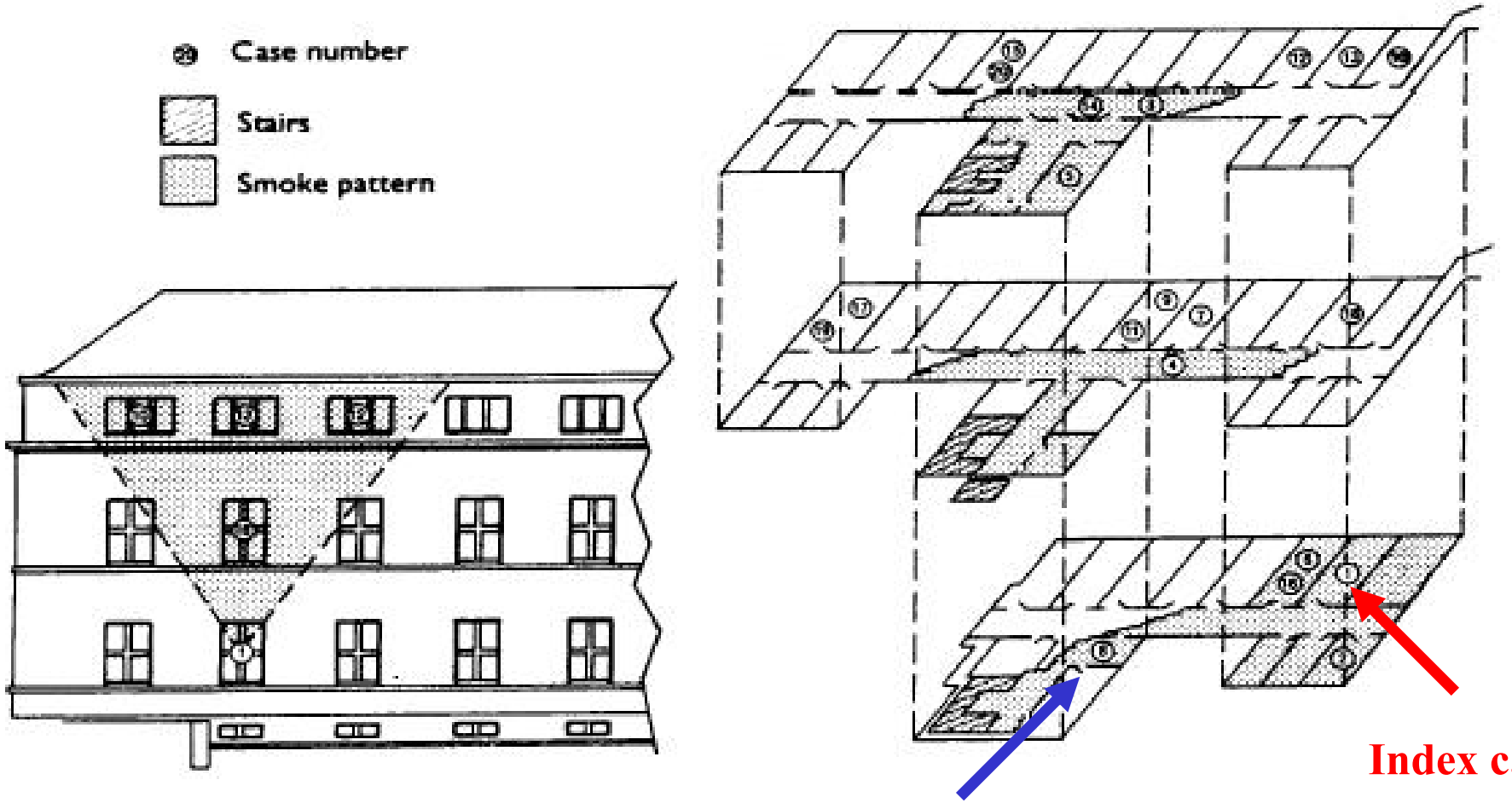


From Fenner, F. et al. 1988

Meschede Outbreak

- Index patient admitted 1 day after start of fever
- Rash starts on day 3-4, transferred on day 6
- Did not leave room, only 2 staff cared for
- Linens were not mixed
- Patient noted to have significant pulmonary involvement and cough
- Smallpox confirmed on day 6, all patients/staff vaccinated

Meschede Outbreak



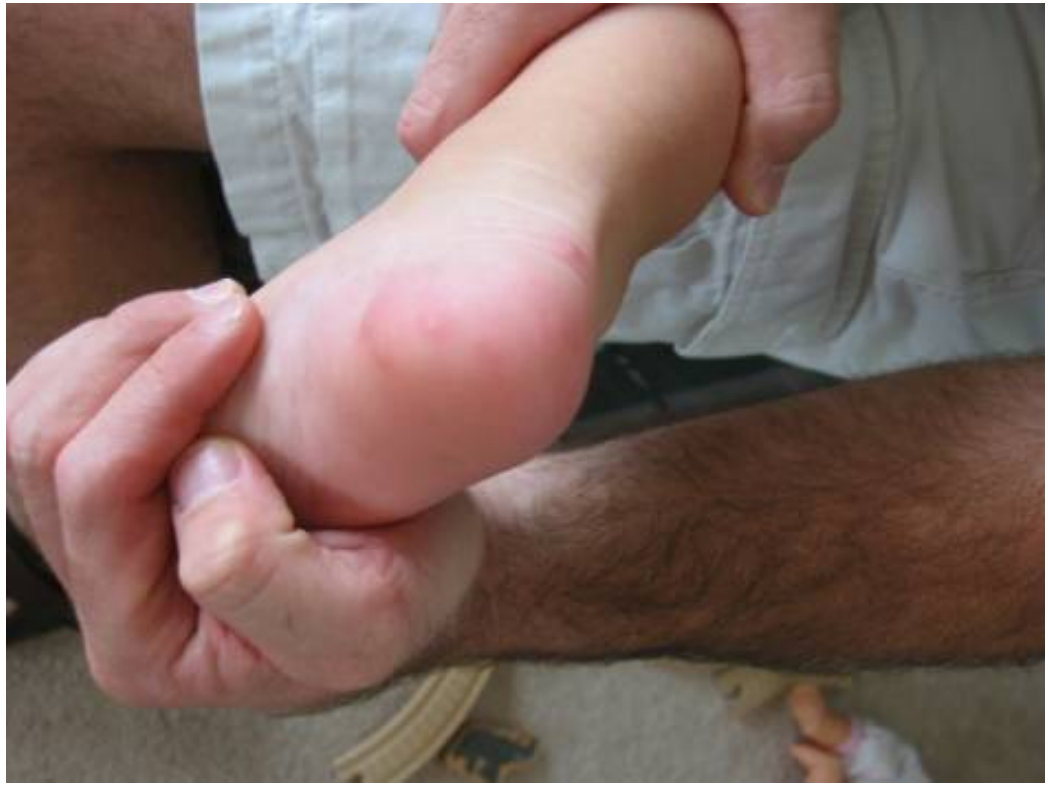
From Wehrle et al. 1970

Diagnosis

- **Clinical diagnosis!**

Differential diagnosis

- Varicella
- Disseminated herpes zoster/simplex
- Impetigo
- Drug eruptions
- Allergic contact dermatitis
- Erythema multiforme
- Hand, foot and mouth disease
- Rickettsialpox
- Other *Orthopoxvirus*



Differential Diagnosis

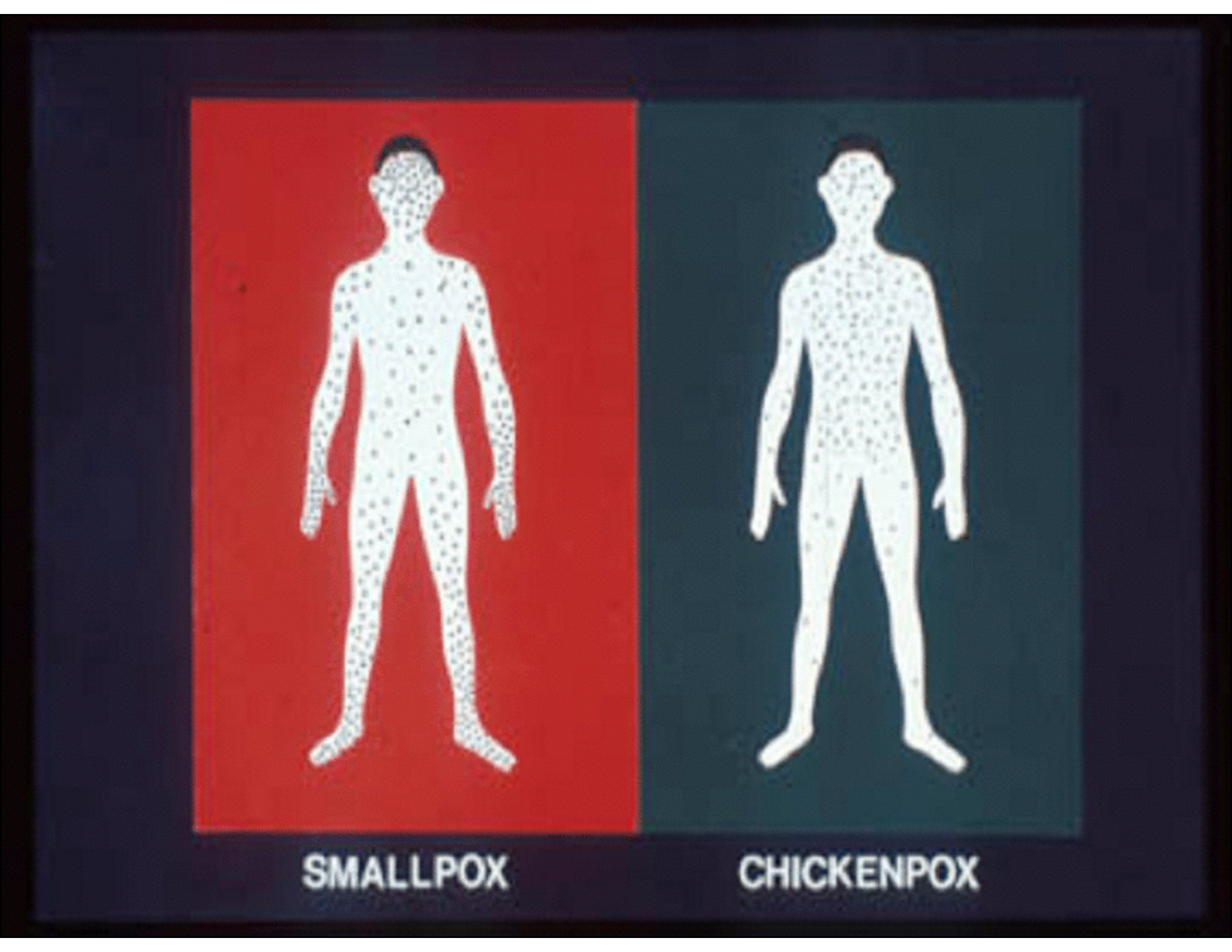
SMALLPOX

- Deep, firm lesions
- Round borders, well defined
- Lesions can touch, have dimples
- Lesions at same stage of development

CHICKEN POX

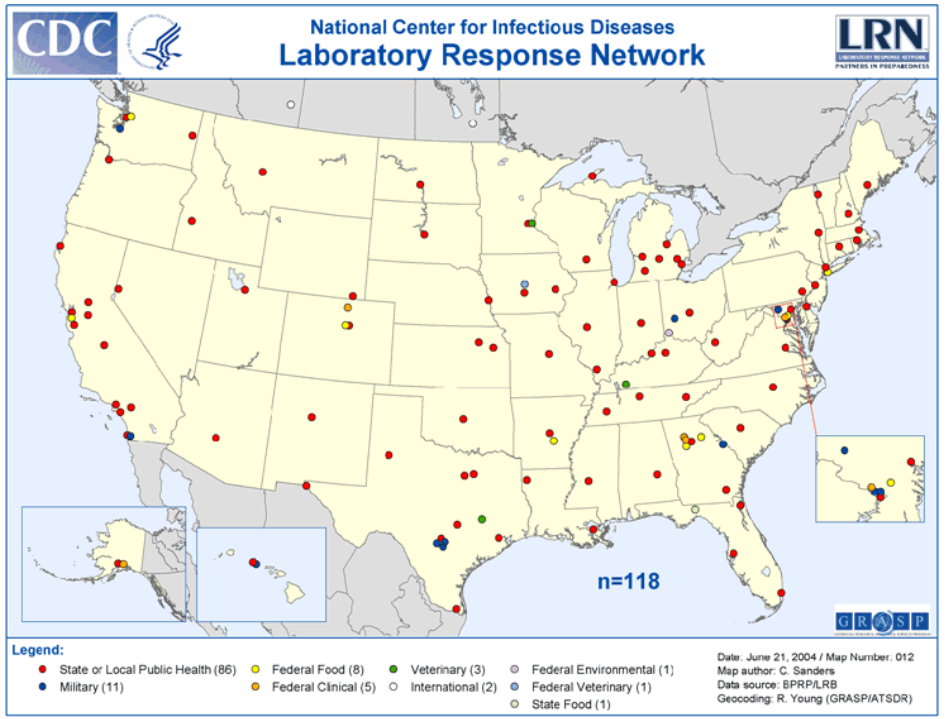
- Superficial lesions
- Ill defined borders
- Lesions do not touch each other or have dimples
- Lesions at different stages of development

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION


SMALLPOX
CHICKENPOX

Laboratory Diagnosis

- Lesion swab or tissue
- Gold standard = culture in chorioallantoic membrane of chick
  - Not practical
- Real-time PCR available at laboratory response network labs
  - And forward DoD labs
- Confirmation/strain identification etc. at CDC or USAMRIID



Management

- Cause of death in smallpox?
- Supportive care
  - Modern critical-care management may make large difference
    - Fluid/electrolyte balance
    - Treat severe cases similar to septic shock
      - Immune modulators?
  - Burn treatment strategies?
- Diligence watching for secondary infection
  - Pneumonia and soft tissue infection
- Analgesics
  - Lesions are extremely painful

Management – Infection Control

- Patient in respiratory isolation
- Previously vaccinated staff
  - Boost if > 3 years
  - Contact precautions (N-95 if pt coughing – probably prudent)
- May use previously unvaccinated staff
  - Vaccinate immediately
  - Contact and respiratory precautions
- Safe disposal of waste (biohazard bags - autoclave)
- Autoclave laundry

Treatment - Drugs

- **Thiosemicarbazones – developed in 1950's**
  - **Not effective as tx (minimally as prophylaxis)**
- **Vaccinia Immune Globulin (VIG)**
  - **Only effective for post-exposure prophylaxis**
  - **Very small supply reserved for vaccine complications**

Treatment - Antivirals

- **Cidofovir – nucleotide analog**
  - **Already approved for human use for CMV infection**
  - **Effective in animal models of poxvirus infections**
  - **IND for smallpox treatment held by DOD**
- **ST-246 – targets *Orthopoxvirus*-specific protein**
  - **Currently in Phase I trials**
  - **Several logs more effective than cidofovir in vitro**
  - **Compassionate use case**

VIG – Post Exposure Prophylaxis

- Randomized trial in Madras, India
  - Index case ID'd and randomized
  - Contacts get vaccine + VIG (n= 326) or vaccine alone (n = 379)
    - Vaccination histories similar
- Results
  - VIG = 5 cases of smallpox
  - No VIG = 21 cases
    - .025 < p < .05 (Chi-square by me)

Kempe et al. *Bull WHO*. 1961;25:41-8

Therapy: Desperate times…

HOMEOPATHIC SMALLPOX for use post exposure bioterrorism

Item number: 5608870218

Seller:	devkeunescats (805 ★) **Positive Feedback: 99.8%** Member since Jan-14-01 in United States
Starting bid:	**US $9.95**
Time left:	**2 days 22 hours** 7-day listing Ends Aug-28-05 18:04:10 PDT
Item location:	beautiful Norwalk Connecticut United States
Ships to:	Worldwide

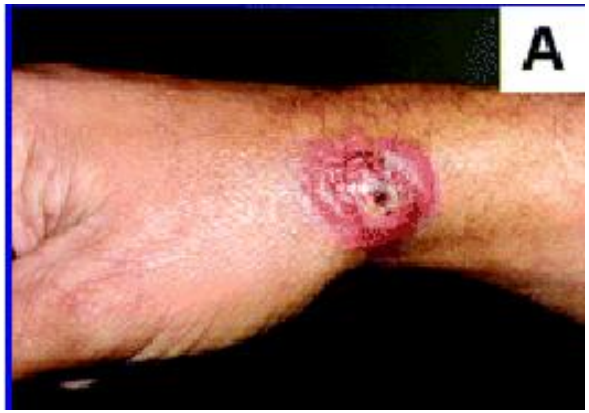
Summary

Up for your bidding consideration is a BRAND NEW--FACTORY SEALED--60 ml. bottle of homeopathic SMALL POX (Variola 12, 30 , 60X and Variolinum 30x, 60X) in a base of 20% alcohol and purified water. In case of a bioterrorist attack, there is enough remedy in this bottle for your family and you. Would you ever actually need this? I hope not but one can just not be sure these days..... ALWAYS REMEMBER TO CONSULT YOUR DOCTOR OR QUALIFIED HOMEOPATH BEFORE TAKING AND HEALTH SUPPLEMENTS!!! Thanks for looking and Good Luck!! It

SMALLPOX VACCINATION

Vaccinia Virus

- Became predominant vaccine by 20th century
- Debate of origins of vaccinia
  - Wild type virus?
    - Horsepox?
    - Buffalopox is sub-clade
    - Zoonotic vaccinia infections in Brazil
  - Lab mutant?
- Different strains used for vaccine
  - US used NY City Board of Health (NYCBH) strain



deSouza Trinidad et al. JCM 2007

Traditional Vaccine Production

- Seed
- Vaccinifer
- Scarification and incubation
- Harvest of pulp
- Lymph
- Stabilization

Nat Museum Health and Med

Vaccine Protection

Moo

- Smallpox mortality 3% in prior vaccinees (vs. 30% in unvacc.)
- Post-exposure vaccination
  - Significant immunity if given in first 3 days
  - Protective effect if given within 1 week
- Duration of immunity
  - Party line is 3 years
  - Probably protection even > 20 years

Vaccine Imparted Immunity

Table 11.25. Rate of protection afforded by vaccination

Location of outbreaks	Vaccination scar	Total number of contacts	Contacts developing smallpox: Number	Contacts developing smallpox: %	Rate of protection by vaccination[a] (%)	Reference
Madras, India	–	103	38	36.9	96.7	Rao et al. (1968a)
	+	1 146	14	1.2		
Punjab Province, Pakistan	–	45	33	73.3	95.7	Heiner et al. (1971a)
	+	190	6	3.2		
Punjab Province, Pakistan	–	22	10	45.5	97.1	Heiner et al. (1971b)
	+	238	3	1.3		
Sheikhupura District, Pakistan	–	43	38	88.4	91.9	Mack et al. (1972a)
	+	180	13	7.2		
Calcutta, India	–	80	61	76.3	90.7	Mukherjee et al. (1974)
	+	661	47	7.1		

[a] Rate of protection by vaccination = $100\left(1 - \frac{\text{percentage of vaccinated contacts with smallpox}}{\text{percentage of unvaccinated contacts with smallpox}}\right)$.

Table 11.26. Effect of vaccination after exposure on occurrence of smallpox in family or household contacts

Vaccination status of contacts	Number of contacts	Cases of smallpox: Number	Cases of smallpox: %	Reference
Primary vaccination after exposure	61	18	29.5	Rao et al. (1968a)
Never vaccinated	42	20	47.6	
Primary vaccination within 10 days of exposure	16	12	75.0	Mack et al. (1972a)
Never vaccinated	27	26	96.3	
Vaccinated or revaccinated within 7 days of exposure	52	1	1.9	Heiner et al. (1971b)
Never vaccinated	412	90	21.8	

From: Fenner 1988

Vaccine protection – Mack

- **Mack's study of imported cases into Europe 1950-1971**
  - **Vaccine protective for > 20 years**
  - **Mortality in unvaccinated > 50%**

Table 6. Age and vaccination status of all cases of variola major.

Successfully vaccinated	Age 0–9	10–49	50+	Unknown	Total
Never	30 (12)*	37 (18)	11 (10)	1 (1)	79 (41)
Only after exposure	20 (4)	41 (13)	9 (3)	0	70 (20)
0–10 years before exposure	18 (0)	48 (1)	5 (0)	1 (0)	72 (1)
11–20 years before exposure	. . .	40 (3)	3 (0)	0	43 (3)
20+ years before exposure	. . .	187 (8)	96 (25)	14 (0)	297 (33)
Unknown	24 (2)	50 (4)	24 (5)	21 (0)	119 (11)
Total	92 (18)	403 (47)	148 (43)	37 (1)	680 (109)

* Number of cases (number of deaths).

From: Mack 1972

Duration of Protection

- **Detection of memory T-cells 35-50 years after vaccination**

TABLE 1. Vaccinia virus-specific cytotoxic activity in PBMC of asymptomatic HIV-1-seropositive donors[a]

Donor no.	% Specific lysis of target cells			
	Uninfected B-LCL cells	Vaccinia virus-infected B-LCL cells	V/gp160-infected B-LCL cells	Uninfected K562 cells
2	17.2	29.8	23.8	1.8
11	8.9	31.8	29.0	1.7
12	10.1	25.9	18.4	2.3
13	2.8	13.5	15.4	2.1
26	7.0	17.4	13.1	2.5
27	2.3	17.8	15.4	3.3

[a] Unstimulated PBMC were tested directly in a CTL assay. Cytotoxicity was determined in a 6-h ^{51}Cr release assay effector-target cell at an ratio of 50:1. ^{51}Cr release was calculated by the formula 100 × (mean experimental release − mean spontaneous release)/(mean total release − mean spontaneous release). The results of an assay were excluded if the mean level of spontaneous release was >30%.

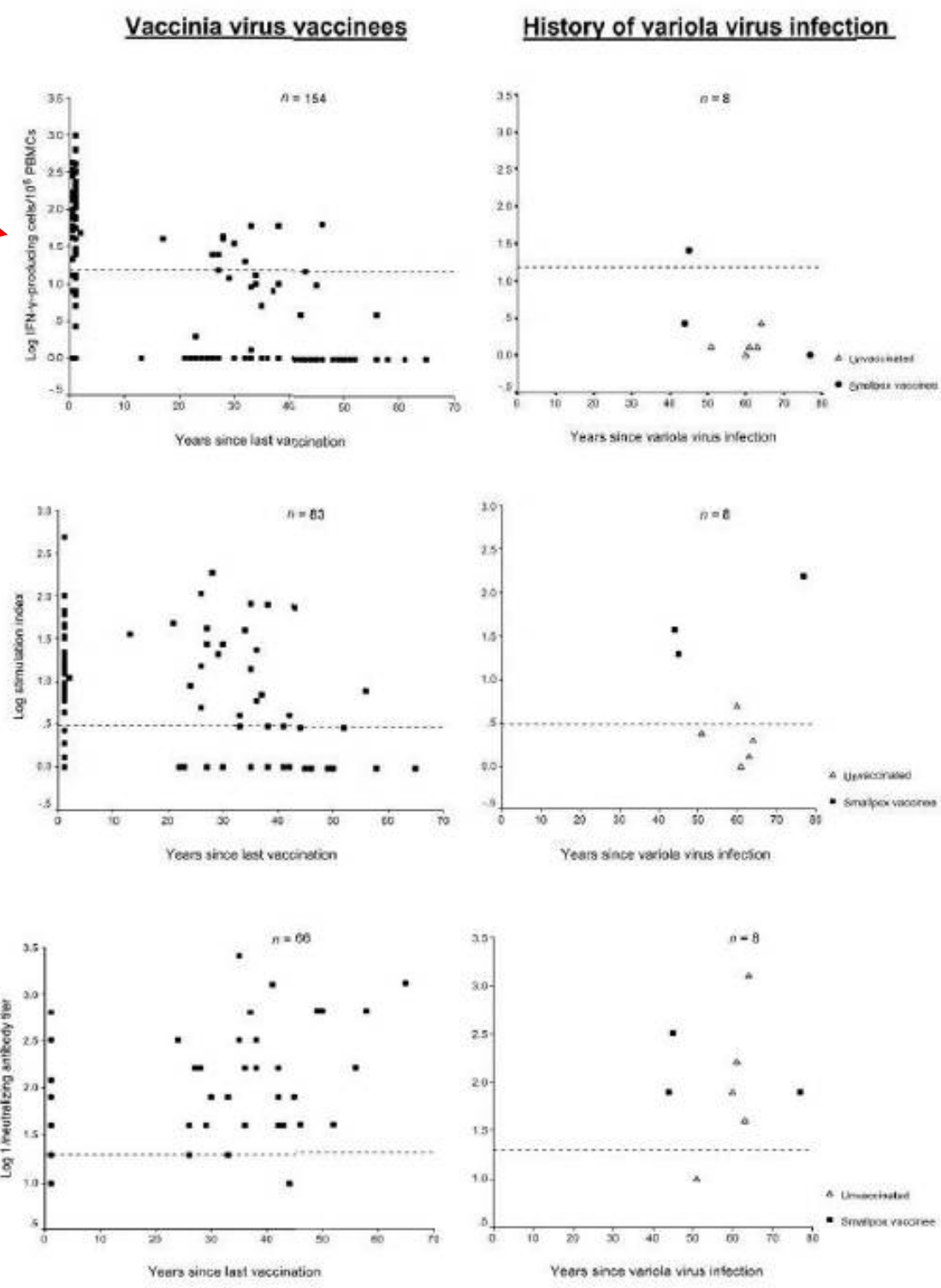
From: Demkowicz 1996

Duration of Protection

IFN-γ ELISPOT

Lymphoproliferation

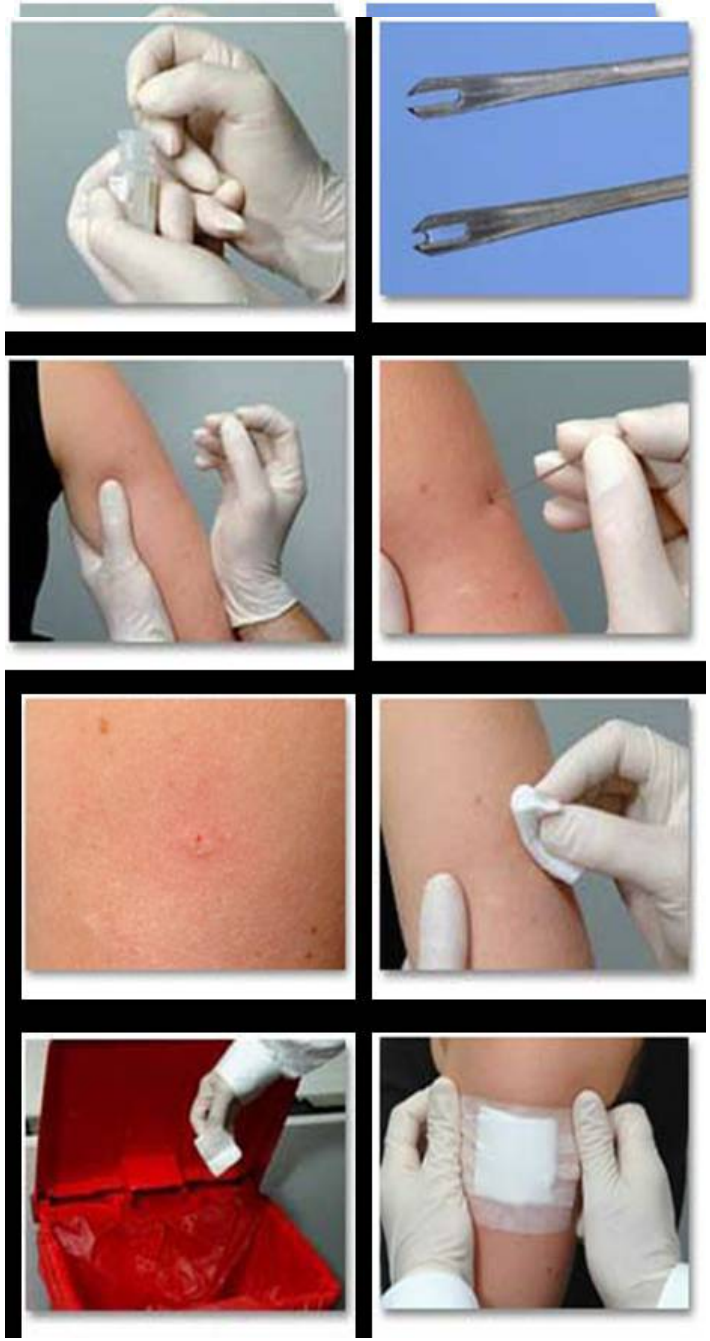
Neutralizating Ab



from Sivipalasingam et al. *JID* 2007.

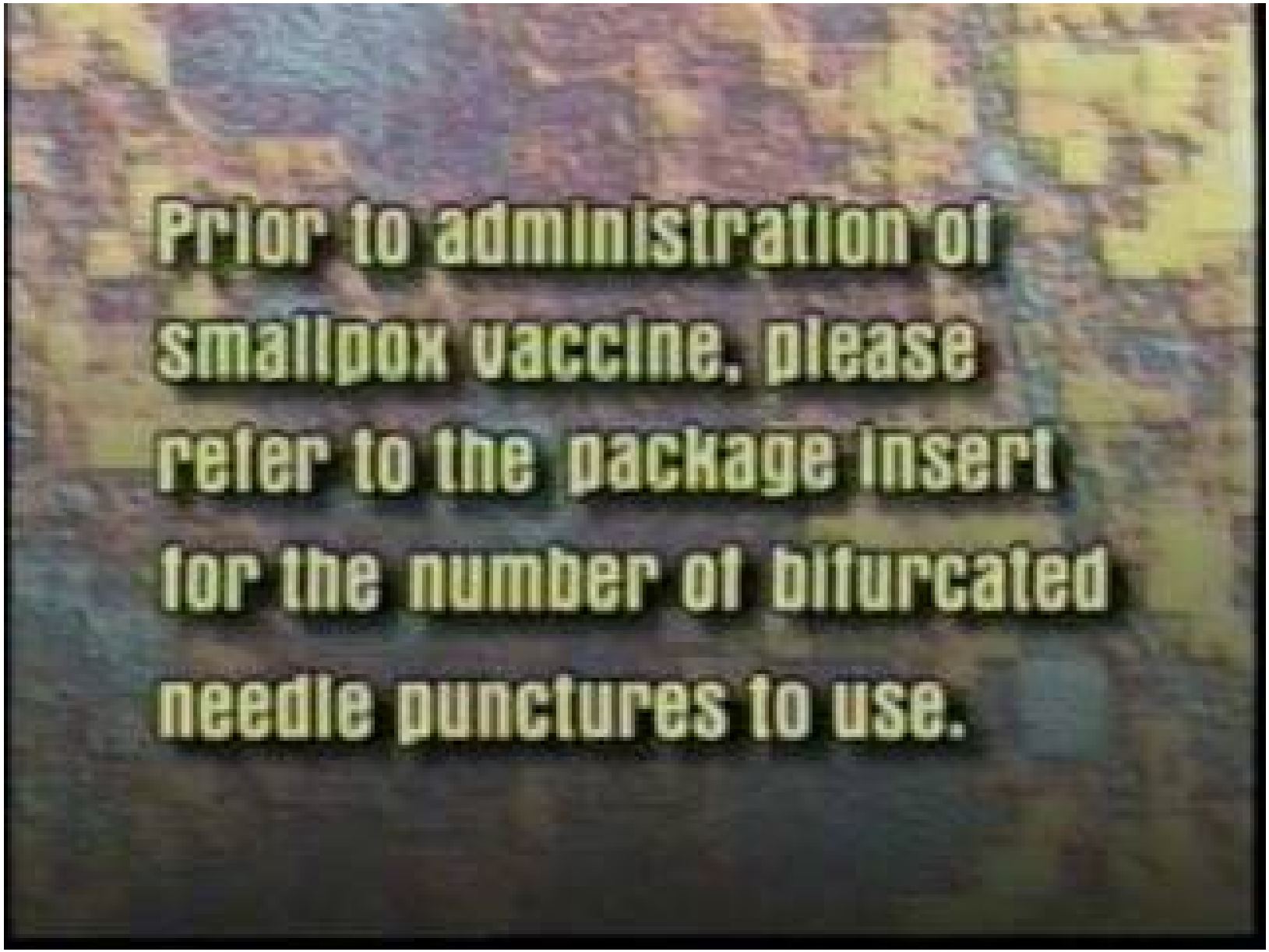
Vaccination

- Intradermal innoculation with bifurcated needle (scarification)
- "Major reaction"- also called "take"
  - ***ONLY PROVEN CORRELATE OF IMMUNITY!!!***
- Low grade fever, axillary lymphadenopathy
- Scar constitutes permanent record of successful vaccination

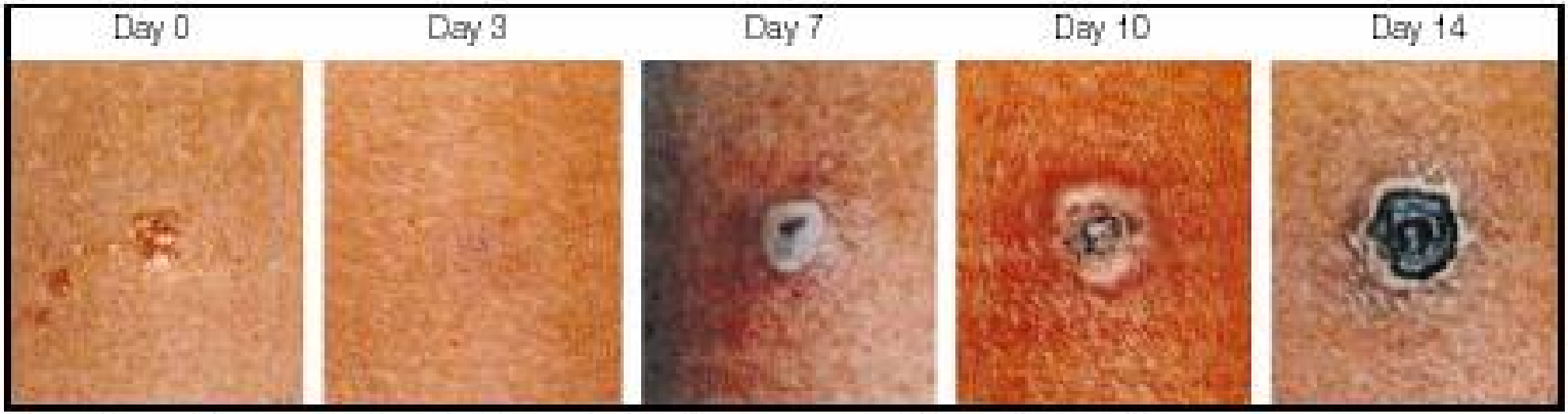


http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/vac_method.html

Vaccination Video



Major Reaction (Normal Response to Vaccination)



Reproduced with permission from the Centers for Disease Control and Prevention.[3]

Vaccine Complications

- Inadvertent inoculation 529
- Generalized vaccinia 241
- Eczema vaccinatum 38.5
- Progressive vaccinia 1.5
- Encephalitis 12.3
  - Numbers per million first-time vaccinees
- Fatal outcome in 1 in 1,000,000
- Myopericarditis (US Military 67/540,000)

From Lane et al 1970, Eckart et al 2004

Complications of Vaccination: CDC Survey

TABLE 3. Rates of reported complications* associated with vaccinia vaccinations† (cases/million vaccinations)

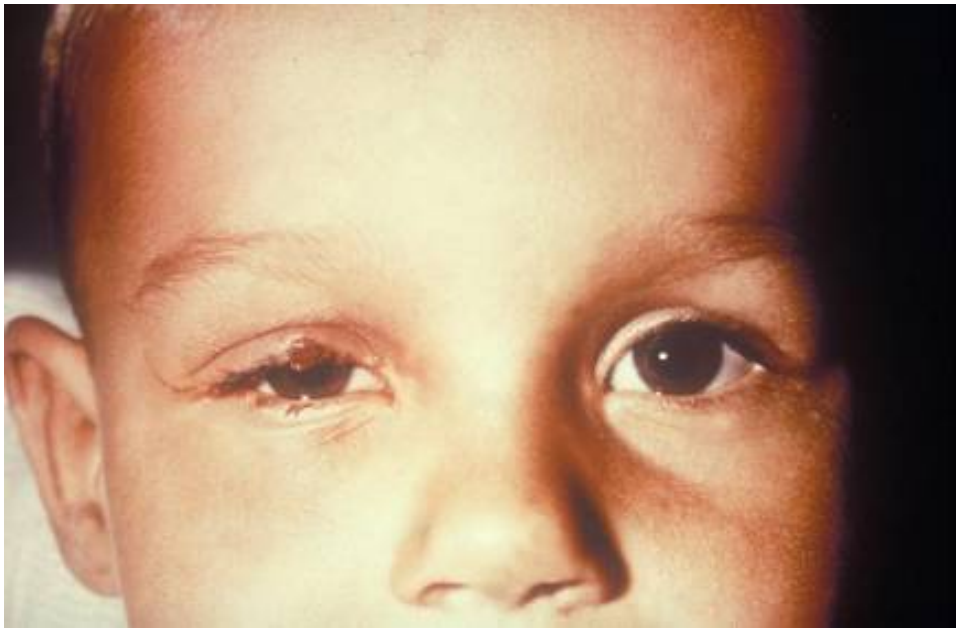
Age (yrs) and status	Inadvertent inoculation§	Generalized vaccinia	Eczema vaccinatum	Progressive vaccinia¶	Postvaccinial encephalitis	Total**
Primary vaccination						
<1	507.0	394.4	14.1	—††	42.3	1549.3
1–4	577.3	233.4	44.2	3.2	9.5	1261.8
5–19	371.2	139.7	34.9	—††	8.7	855.9
≥20	606.1	212.1	30.3	—††	—††	1515.2
Overall rates††	**529.2**	**241.5**	**38.5**	**1.5**	**12.3**	**1253.8**
Revaccination						
<1	—††	—††	—††	—††	—††	—††
1–4	109.1	—††	—††	—††	—††	200.0
5–19	47.7	9.9	2.0	—††	—††	85.5
>20	25.0	9.1	4.5	6.8	4.5	113.6
Overall rates§§	**42.1**	**9.0**	**3.0**	**3.0**	**2.0**	**108.2**

* See text for descriptions of complications.
† Adapted from Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. J Infect Dis 1970;122:303–9.
§ Referenced as accidental implantation.
¶ Referenced as vaccinia necrosum.
** Rates of overall complications by age group include complications not provided in this table, including severe local reactions, bacterial superinfection of the vaccination site, and erythema multiforme.
†† No instances of this complication were identified during the 1968 10-state survey.
§§ Overall rates for each complication include persons of unknown age.

From Lane et al. JID 1970

Complications of Vaccination: Auto-inoculation

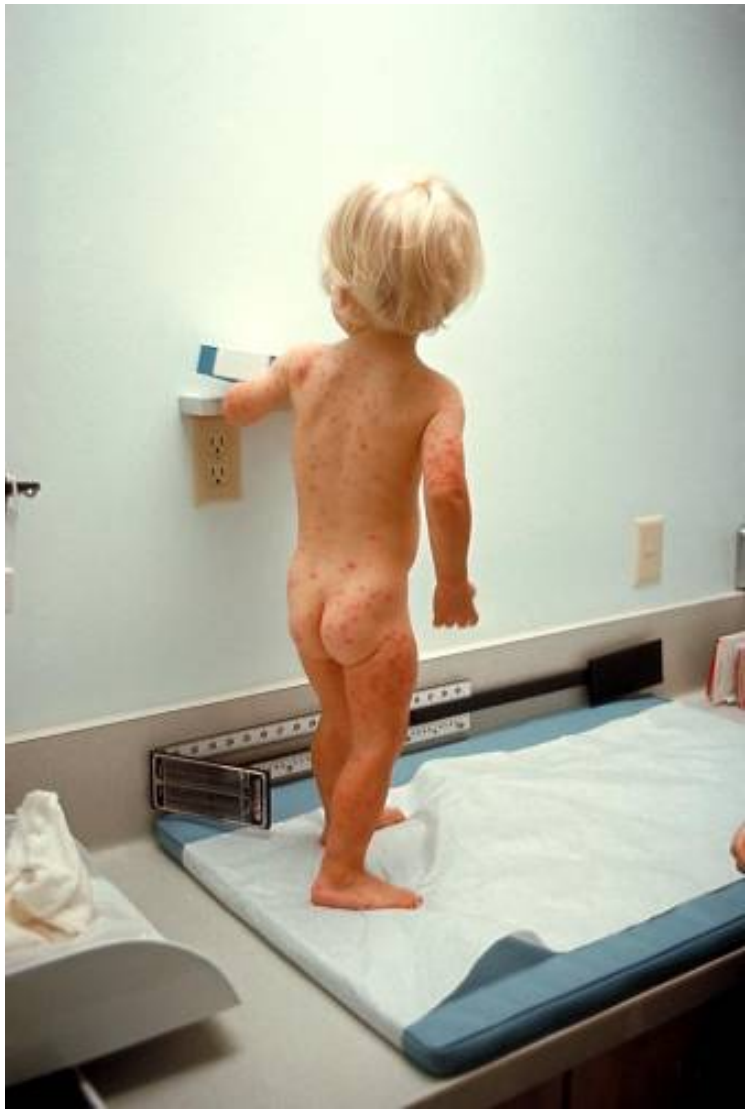
- **Mild side-effect unless eye involved**
- **VIG can be used in ocular auto-inoculation, except in keratitis**



CDC

Complications of Vaccination:
Generalized Vaccinia

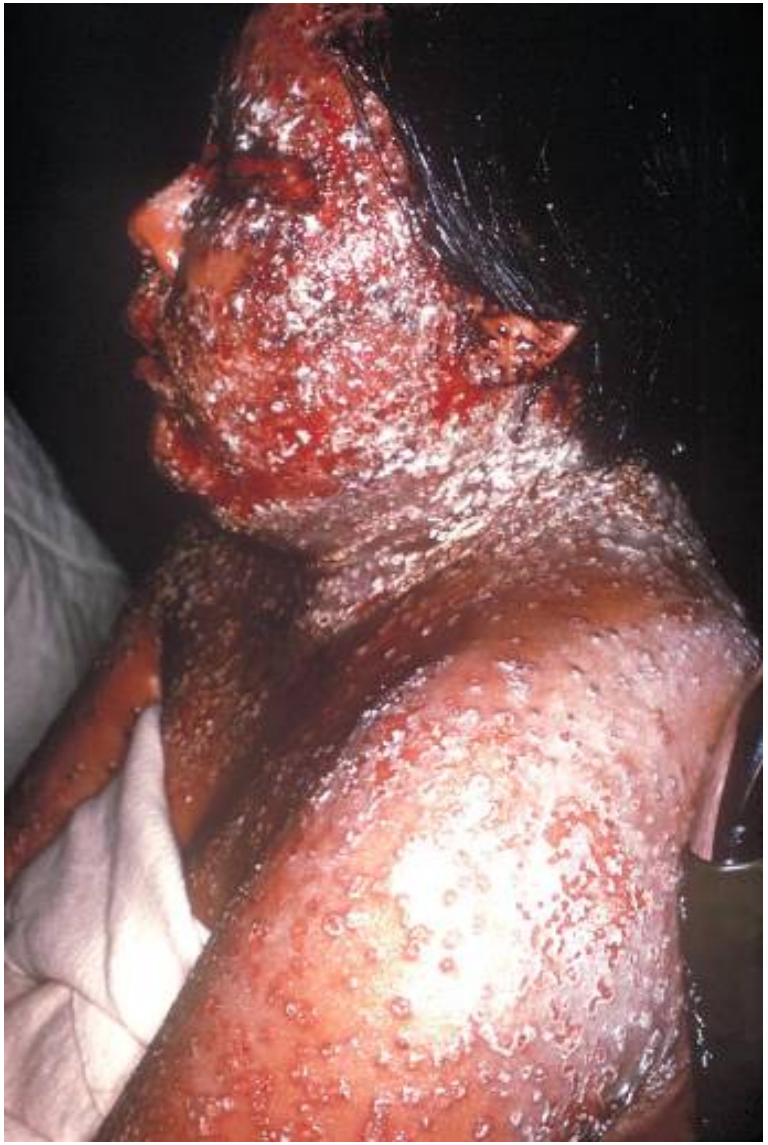
- **Usually occurs in normal hosts**
- **Self-limited**



CDC

Complications of Vaccination:
Eczema Vaccinatum

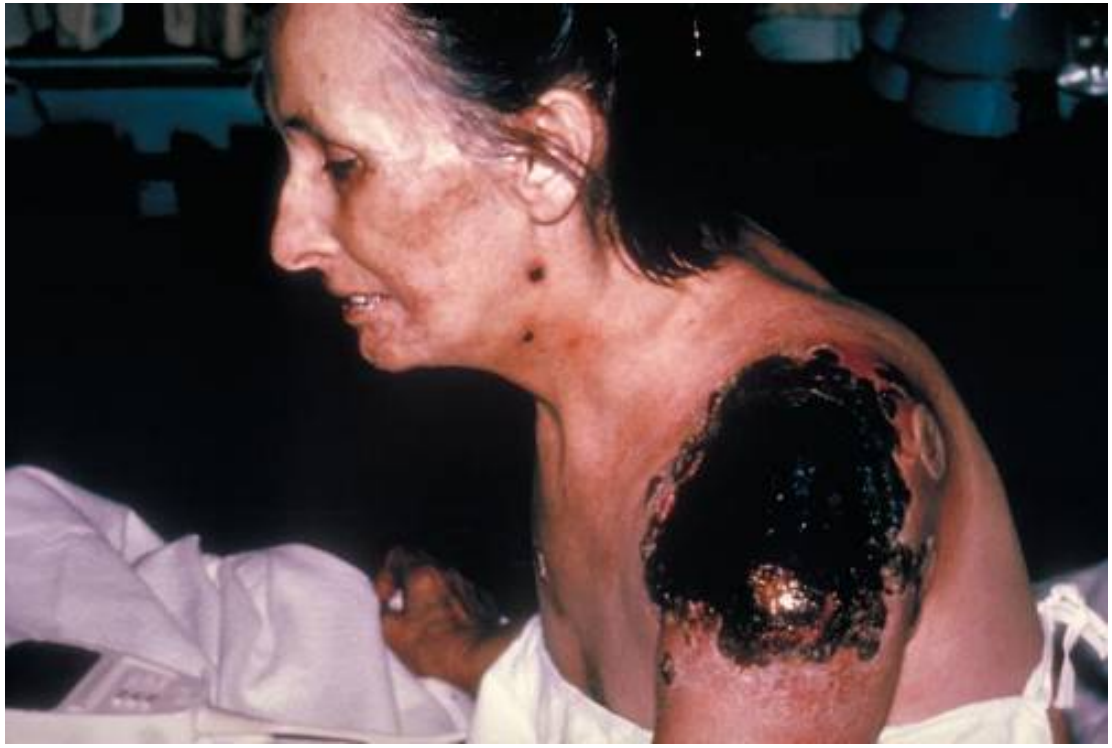
- Seen in vaccinees and contacts
- Current state of eczema not predictive
- VIG improves outcome



CDC

Complications of Vaccination: Vaccinia Necrosum

- Also known as progressive vaccinia
- Occurs in immuno-compromised
- High mortality
- VIG less effective



CDC

Complications of Vaccination: Postvaccinial Encephalitis

- More likely after 1^0 vaccination
- 25% mortality
- VIG only effective in prophylaxis
- Incidence depends on vaccinia strain

Table 11.20. Classification of strains by degree of pathogenicity[a]

Country of origin	Strain
High pathogenicity	
China	Temple of Heaven
Denmark	Copenhagen
France	Paris
Hungary	Budapest
Japan	Dairen, Ikeda
USSR	Gam, MRIVP, Per, Tashkent, TBK, Tom
Moderate pathogenicity	
Federal Republic of Germany	Bern
India	Patwadangar
USSR	BIEM, B-15
United Kingdom	Lister
Low pathogenicity	
USSR	EM-63
USA	New York City Board of Health

[a] Based on Marennikova et al. (1969).

From: Fenner 1988

Treatment of Complications

- VIG – effective for ectopic/contact cases, GV, EV, less effective for VN or encephalitis
  - intravenous licensed
- Cidofovir – consider for serious/life threatening
  - IND
- ST-246 – if you use cidofovir, you should strongly consider
  - Emergency use authorization from FDA req'd

Myocarditis/Pericarditis

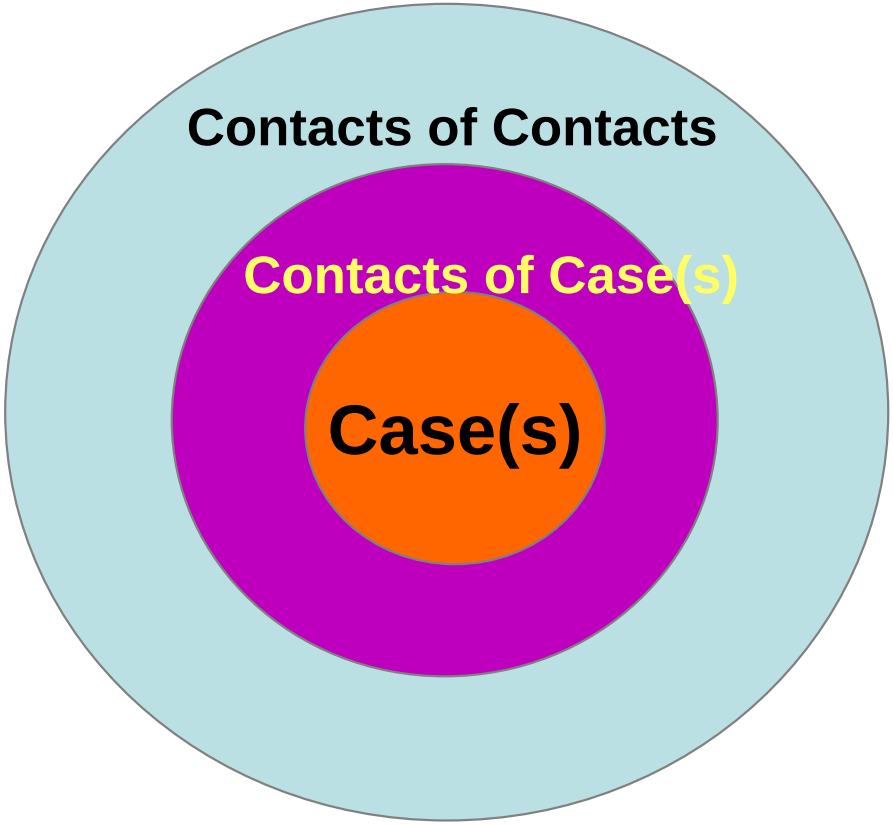
- Recent military vaccination experience: 67/541,000 (rate ~ 1/8,100)
  - Some with significant systolic dysfunction
  - Almost all recovered without sequelae
- Numerous case series in older literature
- No apparent link between vaccine and coronary disease

Vaccinia Contraindications

- **Avoid in immune disorders, pregnancy, eczema/other major skin conditions**
  - **And close contacts of above**
- **Avoid in breast-feeding, infants (<1), acute illness, allergy to vaccine components, heart disease (????)**
- **CONTRAINDICATIONS DO NOT APPLY IN CASE OF OUTBREAK**
  - **May need to do something to reduce likelihood of complications in high-risk individuals**

Surveillance and Containment Strategy (Ring Vaccination)

- Search for cases
- Provide a ring of immunity around each case
  - Used to eradicate smallpox
    - required to control disease <u>even with</u> 'routine or large-scale' immunization



Ring Vaccination Video

Dryvax…RIP

CDC | CDC Home | Search | Health Topics A-Z

MMWR Weekly

February 29, 2008 / 57(08);207-208

Notice to Readers: Newly Licensed Smallpox Vaccine to Replace Old Smallpox Vaccine

CDC has begun distribution of a new-generation smallpox vaccine, ACAM2000™ (Acambis, Inc., Cambridge, Massachusetts), to civilian laboratory personnel, the military, and state public health preparedness programs. ACAM2000 is a live, vaccinia virus smallpox vaccine that was licensed for use in the United States by the Food and Drug Administration in August 2007 (*1*).* ACAM2000 will be replacing Dryvax® smallpox vaccine (Wyeth Pharmaceuticals, Inc., Marietta, Pennsylvania) because of withdrawal of the Dryvax license. ACAM2000 is a live vaccinia virus derived from plaque purification cloning from Dryvax. The safety data available from the ACAM2000 clinical trials indicate a similar safety profile to Dryvax.

Wyeth intends to withdraw the Dryvax license and asks that all remaining quantities of vaccine held by civilian and military users be quarantined by February 29, 2008, for the purpose of destruction. This withdrawal is not necessitated by any safety, purity, or quality concerns with the product but rather is consistent with a contract agreement between CDC and Wyeth.† All lots of Dryvax vaccine will expire on February 29, 2008, and should not be used after that date.

All Dryvax vaccine should be destroyed on site. Vaccine vials can be 1) dropped into the hospital sharps container and autoclaved or 2) disposed of following the procedure for all other biohazard materials. In sites where medical waste is buried, soaking the medical waste in a 1:10 dilution of bleach for at least 10 minutes before disposal is advised. All programs that hold supplies of Dryvax vaccine must provide documentation of Dryvax vaccine destruction to the CDC Drug Service by March 31, 2008. These programs are advised to use the Dryvax vaccine destruction form.§

CDC will continue to provide ACAM2000 smallpox vaccine to protect responders as part of state public health preparedness programs (*2*) and civilian laboratory personnel who risk exposure to orthopoxviruses (*3*). Unlike Dryvax, ACAM2000 expires 18 months after release from the CDC Strategic National Stockpile. Requests for smallpox vaccine should be directed to the CDC Drug Service by e-mail (drugservice@cdc.gov) or telephone (404-639-3670).

References

1. Food and Drug Administration. Product approval information. Available at http://www.fda.gov/cber/products/acam2000.htm.
2. CDC. Recommendations for using smallpox vaccine in a pre-event vaccination program: supplemental recommendations of the Advisory Committee on Immunizations Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR 2003;52 (Dispatch).
3. CDC. Smallpox vaccine available for protection of at-risk laboratory workers. MMWR 1983;32:543--4.

Current U.S. Vaccines

- Acambis ACAM 2000 cell culture (vero cell) vaccine (licensed Aug 2007)
  - Plaque-picked NYCBH
- Aventis pasteur smallpox vaccine (unlicensed)
  - Found 85M doses in 2002
- Enough vaccine in SNS to vaccinate every person in the United States

Future Vaccines

- Decreased virulence is goal
- MVA
  - Used in several countries prior to eradication
  - Bioshield purchase of 20M doses from Bavarian Nordic, June 2007
    - Currently in Phase II trials
- LC16m8
  - Used in Japan, good prelim data
  - VaxGen development on hold as of June 2007

MVA

Table 2
Self-assessed local and systemic reactogenicity are represented

Part A: Reactogenicity following placebo or MVA injection series	Vaccinia-naive		Vaccinia-immune	
	Placebo $N=11$	All MVA $N=54$	Placebo $N=30$	All MVA $N=45$
$T>100$	0 (0.0%)	2 (3.7%)	0 (0.0%)	0 (0.0%)
Headache	4 (36.4%)	17 (31.5%)	8 (26.7%)	7 (15.6%)
Malaise	6 (54.5%)	22 (40.7%)	5 (16.7%)	13 (28.9%)
Myalgia	3 (27.3%)	16 (29.6%)	5 (16.6%)	10 (22.2%)
Chills	3 (27.3%)	3 (5.6%)	1 (3.3%)	1 (2.2%)
Nausea	4 (36.4%)	7 (13.0%)	4 (13.3%)	1 (2.2%)
Pain at site	2 (18.2%)	26 (48.1%)	5 (16.7%)	19 (42.2%)*
Underarm pain	1 (9.1%)	6 (11.1%)	0 (0.0%)	5 (11.1%)
Underarm swelling	2 (18.2%)	1 (1.9%)	0 (0.0%)	2 (4.4%)
Pruritus[a]	1 (9.1%)	3 (5.6%)	1 (3.3%)	6 (13.3%)

Part B: Reactogenicity following Dryvax® challenge	Vaccinia-naïve		Vaccinia-immune	
	Dryvax® only[b] $N=20$	All MVA/Dryvax® $N=43$	Dryvax® only $N=28$	All MVA/Dryvax® $N=39$
$T>100$	3 (15.0%)	0 (0.0%)**	2 (7.1%)	0 (0.0%)
Headache	7 (35.0%)	18 (41.9%)	11 (39.3%)	8 (20.5%)
Malaise	15 (75.0%)	23 (53.5%)	11 (39.3%)	8 (20.5%)
Myalgia	10 (50.0%)	19 (44.2%)	6 (21.4%)	8 (20.5%)
Chills	4 (20.0%)	1 (2.3%)**	3 (10.7%)	2 (5.1%)
Nausea	9 (45.0%)	10 (23.3%)	1 (3.6%)	1 (2.6%)
Pain at site	16 (80.0%)	22 (51.2%)**	5 (17.9%)	15 (38.5%)
Underarm pain	16 (80.0%)	20 (46.5%)**	5 (17.9%)	4 (10.3%)
Underarm swelling	12 (60.0%)	13 (30.2%)**	2 (7.1%)	1 (2.6%)
Pruritus[a]	18 (90.0%)	39 (90.7%)	25 (89.3%)	26 (66.7%)**
Mean diameter of peak erythema (cm)	3.665	1.860**	2.386	1.223**
Mean diameter of peak induration (cm)	2.565	1.818**	1.643	1.192**
Mean diameter of peak lesion size (cm)	1.145	0.820**	0.821	0.692

The number and percent of volunteers with systemic rated as mild, moderate, or severe are included following primary injection series of either MVA or placebo (panel A) and following Dryvax® (panel B).

[a] Indicates local reactogenicity.

[b] Includes 11 volunteers in the placebo group after Dryvax® challenge and 9 volunteers in two dose Dryvax® group after their first Dryvax® vaccination.

* p-Value < 0.05 comparing all MVA to placebo, two-sided Fisher's exact test.

** p-Value < 0.05 comparing all MVA/Dryvax® to Dryvax® only, two-sided Fisher's exact test.

- **MVA safer, likely less effective than NYCBH (DyVax)**
- **Prime-boost strategy may be best of both worlds**

From Parrino et al.
Vaccine 2007

Vaccine Adverse Events

- DoD Vaccine Clinical Call Center at 1-866-210-6469 (business hours)
- CDC hotline 800-CDC-INFO
- USAMRIID 800-USA-RIID

Questions????

jlawler@who.eop.gov

USAMRIID

Laboratory Identification of BioWarfare & Terrorism Agents

MAJ Jeanne A. Geyer, Ph.D.

Chief, Systems Development Branch
Diagnostic Systems Division, USAMRIID
jeanne.geyer@us.army.mil

Botulinum Toxin

How easy is it to identify/confirm?

Botulinum Toxins

- **7 antigenically distinct toxins (A through G)**
  - Types A, B, E, and rarely F, cause human disease
  - Types C and D cause disease in birds in mammals
  - Type G not shown to cause disease in animals or humans
- **Produced by *Clostridium botulinum* as well as a few other Clostridium species**
- **Toxin is on a "mobile genetic element" (i.e., phage, plasmid)**
- **~150 KD protein**
- **Neurotoxin – prevents acetylcholine release from synaptic terminals at the motor neurons**
- **1000 times more toxic than VX (lethal at 0.001 μg/kg)**

Botulinum Toxins

- PCR will ID the genetic component that makes the toxin, not the toxin itself….how much will actually remain in the sample?
- Requires 7 PCR targets to test for all types.
- Requires a mixture of antibodies to identify all 7 types.

- ….or does it?

Botulinum Toxins

Bottom line. Testing is more complex than it appears on the surface.

Agenda

- USAMRIID/DSD Mission
- Key Considerations
- Introduction to the Agents and the Battlefield
- Common Technologies Used by Labs for Agent Identification on the Battlefield
- Scenarios
- Conclusion

USAMRIID MISSION

Conduct research to develop strategies, products, information, procedures, and training for **medical defense against biological warfare agents** and naturally occurring agents of military importance that require special containment.

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DSD MISSION STATEMENT

Conduct research to develop diagnostic strategies, products, information, procedures, and training for medical defense against biological warfare agents and naturally occurring agents of military importance that require special containment.

Key considerations

- Why are you testing the sample?
- What do you test for?
- What testing and technology is sufficient to call something positive…or negative?
- How does technology help?

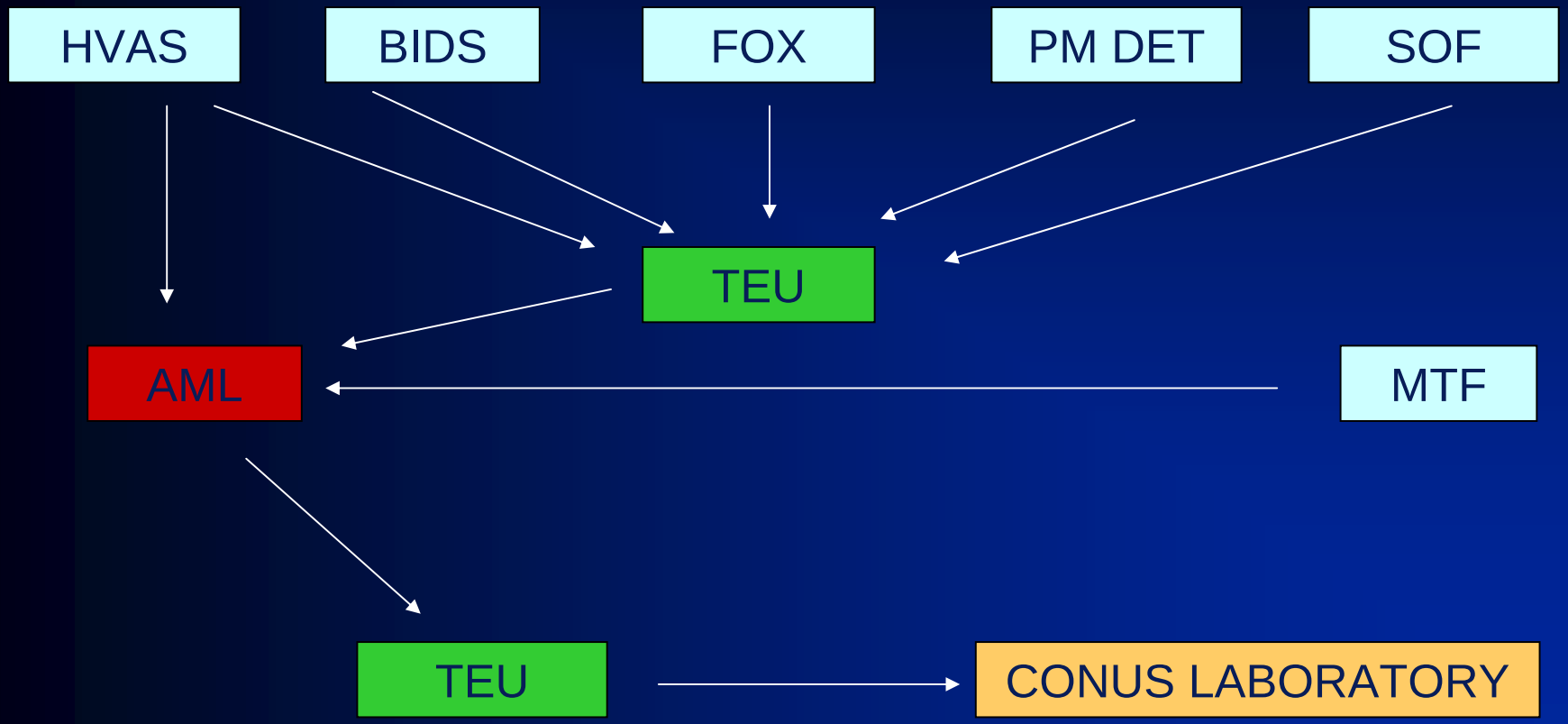
Soviet BW Priorities
"Agents Likely to be Used"

Smallpox	26
Plague	23
Anthrax	21
Botulism	21
VEE	20
Tularemia	20
Q Fever	20
Marburg	18
Influenza	17
Melioidosis	17
Typhus	15

Vorobjev, A., et.al., "Criterion Rating" as a Measure of Probable Use of Bioagents as Biological Weapons, International Symposium, Severe Infection Diseases, Kirov, June 1997

USAMRIID

Integrated Battlefield

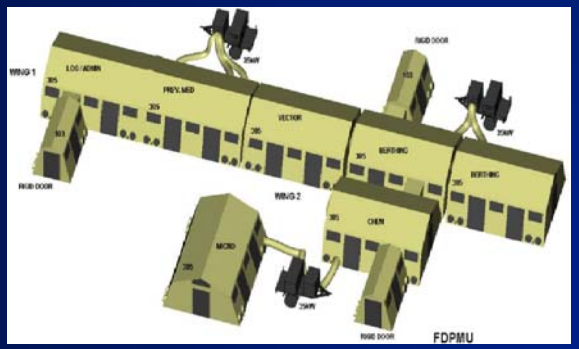


USAMRIID

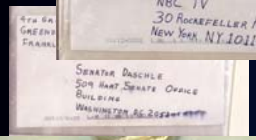
Integrated Battlefield

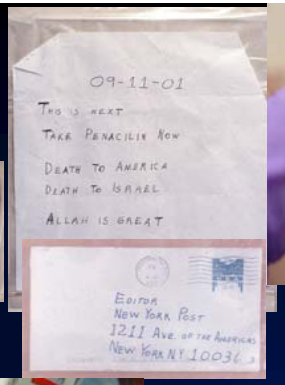
Army Area Medical Laboratory (AML)

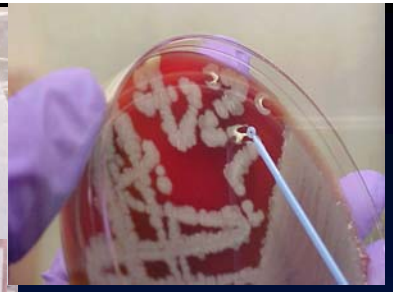
Navy Forward Deployed Preventive Medicine Unit (FDPMU)



Air Force Biological Augmentation Team (BAT)







Laboratory

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Aerosol Detectors

Biological Integrated Detection System (BIDS)
Semi-automated biological detection/identification

JSLNBCRS

Joint Biological
Point
Detection
System (JBPDS)

Joint Portal Shield

M93A1 FOX Reconnaissance System
Nuclear and Chemical detection
and Biological sampling

Dry Filter Unit
(DFU)

KEY POINT ON DETECTORS

Biological agent detectors are detect to

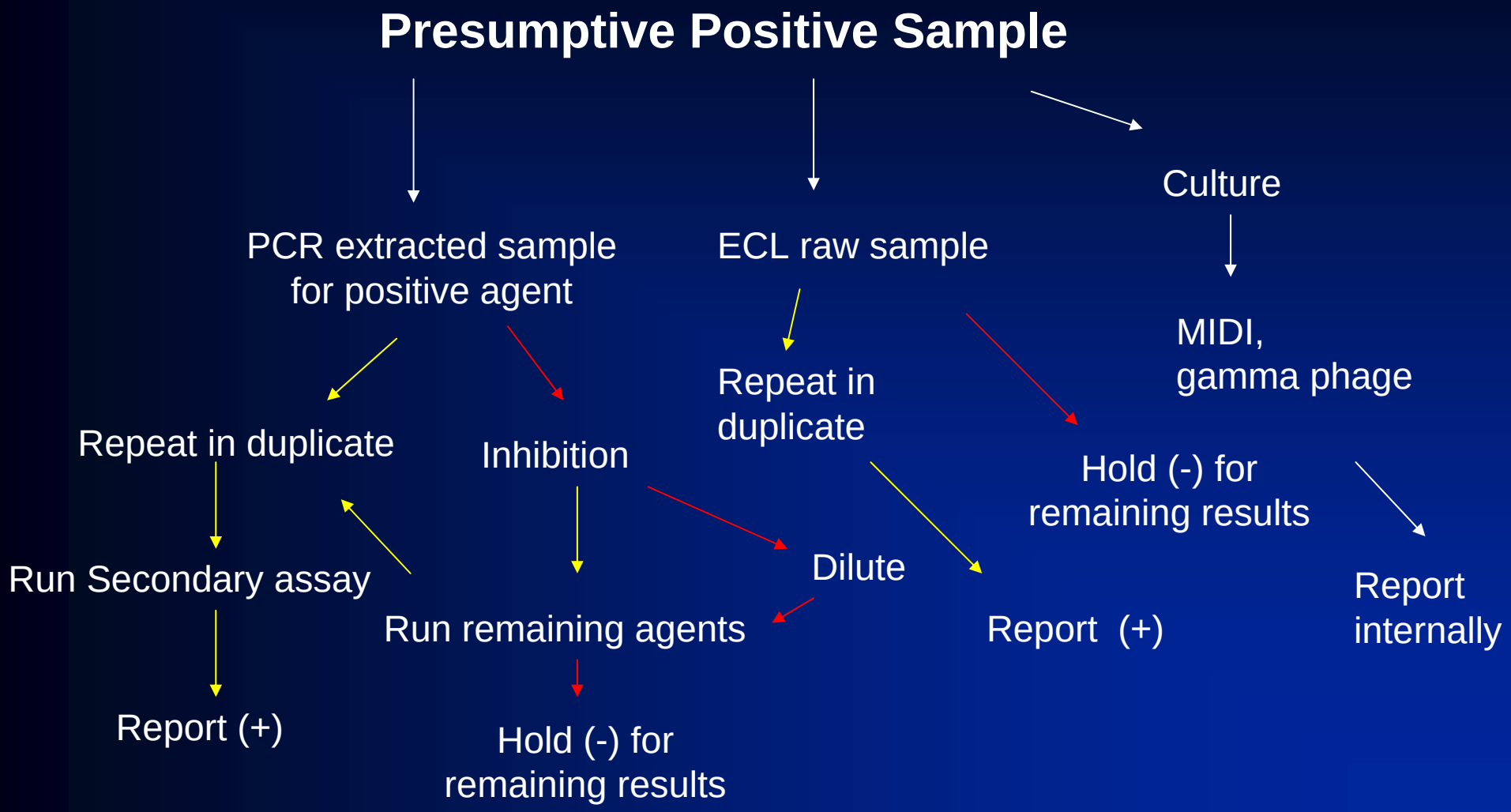
TREAT NOT WARN

Proper Use of Technology leads to Confidence…not the technology itself

Minimize Potential for False Positive and False Negative Test Results

- low detection limits
- highly sensitive and specific mature technologies
- utilize multiple technologies
- utilize appropriate positive and negative controls
- utilize processing controls
- test for the presence of inhibitors
- minimize potential for sample contamination

Integrated Testing strategy



Operation Desert Thunder (1997)

USAMRIID

Operation Iraqii Freedom (2003)

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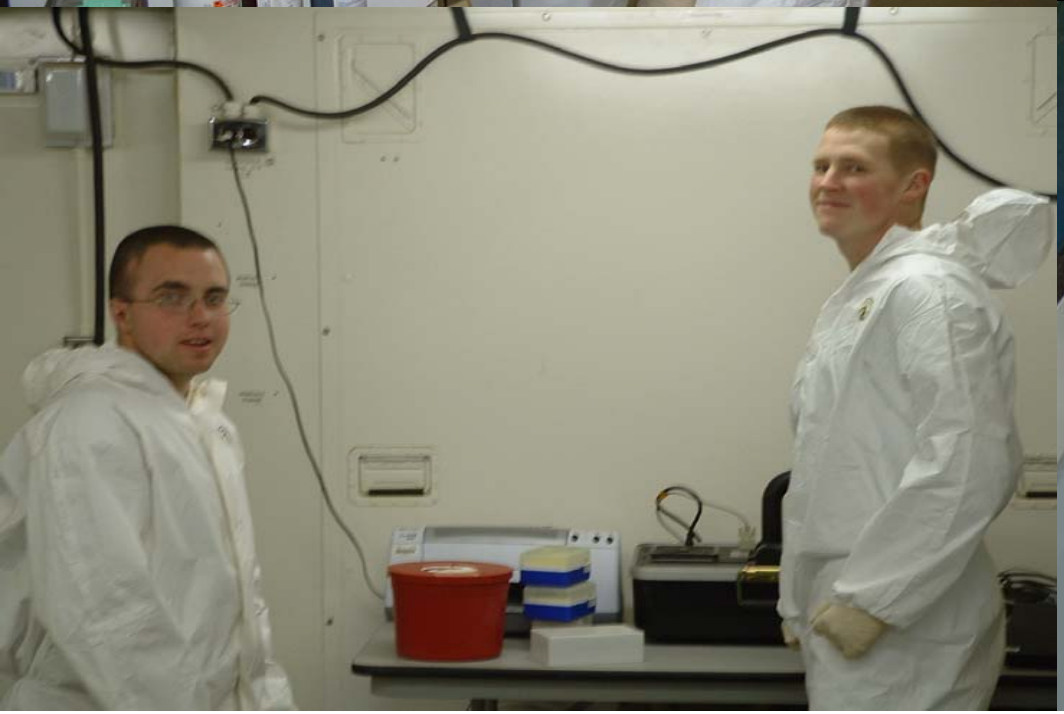
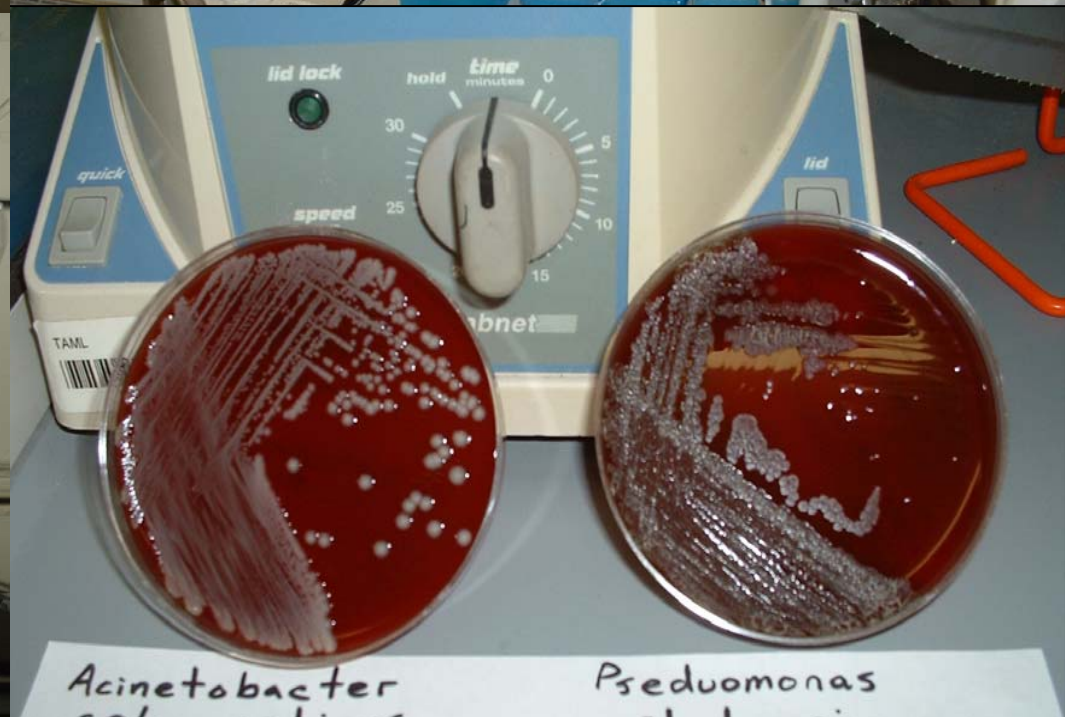
WELCOME
TO
IRAQ
LANE
5




lid lock
hold
time
minutes
0
5
10
15
30
25
quick
speed
lid
TAML
Acinetobacter
Pseduomonas

Current Field Capabilities

Culture

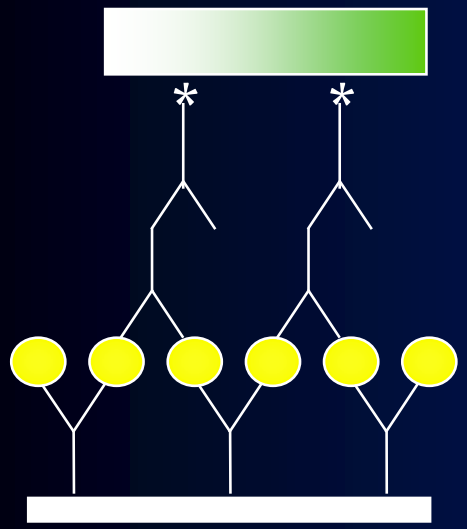
Microscan Autoscan

MIDI Sherlock

Common "Advanced" Technologies for Identifying Agents on the Battlefield

- Immunoassays (ECL)
- Real-Time Polymerase Chain Reaction

Electrochemiluminescence is similar to the Enzyme-linked Immunosorbent Assay (ELISA)



Substrate

Anti-Species B Conjugate

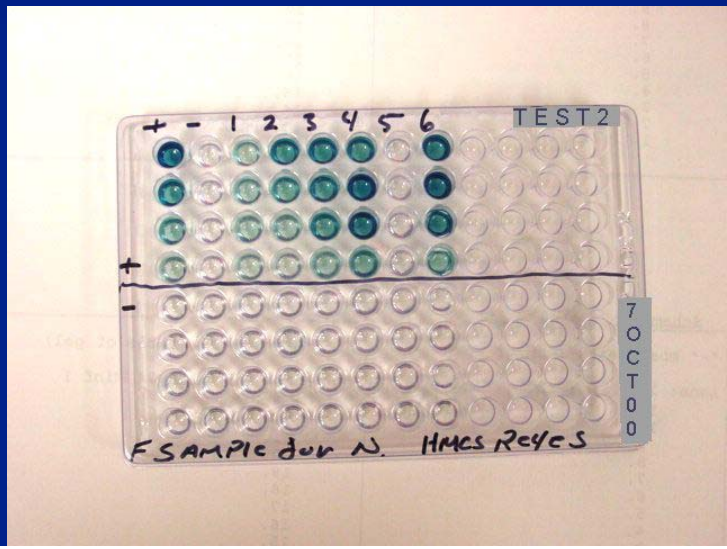
Detector antibody Species B

Test Sample (suspect antigen)

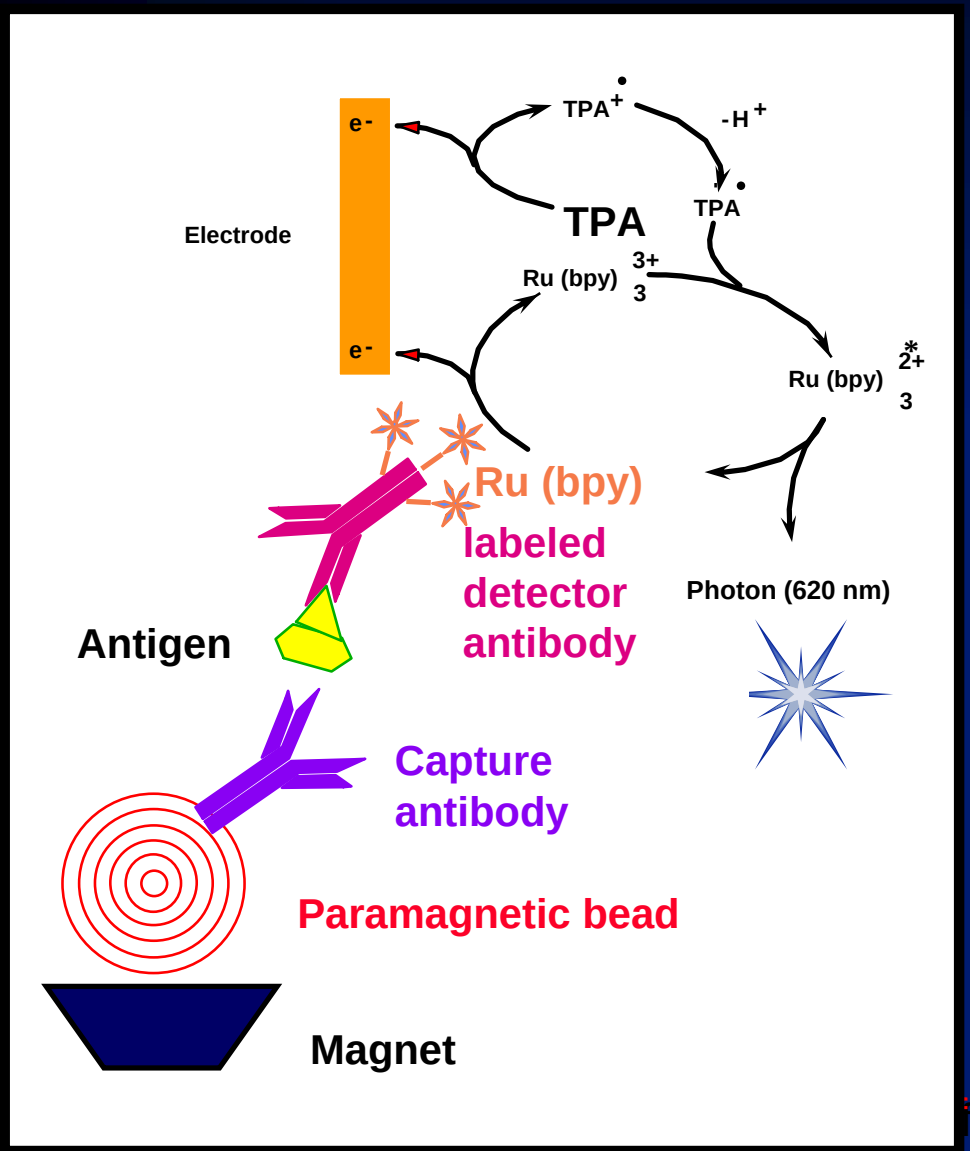
Capture Antibody Species A

Solid Phase (PVC u-bottom plate)

4-16 hours



Electrochemiluminescence “ECL” Immunoassay



- High sensitivity
- Wide dynamic range
- 30 min assay
- Stable reagents
- No sample manipulation

ECL Weaknesses

- Requires good antibodies (a problem for all immunoassays)
- Matrix dependent
- Not as sensitive as some methods (such as culture and PCR)
- Generally does not determine viability
- Does not tell you what it isn't

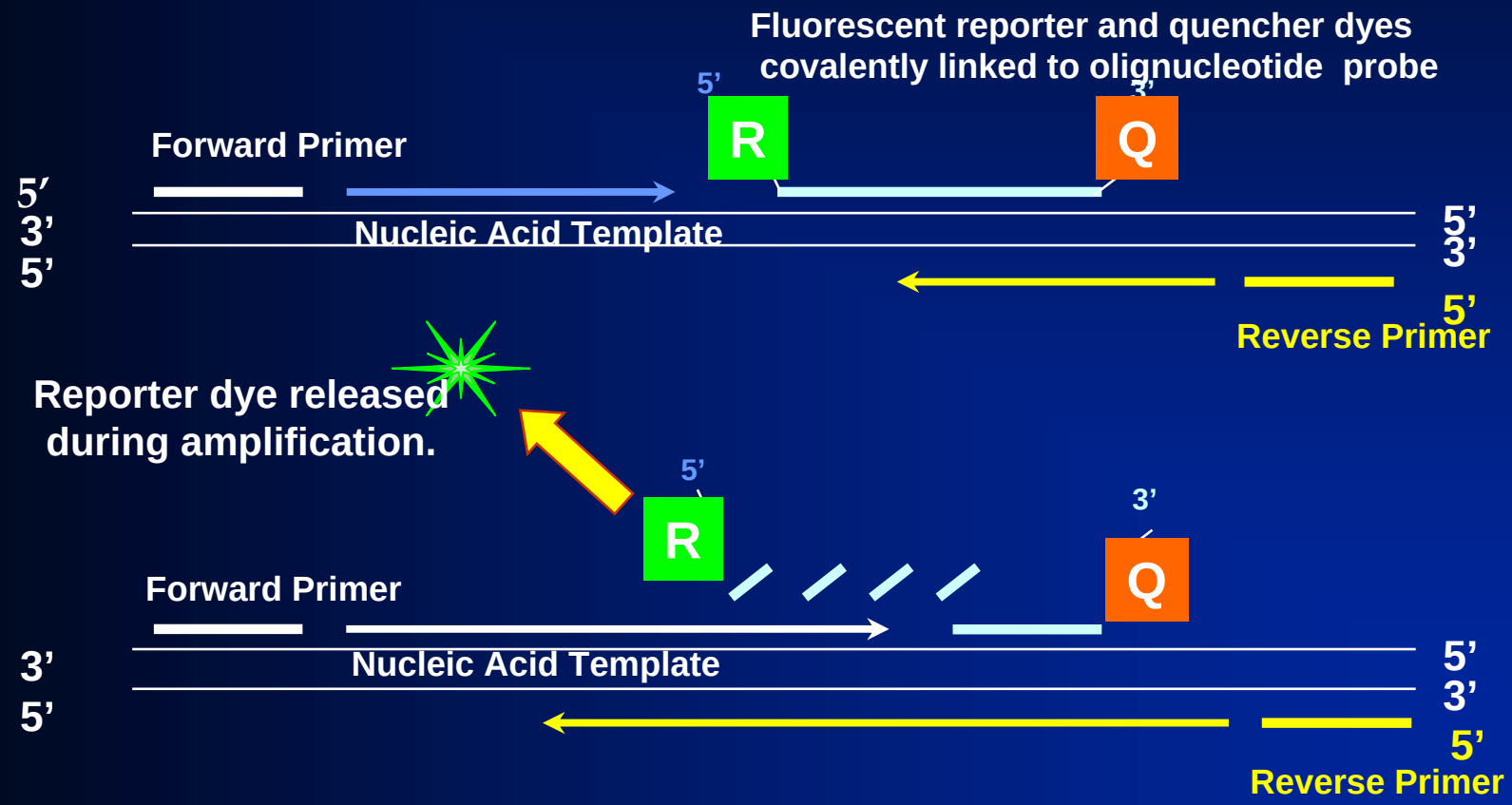
Polymerase Chain Reaction "PCR"

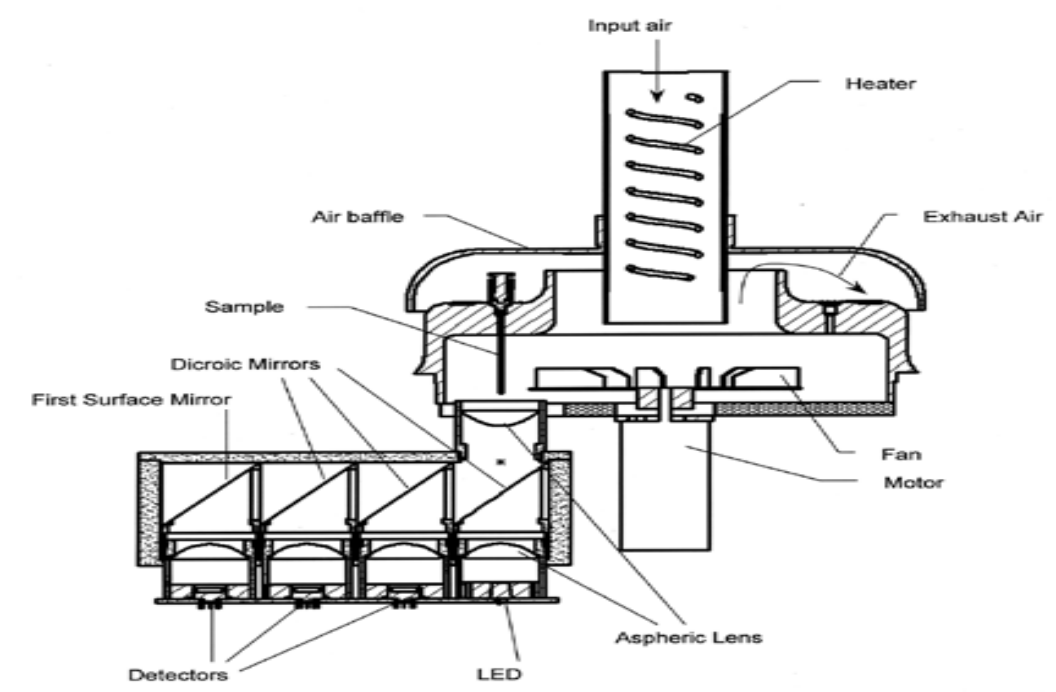
- **DNA**
  - amplification and identification
- **RNA**
  - reverse transcription followed by DNA amplification and identification

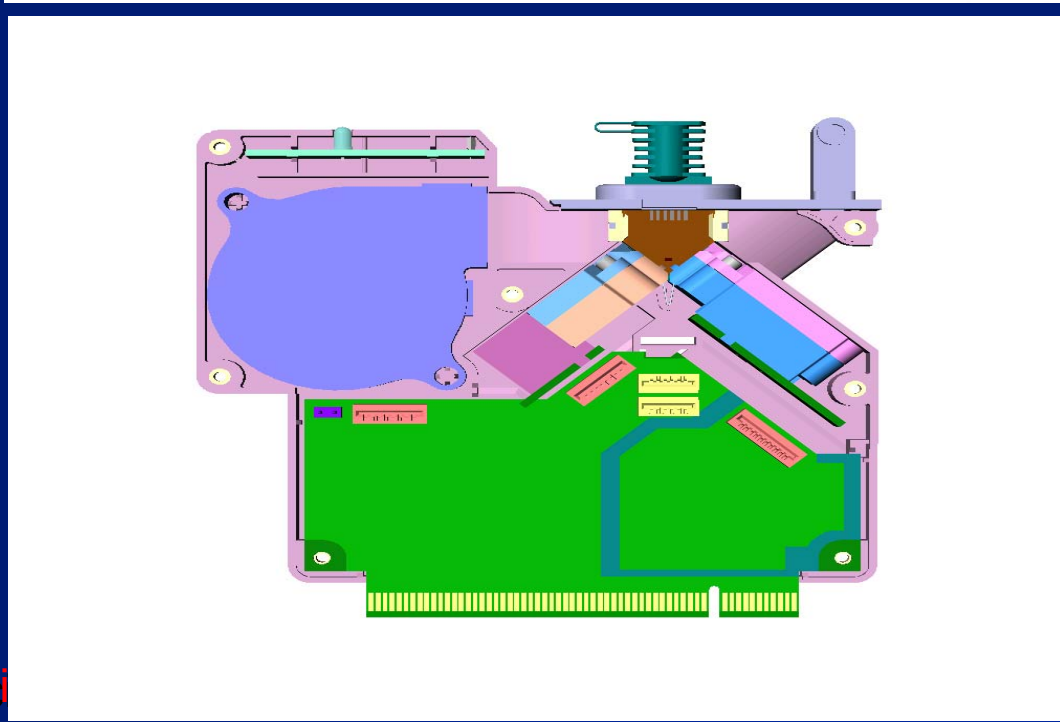
Principle of PCR

- Targets a unique segment of DNA
- Uses the target segment of DNA as a template to produce billions of copies of DNA
- Therefore, making the target easier to detect
- Real-time PCR uses a probe specific for part of the sequence that is in between the primers (this adds another layer of specificity to the target)

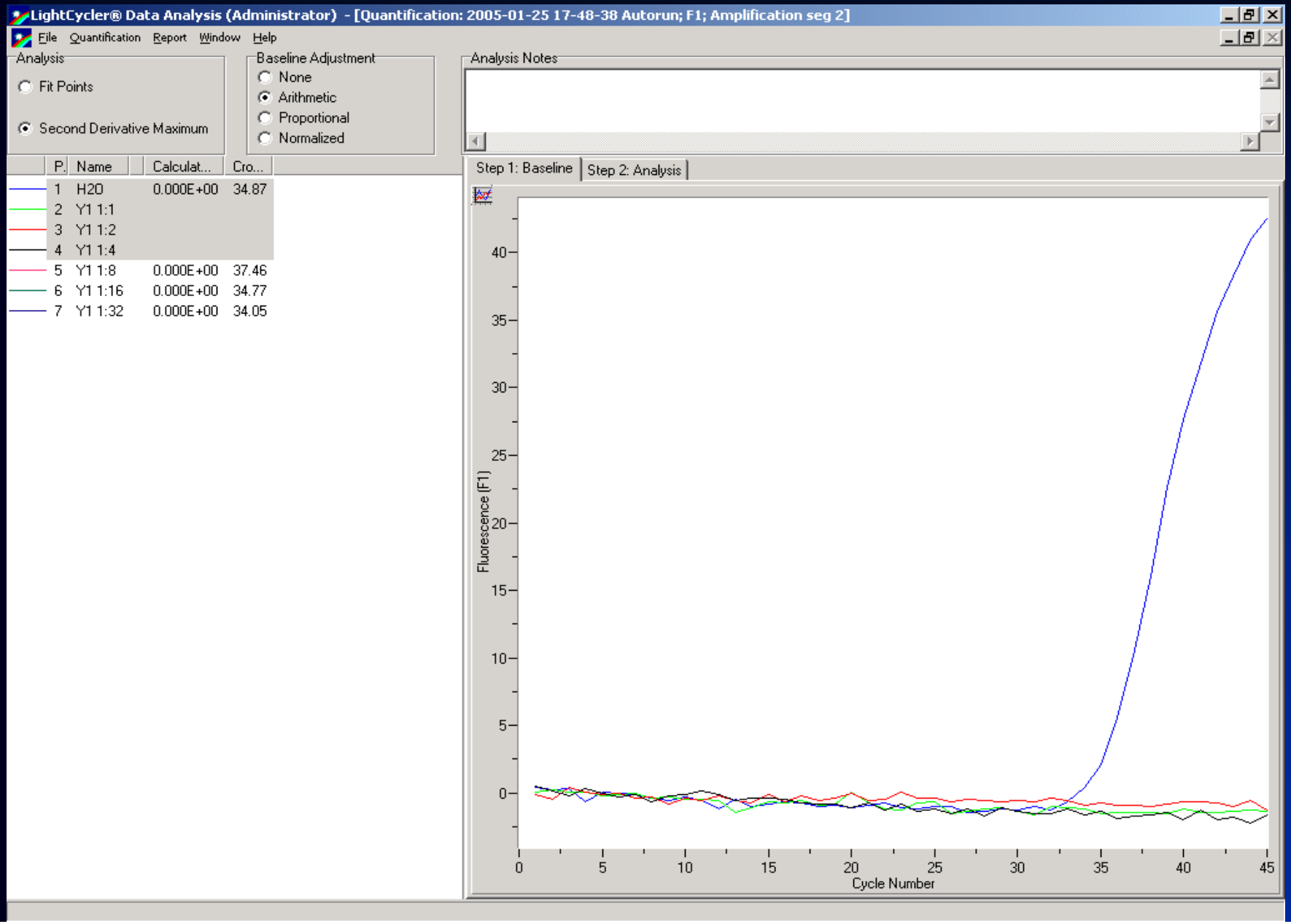
Real-Time PCR
Common Gene Amplification Chemistry
5’ Nuclease Fluorogenic PCR (Taqman™)



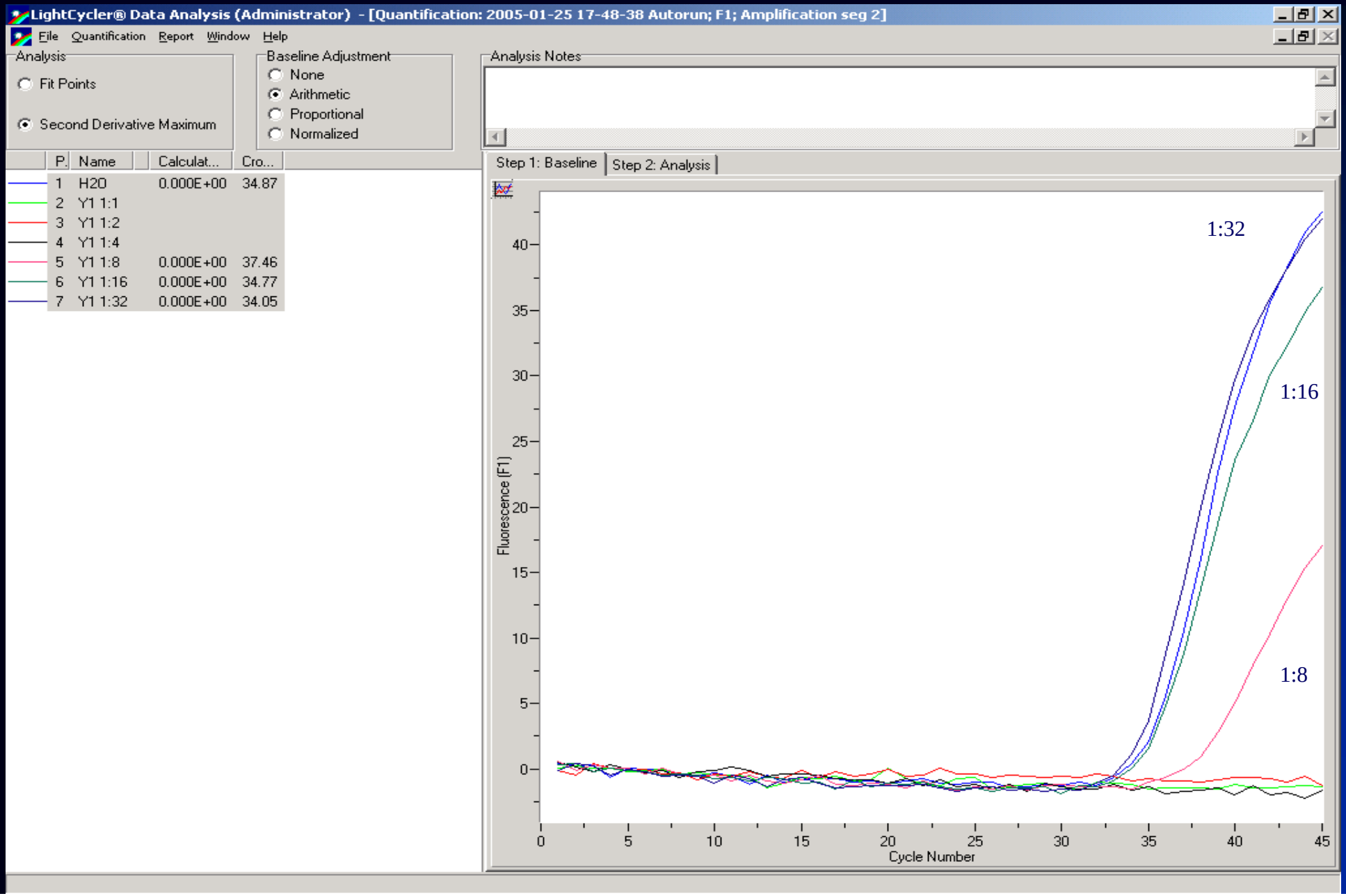

Input air
Heater
Air baffle
Exhaust Air
Sample
Dicroic Mirrors
First Surface Mirror
Fan
Motor
Aspheric Lens
Detectors
LED



Real-time Detection Using Taqman Assays



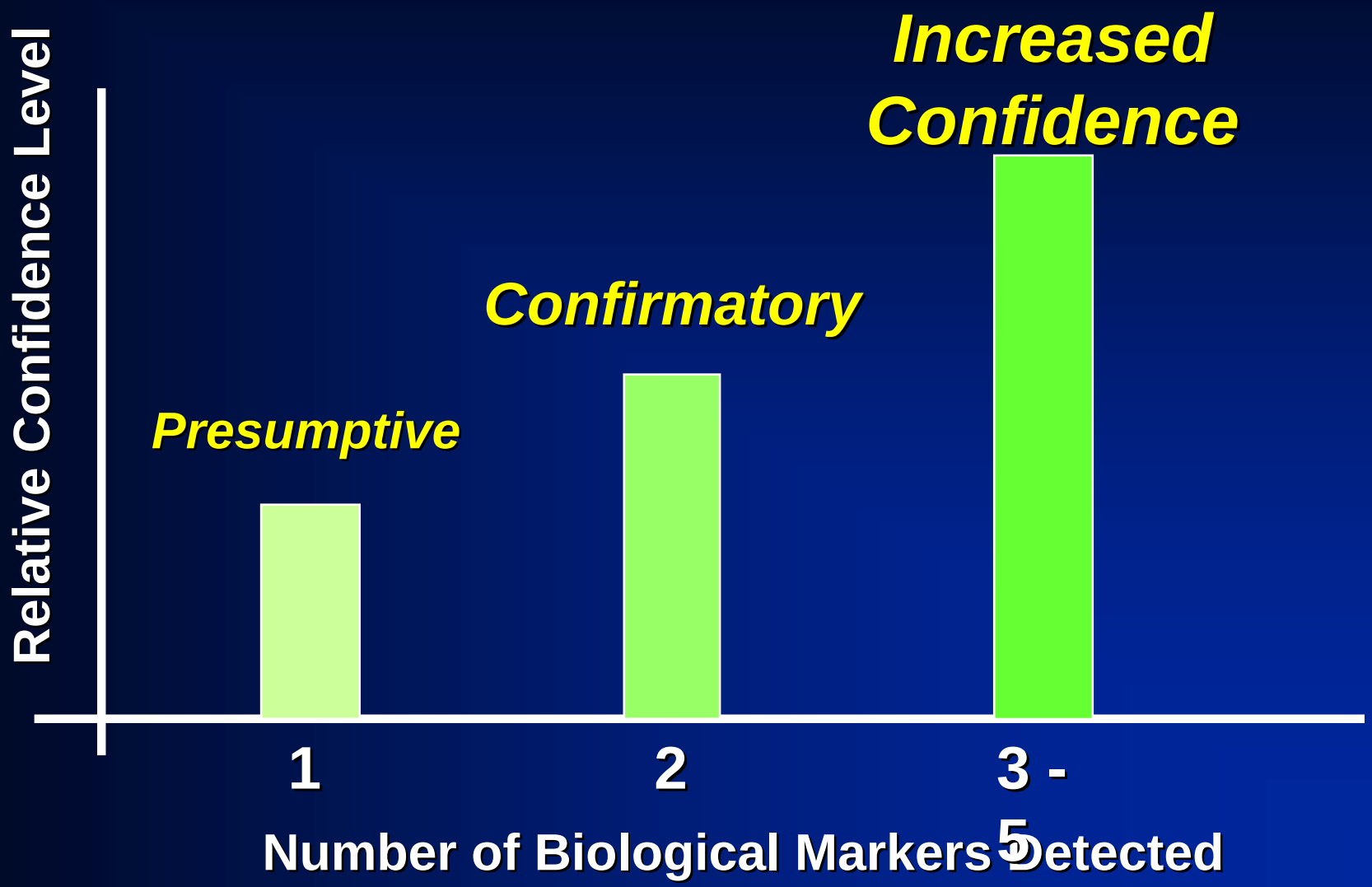
Inhibition Completely Overcome at 1:16 and 1:32



PCR Weaknesses

- Prone to contamination (theoretically, all you need is one copy for a false positive)
- Requires more controls (particularly inhibition)
- Does not determine viability
- Environmental samples must be extracted
- Does not tell you what it isn't
- Reagent stability

Requirement for Diagnostic Systems

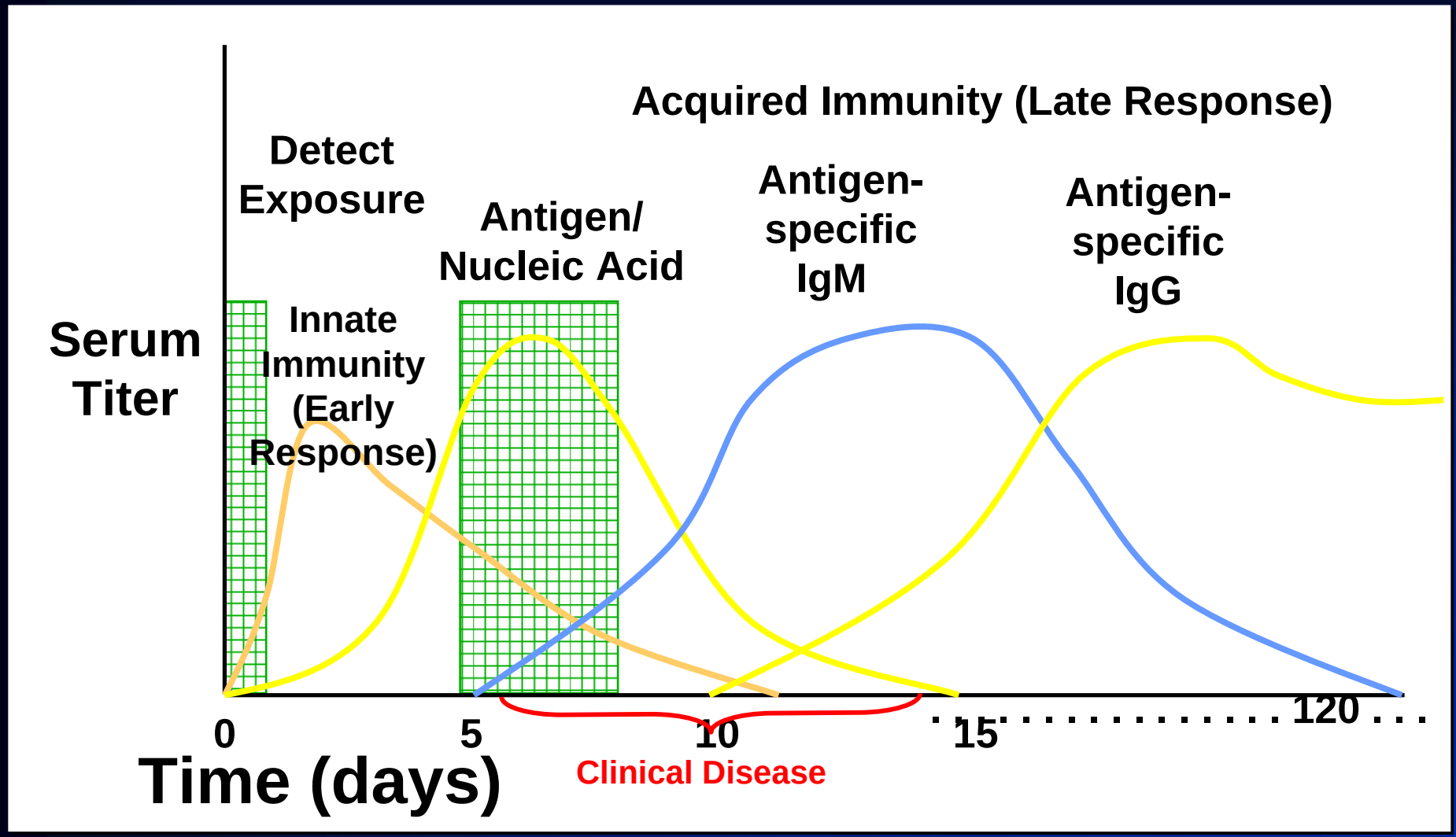


USAMRIID

Joint Biological Agent Identification and Diagnostic System (JBAIDS)

For Official Use Only

Infection/Response Time Course



Scenario 1

You have received a sample for testing. After initial testing it was determined that the first target for Yersinia pestis was positive. Follow on testing showed that all 3 targets for Yersinia pestis were positive. How confident are you in that the organism is present?

Scenario 2

Hostilities with Iraq are escalating. The civilian population along the Kuwaiti border has been evacuated. U.S. convoys have been traveling extensively along the major north-south highway as coalition forces increase their presence along the border. A large number of dead animals have recently been reported along the road. You have been asked to investigate.

What is Your Plan?

- What should you consider?
- What samples should be collected?
- Who can/should collect those samples?
- Who should be contacted/notified?
- Any peripheral issues

Clinical Detection

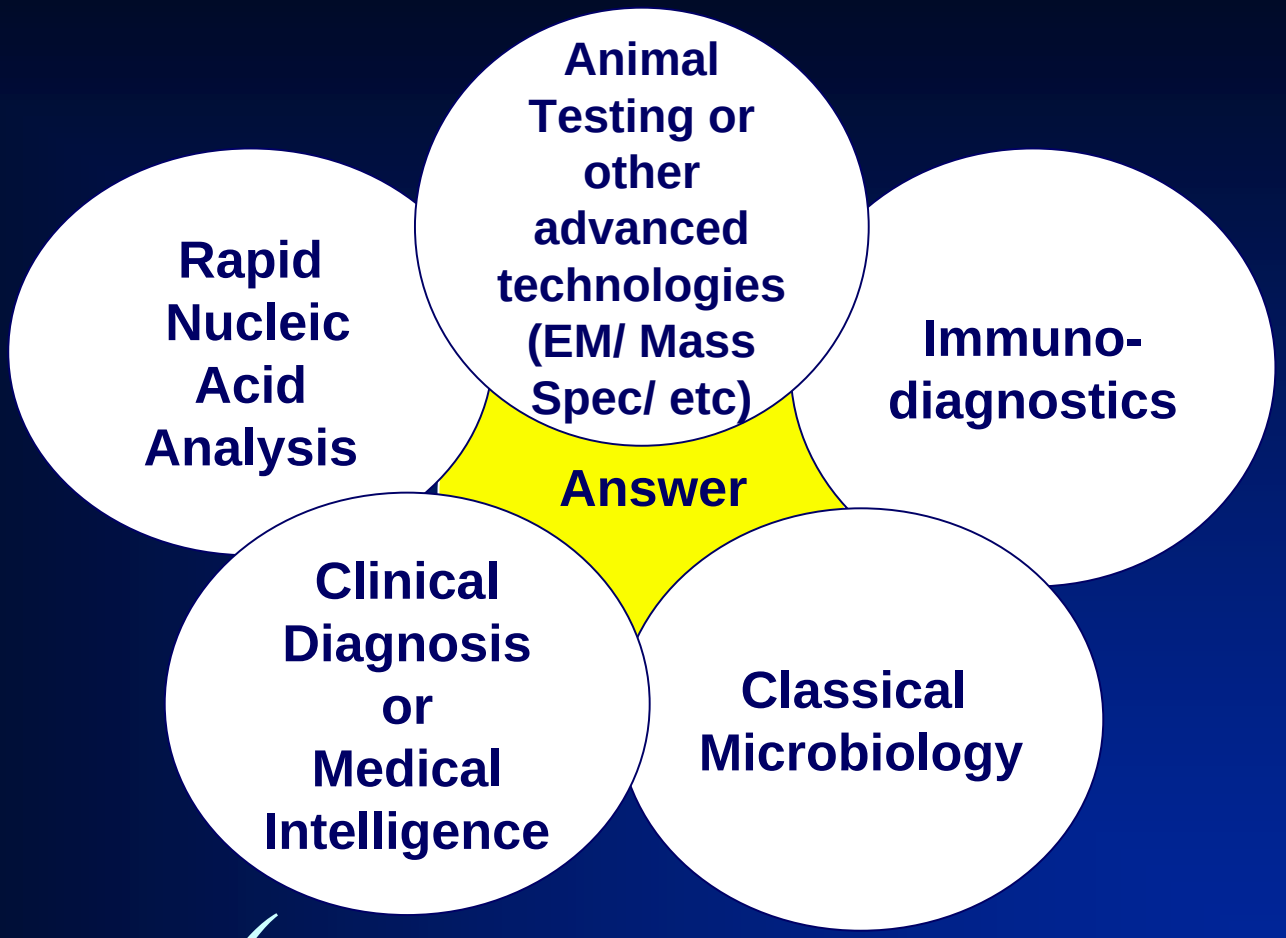
- Signs & Symptoms
  - Assessment
    - History

- Maintain an INDEX OF SUSPICION!!!

-- Just one leg of the orthogonal approach

-- Lab results don't perform well for screening

Integrated Identification and Diagnostics



✓ ***Biological agent diagnosis requires integrated identification technologies.***

Know Your Plan

- Be aware of who and what supports your facility
- Test your plan, and keep it updated
- Provide training / in-service to your staff
- Know whom to call
- Know chain of custody requirements
- Know sample collection and shipping requirements !!!!
  - POCs
    - Technical Escort Unit
    - AFMIC
    - 1st or 9th AML
    - USAMRIID
    - WRAIR
    - CDC

Conclusion

- No single technology is sufficient to identify biological warfare threats
- Technology is only good if you now how to use and interpret the results properly
- The wrong answer fast is still wrong


Questions?

USAMRIID

Psychological Aspects of Biological Warfare and BioTerrorism

Ross H. Pastel, Ph.D.
Lieutenant Colonel, MS, U.S. Army
US Army Medical Research and Materiel Command
Program Manager, Military Population Research Coordinating Cell,
DoD Blast Injury Research Program Coordinating Office and
Medical Liaison Officer to Program Executive Office (PEO) Soldier

CDC Category A Biological Agents

- Bo – Botulinum toxin
- P - Plague
- A - Anthrax
- S - Smallpox
- T - Tularemia
- E - Ebola/Marburg
- L - Lassa/Junin

September 11, 2001

- National telephone survey
  - 44% had one or more substantial stress symptoms
  - Sleep difficulties
  - Irritability and anger
  - Difficulty concentrating
  - Disturbing thoughts, memories, dreams

Pre-exposure Psychological Effects
Protective Equipment

Symptoms during MOPP training exercises

- **Shortness of breath (33%)**
- **Rapid breathing (7-33%)**
- **Anxiety (14-20%)**
- **Claustrophobia (1-20%)**
- **Irritability (10%)**
- **Panic (1-10%)**
- **Poor concentration (8%)**

Pre-exposure Psychological Effects
Anthrax Vaccination

- **Fears of Gulf War illness, sterility and other health effects**
- **News reports of significant reactions and side effects**
- **Resignations, courts-martial, less than honorable discharges rather than receive vaccination**
- **Increased attrition of pilots in Air Reserve and National Guard reported**

Psychological Effects

- **Risk communication & risk perception**
- **Mass panic**
- **Normal disaster behavior**
- **Estimating psychological casualties**
- **“Worried well?”**
- **Triage and differential diagnosis**
- **Hyperventilation**

Psychological Effects

- **OMUS – outbreaks of multiple unexplained symptoms**
- **Role of Media**
- **Historical examples**
- **Range of psychological effects**
- **At-risk populations**
- **5-R's**

Risk Communication and Risk Perception

- Risk = Hazard + Outrage

- Risk Perception
  - Not completely understood
  - Important driver for outrage portion of risk communication
- Media
  - Important driver for risk perception and outrage

Risk Communication

Risk Perception
BW Agent Characteristics

- Invisible, odorless
- Ubiquitous symptoms
- Uncertainty
- Novelty (Unfamiliarity)
- Grotesqueness
- Magical thinking

Mass Panic

- **Acute fear reaction marked by loss of self-control and followed by nonsocial and unreasoning flight**
- **Perceived imminent threat**
  - **Especially limited escape routes**

Mass Panic
Most Common Scenarios

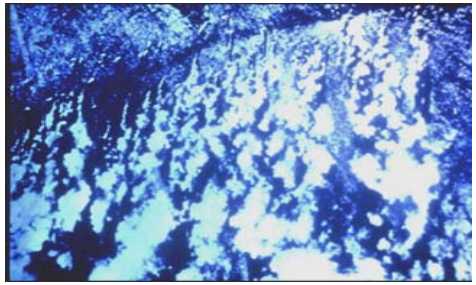
- Fires
- Mine explosions and collapses
- Sinking ships
- War

MASS PANIC
FIRST USE OF CHLORINE GAS

Ypres, Belgium, on 22 April 1915

"***As the cloud of gas drew close to the French lines, some men began dropping their rifles and running toward the rear, even before the cloud engulfed them. Officers were powerless to hold them. A full-blown, blind, contagious panic swept portions of the line.***"

G. Hammerman, Proceedings of the DNA Symposium / Workshop on the Psychological Effects of Tactical Nuclear Warfare, DNA-TR-87-209, 1987.



MASS PANIC
WORLD WAR I GAS EXPERIENCE

- Ypres: April, 1915
  - Where gas was thickest, panic and flight
  - Where there was little or no gas, no panic or flight
- April and May 1915
  - Six chlorine gas attacks that followed
  - No widespread panics
- June 1915 through November 1918
  - Only four other examples of widespread gas panic
  - All accompanied by heavy artillery bombardment

MASS PANIC

- Historically, only limited number of situations produce panic
- Even in those situations, mass panic is *not* common

Normal Disaster Behavior

- Cool and collected (12-25%)
- Stunned and bewildered (50-75%)
- Disorganized behaviors (10-25%)
  - Confusion
  - Overly-active
  - Anxiety
  - Panic (*rare*)
- Example – Loma Prieta earthquake, 1989

Medical Planning
Psychological Casualties

- **Planners estimate battle fatigue casualties (BFC) as proportion of wounded in action (WIA), i.e., BFC:WIA**

- **Highest rates in World War 2**
  - **1 BFC to 2 WIA**
  - **Okinawa, Gothic line**

Persian Gulf War
Scud Missile Attacks on Israel

	Injuries	Stress reactions	Unnec Atr injections
Total[1]	286	544	230
1st Attack[2]	22	172	171

Persian Gulf War
Psychological Casualties
Israeli Civilian Population

BFC: WIA

(Stress + unnecessary Atropine injection): Injuries

Total	**3:1**
1st Attack	**16:1**


Worried Well?

"Worried Well" – A Bad Term!

- **"Worried"**
  - **Maybe a good reason to be**
  - **Uncertainties, potential effects**
- **"Well"**
  - **Symptoms are real**
  - **Symptoms are painful**

Medical or Psychological Effects?

- **Prodromal Symptoms of BW Agents**
  - **Fatigue**
  - **Headache**
  - **Nausea**
  - **Muscle ache/ Joint ache**
  - **Difficulty breathing**
  - **Dizziness**

Difficulties of Triage and Differential Diagnosis

- **Acute and chronic psychological disorders**
  - **Psychological impact of the event**
  - **Medical characteristics of the agent**
- **Many infected patients will also manifest fear, anxiety, etc.**

Hyperventilation Syndrome

- **Symptoms include:**
  - **Weakness and fatigability**
  - **Numbness and paresthesia**
  - **Palpitations and tachycardia**
  - **Twitching, trembling, convulsions**
  - **Difficulty swallowing, talking and breathing**
  - **Anxiety, panic, depression**
- **Physiology**
  - **Respiratory alkalosis**
  - **Reduced cerebral blood flow**

Outbreaks of Multiple Unexplained Symptoms (OMUS)

- Mass hysteria
- Epidemic hysteria
- Mass psychogenic illness
- Mass sociogenic illness

OMUS Epidemiology
Triggering Factors - Events

- **Localized odor or perception of odor**
- **Environmental event**
  - **Nuclear release**
  - **Smog**
  - **Contamination of water supply**
  - **Mass chemical exposure of community**

OMUS Epidemiology
Enhancement of Outbreak

- Physical/visual proximity to ill
- General excitement
- Presence of media
- Media reporting
- Litigation and/or compensation
- Labeling of illness (diagnosis)
- Persistence of rumors

OMUS Following Perceived Exposure
U.S. Military Recruits, 1988

- *Trigger*: **Suspected toxic gas exposure**
- *Symptoms*: **Cough, pleuritic chest pain, nausea, headache, dizziness, and shortness of breath**
- *Numbers*
  - 1,800 men evacuated from barracks
  - 1,000 with at least one symptom
  - 375 evacuated to hospital for medical evaluation
  - 8 hospitalized
- *Diagnosis:* **No toxic or infectious exposure**

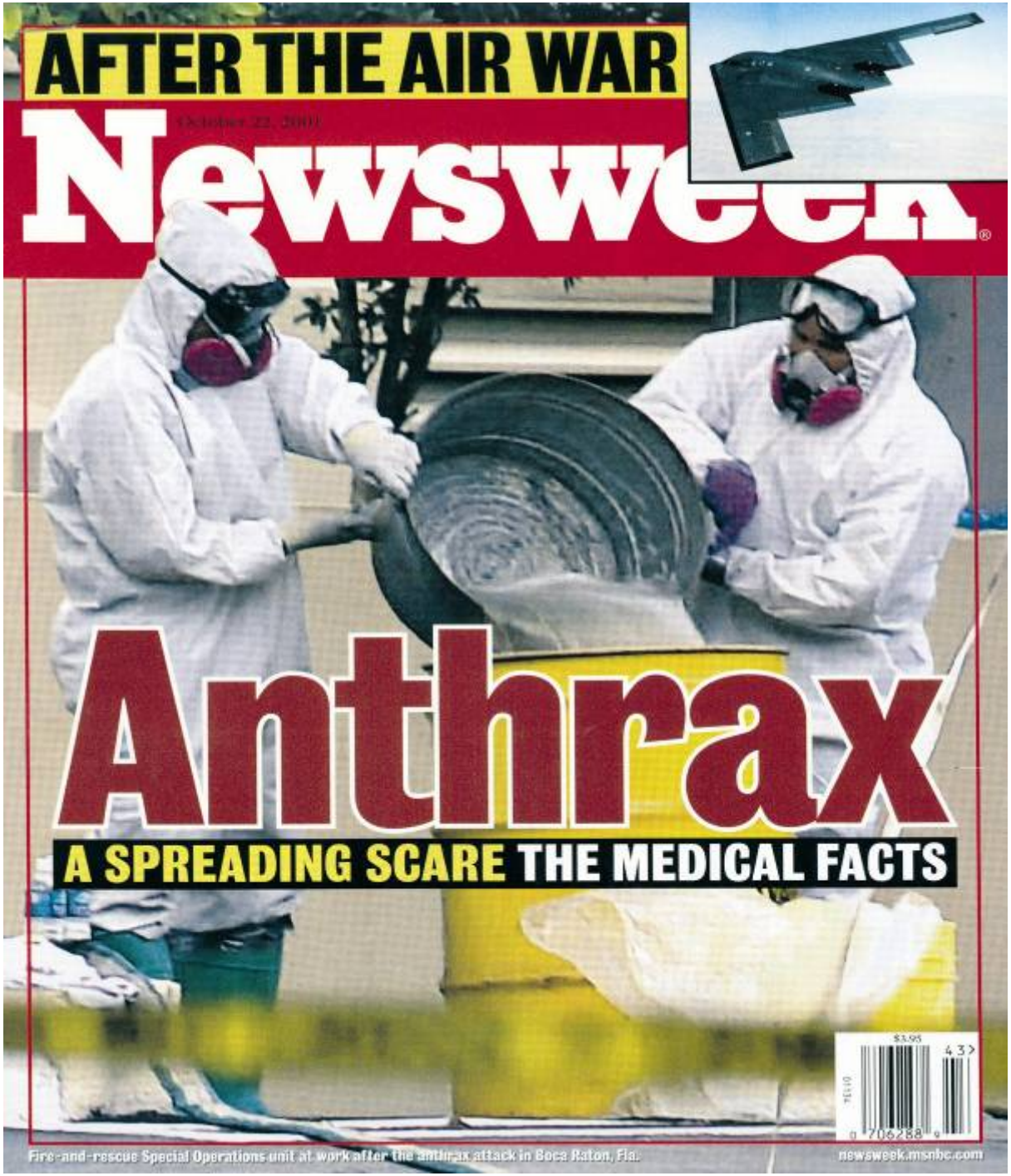
Role of Media

Anthrax Hoax, Fairfax, VA, 1992

The victims started arriving, a few at a time, brought in by EMS units. After an hour, the original 11 cases were joined by 9 of their neighbors, who had seen the police tape and wanted to be checked out and treated as well. . . . Facts were in short supply, rumors were not. . . .

Role of Media
Oklahoma City Bombing, 1995

- **Study of children after OKC bombing**
- **In children without direct physical or emotional exposure to the explosion**
- **High television exposure related to significantly more post-traumatic stress symptomatology**


AFTER THE AIR WAR
October 22, 2001
Newsweek
Anthrax
A SPREADING SCARE THE MEDICAL FACTS
$3.95
Fire-and-rescue Special Operations unit at work after the anthrax attack in Boca Raton, Fla.
newsweek.msnbc.com

Role of Media
Media Information

Believing the Media

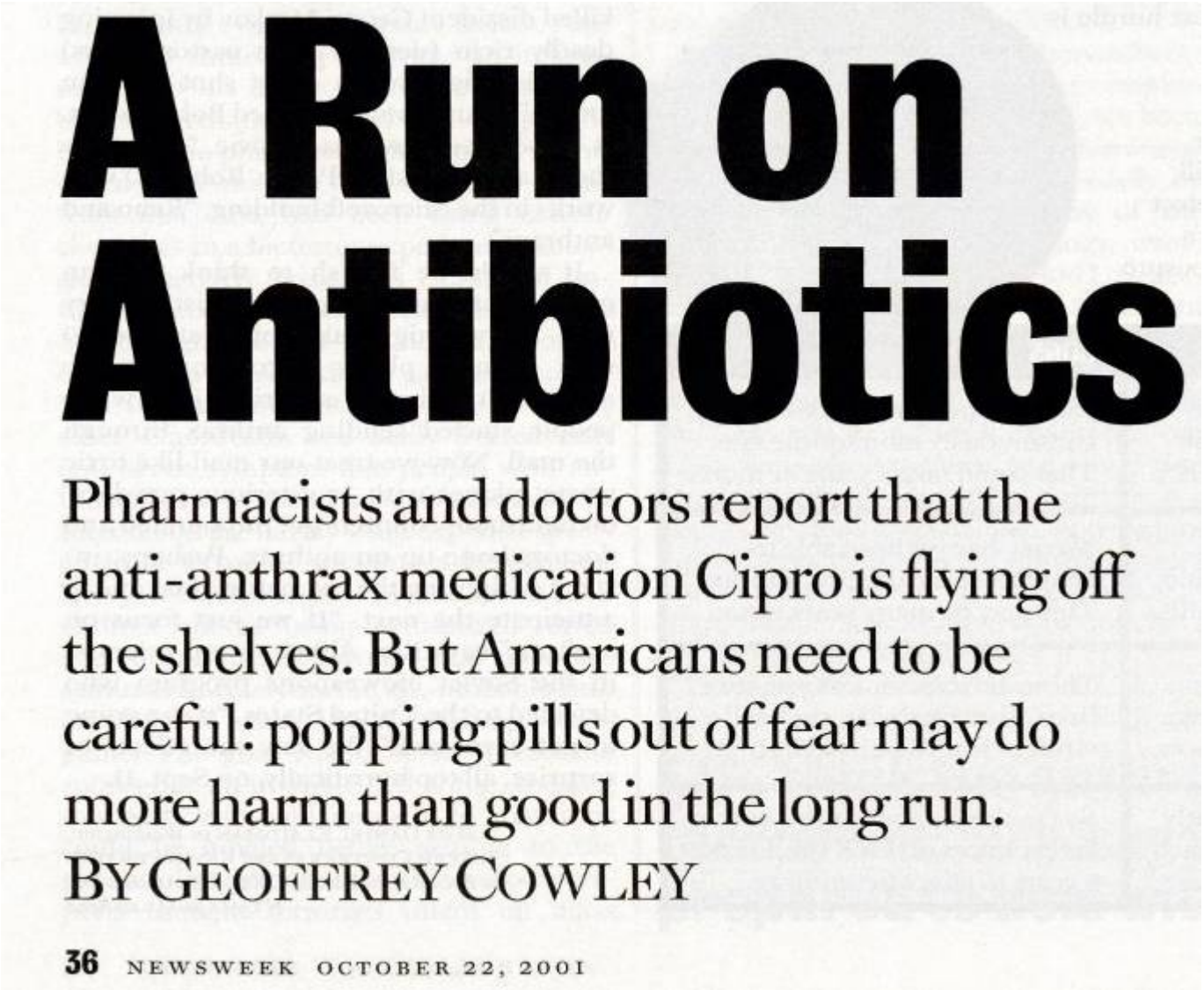
You've Got Mail
Anthrax Letters, October 2001

A few cases do not an epidemic make. But they're unprecedented; worry over what's next is contagious. BY SHARON BEGLEY AND MICHAEL ISIKOFF

Anxious About Anthrax

You've Got Mail
Anthrax Letters, October 2001

You've Got Mail
Anthrax Letters, October 2001



A Run on Antibiotics

Pharmacists and doctors report that the anti-anthrax medication Cipro is flying off the shelves. But Americans need to be careful: popping pills out of fear may do more harm than good in the long run.

BY GEOFFREY COWLEY

You've Got Mail
Anthrax Letters, October 2001

- **11 Inhalational anthrax cases**
  - **7 – postal employees (NJ, DC)**
  - **2 – media employees (FL)**
  - **2 – unknown risk (NY, CT)**
- **12 Cutaneous anthrax cases**
  - **7 – media employees / visitors (NY)**
  - **4 – postal employees (NJ)**
  - **1 – bookkeeper**

Plague Outbreak
Surat, India, 1994

The reappearance of plague . . . Not only created widespread panic and put a severe blow to Surat's economy but had much wider repercussions on the economy of the country as a whole including industrial production, tourism, export, etc. The rough estimates put the loss to industry in Surat in several crores of rupees [~1-2 million dollars].

Sverdlovsk, Russia, 1979

They kept bringing people in. There was nowhere to put them; we had to put them in corridors. . . . It was in the air: infection, infection, infection. . . .

R.K. Gaziyeva, head of admissions, hospital No. 24

Sverdlovsk, Russia, 1979

People were nervous and did not understand things very well. There was fear, innuendoes, panic. . . And, of course, immense sorrow, the tragedy of families. I alone had to bury no less than 50 deceased. . . Witnesses . . . remember . . . the atmosphere of wild fear among the population, the panic, the many alarming rumors. . .

G.A. Lyashchenko, Chief of Funeral Services


Premium Quality
golden circle
SARS
Flavour Burst
375mL

Weather Map: Page 85

Vol. 32 No. 104 ■ THURSDAY, MARCH 27, 2003 120 PAGES ■ torontosun.com

SARS ALARM SOUNDED

As many as 3,000 T.O. residents exposed to deadly illness: Experts

'OUT OF CONTROL'

— Craig Robertson, SUN

A PATIENT is wheeled out of Scarborough Grace Hospital by security yesterday. The hospital is now essentially closed because of a SARS quarantine: No new cases, no visitors, no elective surgeries.

Pages 2-3, 44-46, 104

— Peter Andrews, Reuters

SARS: Health Care Worker (HCW) Casualties

- **HCWs accounted for**
  - **40% of SARS cases in Toronto**
  - **57% of cases in Vietnam**
- **>100 HCWs in Canada developed SARS**
- **3 died of SARS**
  - **2 nurses**
  - **1 physician**

Impact of SARS on Health Care Workers

- People afraid to
  - Go to work in hospitals
  - Care for SARS patients
  - Associate with HCWs, or even spouses of HCWs
- Lingering resentment of colleagues who might not have contributed what was expected
- Feelings of helplessness, anger, and guilt
- Experiences of social isolation and ostracism

SARS: From the Front Lines

- **“Nobody ever thought this was the kind of job they could potentially die from” – ICU nurse**

- **“You cannot appreciate, I don’t believe, what the feeling of isolation was. Physical isolation…you see nothing but people’s eyes for days on end” – I.D. physician**

SARS: From the Front Lines

- **“How terrible it is if you have to look after your own colleagues…[when word came down that several children of sick HCWs had come down with the disease] it broke people’s hearts” – MD**

- **“Emergency would just kind of fall apart because ‘oh no, it’s a staff member’ ”- RN**

Impact of SARS on HCWs

- **Initial unstructured study by Maunder et al:**
  - **Concerns re personal safety, familial transmission and stigmatization**
  - **Responses included fear, anxiety, anger and frustration**
  - **Stressors included caring for colleagues as patients, redeployment to unfamiliar tasks, workload changes**

Impact on HCWs - Doing the SARS hop

Risk Factors for Psychological Effects in SARS Epidemic

1. Care of SARS patients
2. Being a nurse
3. Having children
4. Job stress
5. Perceived social rejection
6. Avoidance of crowds and colleagues
7. Relationship insecurity

Interpersonal Isolation

Mediating Factors

Mediating factors for HCWs having contact with SARS patients and experiencing emotional distress:

1. **Fear for own health/health of others**
2. **Social isolation**
3. **Increased job stress**

Psychological Effects of WMD
Prevention and Mitigation

- **Education**
- **Realistic Training**
- **Practice, Practice, Practice**
- **Information**
  - **Accurate**
  - **Timely**

Positive Psychological Effects

- Heroism
- Emergent leadership
- Baby boom?
- Increase in marriages

Subclinical Post-Disaster Psychological Effects

- Boredom, sensation seeking, recklessness
- Burn-out, job change
- Alcohol / drug misuse (self-medication)
- Family disturbance, abuse, break-up
- Chronic medical complaints & problems
- Overreactions, e.g. obsessive concern with decontamination,
- Anger at government

Clinical Post-Disaster Psychological Effects

- Depression
- PTSD
- Somatization disorders
- Anxiety disorders
- Alcohol / substance abuse

Post-Traumatic Stress Disorder
3 Clusters of Symptoms

- **Re-experiencing of trauma**
- **Avoidance and numbing**
- **Hyperarousal**

Who is at Risk?

- Rescue workers
- Medical personnel
- Leaders
- “Hero”
- Media representatives
- Elderly
- Children
- Single parents
- Injured
- Bereaved

Responses to Traumatic Stress

- Most people experience acute symptoms that will dissipate over time
- Most people do not develop psychiatric disorders
- Magnitude of the trauma is best predictor of psychiatric disorders

Traumatic Stress Reactions

- **Stigma of psychiatric diagnosis**
  - **Depression, somatization disorder, anxiety, PTSD**
- **Military experience - battle fatigue**
  - **Not mentally ill**
  - **Normal reaction to abnormal stimulus**
  - **Positive expectation of recovery**

Psychological Treatment

- Proximity
  - Immediacy
    - Expectancy

Stress Control Measures
The 5 R's

***R*eassure (of normality)**

***R*est (respite)**

***R*eplenish physiologic needs**

***R*estore confidence (work, talk)**

***R*eturn (reunite) to duty & team**

Reducing Psychological Impact of Outbreak by Reducing Job Stress

- Increased mastery
- Increased attention to training and support when redeployment is required
- Dedicated contagious disease wards
- Attention to workload issues
  - Including self-imposed!

Psychological First Aid (PFA) Objectives for Adults

- **Establish safety**
- **Reduce extreme acute stress reactions**
  - **Specific disaster-related stressors**
  - **Arousal reduction**
- **Connect survivors to restorative resources**
  - **Active help with problem solving**
  - **When/how refer to MH services**

Psychological First Aid

- **PFA used with**
  - **Individuals exhibiting extreme acute stress reactions or**
  - **Those with notable risk factors linked to adverse mental health outcomes**
- **PFA does not focus on emotional processing or detailed trauma narratives**

Pre-Disaster Risk Factors

- Female
- Age (40-60 yo)
- Ethnic minority group membership
- Poverty or low SES
- Presence of exposed children in home
- Psychiatric history

Within-Disaster Risk Factors

- Bereavement
- Injury
- Severity of exposure
- Peritraumatic reactions
- Horror
- Life threat

SUMMARY

Please Remember!

- The physical effects seen following exposure to hazardous conditions are REAL
- The uncertainty lies in the origin of the symptoms
  - Physical, psychological, mixed?
- Outbreaks of multiple unexplained symptoms (OMUS) may be common after exposure to CBRNE agents

SUMMARY
Psychological Effects of BW & BT

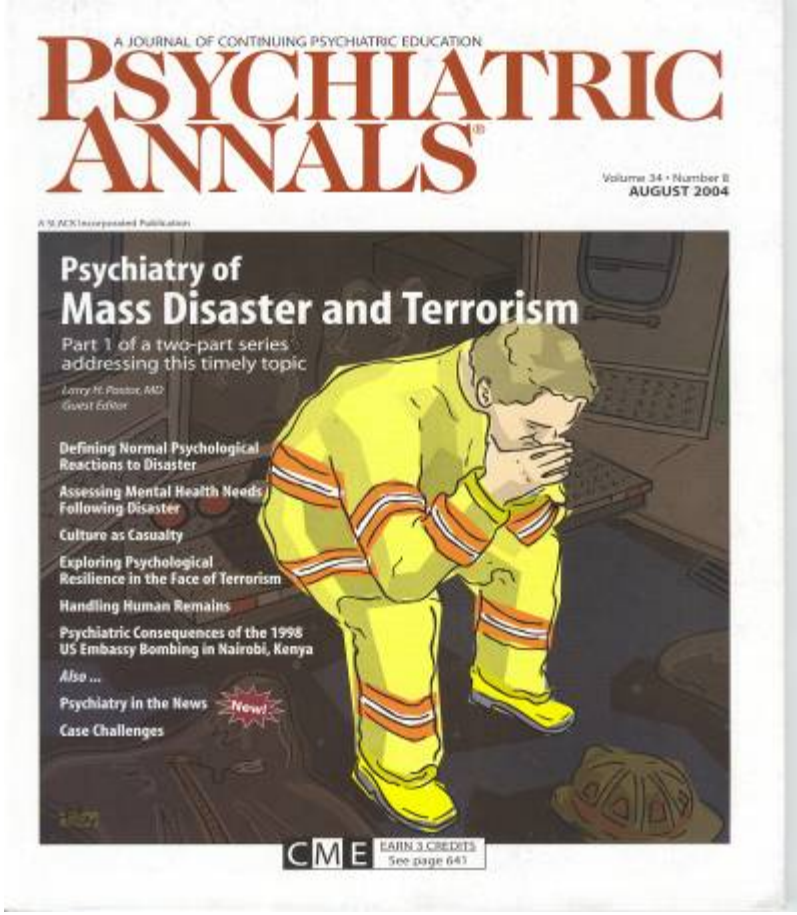
Acute Effects: Expect large numbers of psychological casualties.

Long-Term Effects: Expect high costs for long-term disability health care.

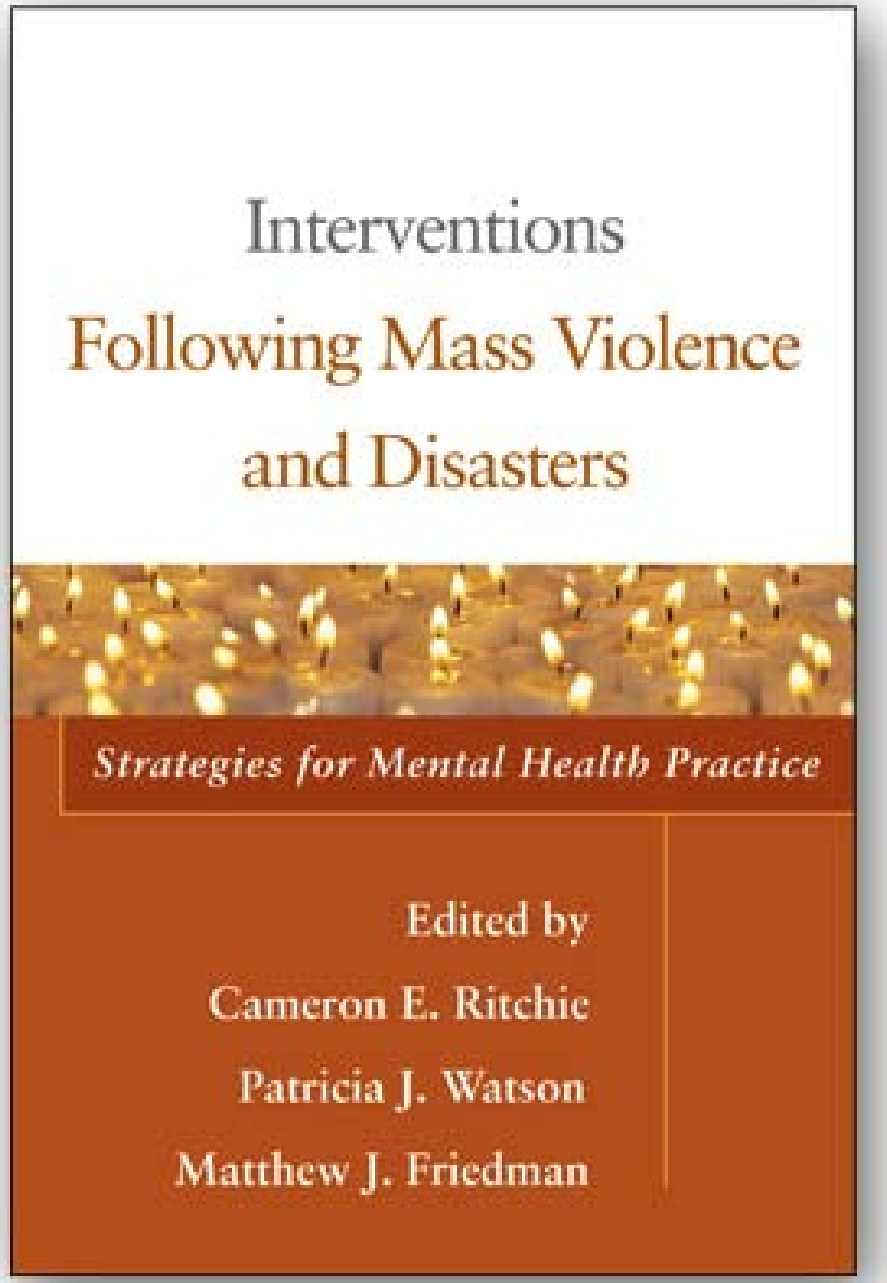
Summary
Psychological Effects of WMD

- **Proximity, Immediacy, Expectancy**
- **Protect yourself**
  - **Stress of dealing with casualties**
  - **Overworked, understaffed**
  - **Sleep deprivation**

Summary
For More Information



Psychiatric Annals: Aug and Sep 2004



Guilford Press
January 2006

Ch.16. Mitigation of Psychological Effects of Weapons of Mass Destruction.

R. Pastel & E. Ritchie

USAMRIID

The Epidemiology of Bioterrorism - Distinguishing Natural from Intentionally Spread Outbreaks

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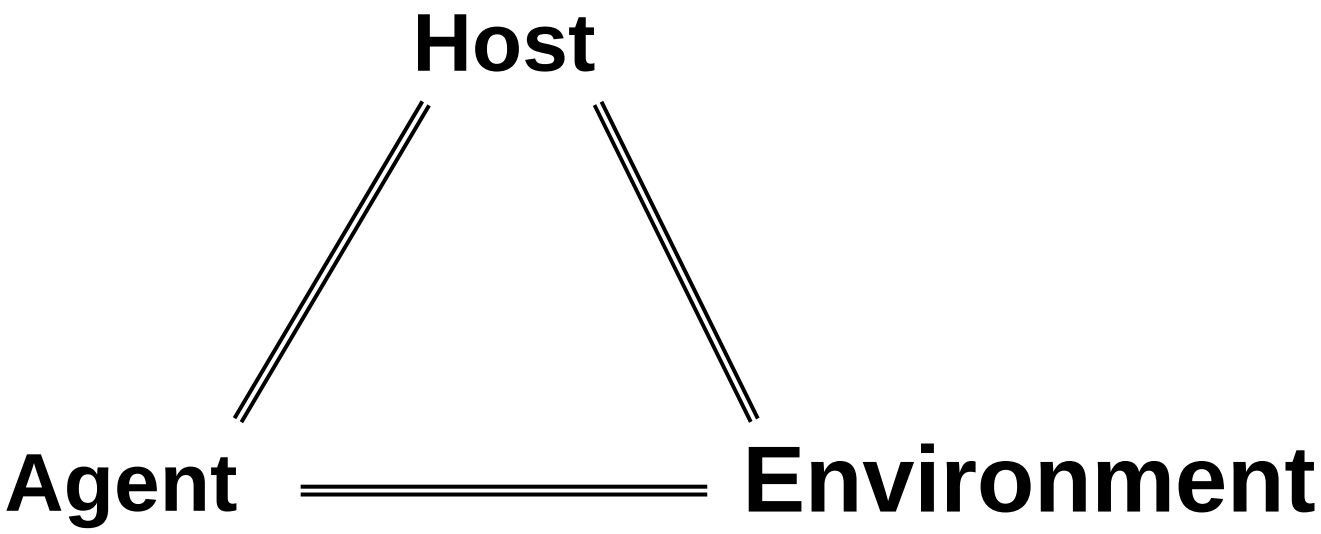
May 2008

BT or Naturally Occurring Disease: Surfing the Ocean with Sharks

Lesson Objectives

- Summarize the steps of an outbreak investigation
- Distinguish steps in an outbreak investigation in which clinicians can play a valuable role
- Differentiate the causes of common epidemics
- List potential clues or signs that might indicate an intentional outbreak
- Describe newer methods of surveillance aimed at identifying large numbers of casualties in a short time frame

Epidemiologic Triangle



Outbreak Causes
Differential Diagnosis

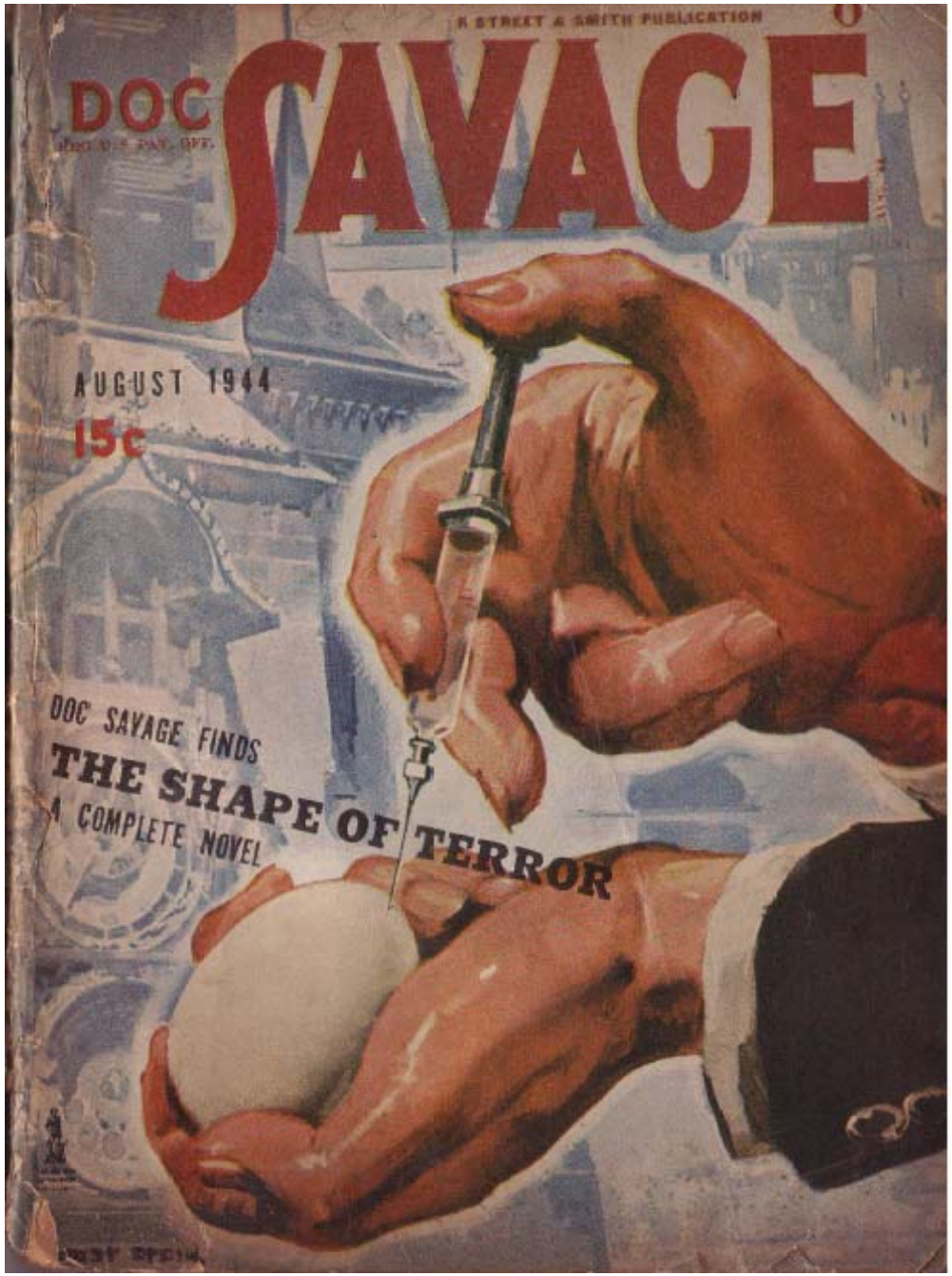
- Spontaneous - known endemic disease
- Spontaneous - new/re-emerging disease
- Lab accident
- Intentional biological attack

Bioterrorism/Biowarfare

Maintain an Index of Suspicion

- The organism may be “wild”
  - naturally occurring - not genetically altered
- Early recognition is key
- A small outbreak may portend a larger one
- All clinicians need basics of epidemiology

Bioterrorism/Biowarfare: New?



Bioterrorism/Biowarfare: New?

“Dip arrows in matter of small pox, and twang them at the American rebels, in order to inoculate them. This would sooner disband these stubborn, ignorant, enthusiastic savages, than any other compulsive measure. Such is their dread and fear of that disorder.”

Military Collections and Remarks. (British) Major Robert Donkin, New York. 1777.

Initial Flu-like Symptoms of Potential Agents

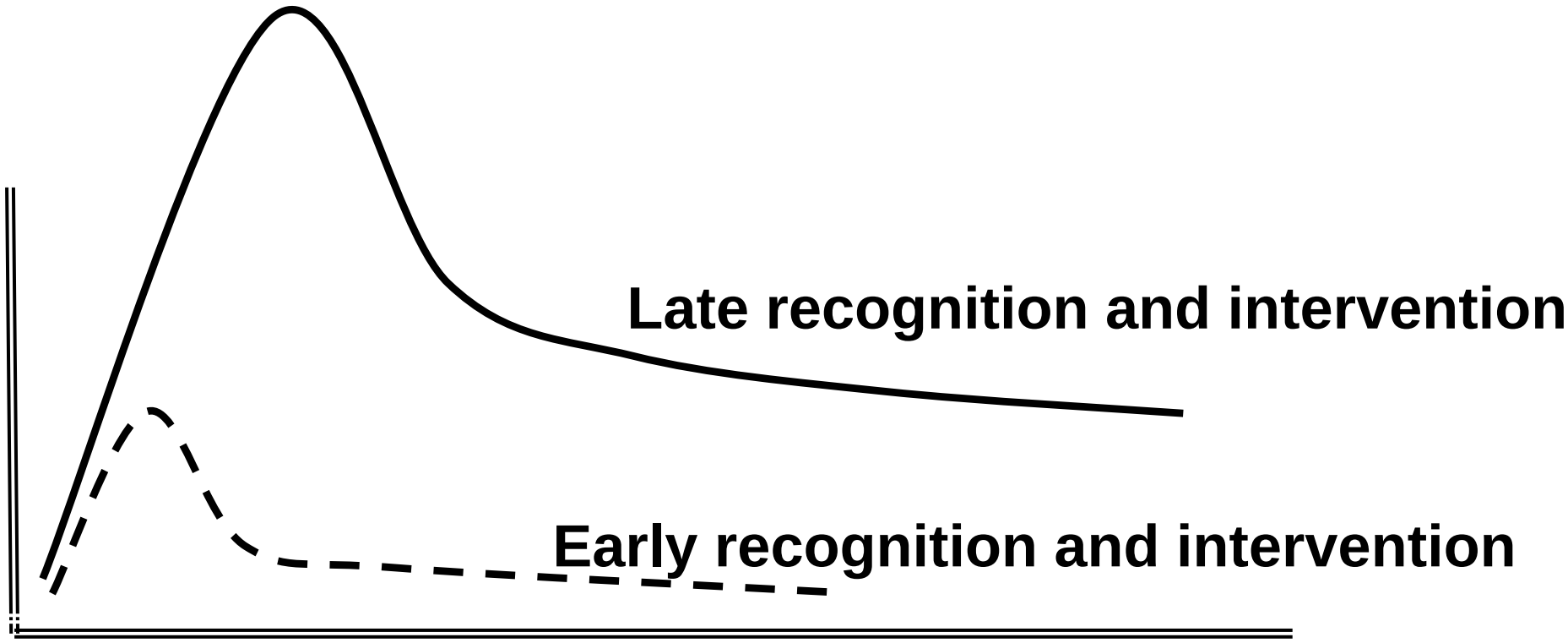
Agent	Clinical Events	Initial Symptoms
Anthrax	Mediastinitis	Fever, Cough, Malaise, Headache
Plague	Pneumonia	
Q fever	Pleuritis, hepatitis	
Tularemia	Pneumonia	
Smallpox	Pustules	

Outbreak Recognition

Who	Trigger	When
Epidemiologist	More admissions/deaths	Late
Clinical laboratory	More specimens Unusual isolate	Late Early
Pharmacy	More prescriptions	Late
Funeral Director	More business	Late
Veterinarian	Animal disease/die-off	Early (natural) Late (intentional)
Clinician	Unusual or rare disease	Early

Bioterrorism/Biowarfare

Early vs Late Recognition



Outbreak Investigation Steps (Epidemiology 101)

1. Develop the diagnosis
   - Two pairs of eyes better than one
   - Initiate labs, if possible
2. Develop a case definition
   - Diagnosis substitute
   - None are perfect
   - Broad at first, more specific as more is known
   - Enables next steps in investigation

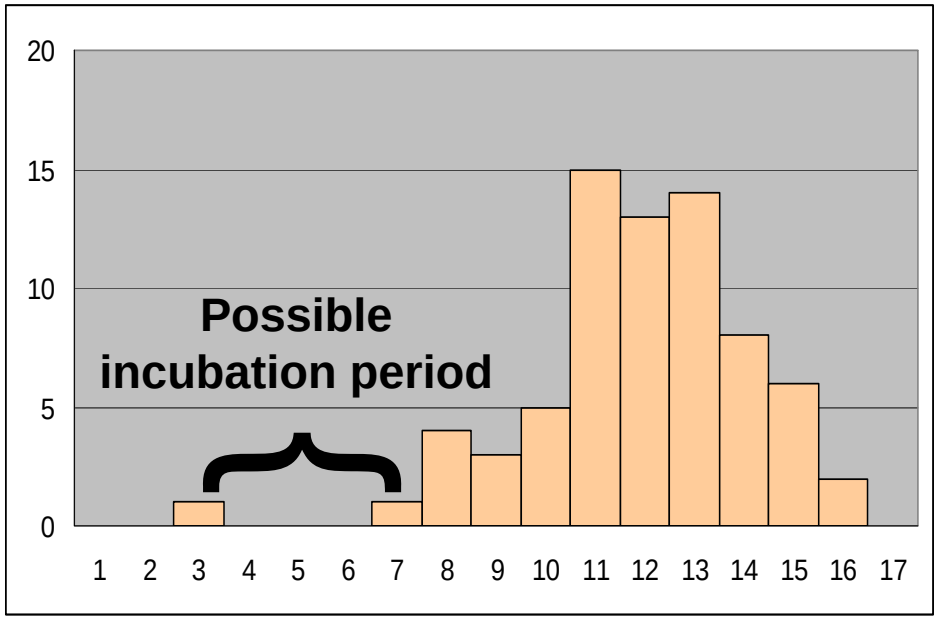
Epidemiology 101

3. Identify and count cases
   - Determine magnitude of exposed
4. Determine presence of an outbreak
   - Based on clinical picture, severity or unusual pattern of illness
   - 1, 10, 100?
5. Key questions: person, place, time
   - Who, where, when?
   - Type of exposure
   - Route of transmission, spread
   - Epidemic curve

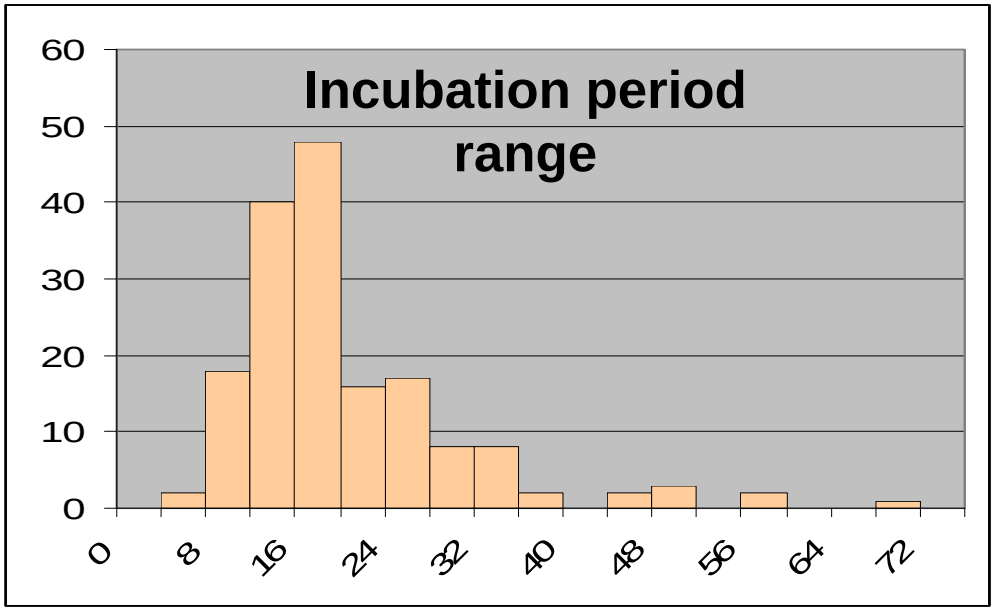
Epidemic Time Curve

- Number of cases by time of illness onset or time since an event of interest
  - Time may be hours, days, weeks
  - May establish incubation period

Unknown time of exposure; 2º transmission



Known time of exposure



Obtain Relevant History

- Others in a group/unit members ill
- Notice unusual munitions or dissemination device, other BW clues
- Uncontrolled/unauthorized food/water sources
- Vector exposure
- Immunization history
- Travel history
- Occupational exposure
- MOPP/Protective status

Epidemiology 101

6. How and why do you think epidemic happened?
   - Source and mode of transmission
   - Pattern of spread
   - Natural or intentional
7. Test the hypothesis
   - Differences between cases and controls?
   - Laboratory analysis
   - Does it fit with facts?
   - Are those who should be ill actually ill?

Epidemiology 101

8. Formulate conclusions and share results
   - Command, public/soldiers, higher echelons
9. Implement control measures
   - Hone initial control measures based on objective information
   - Education, administrative
   - Sanitation, prophylaxis, diagnosis and treatment, vector control
10. Evaluate control measures
   - Were you right or do you re-evaluate?

How BT/BW Agent Investigations Differ

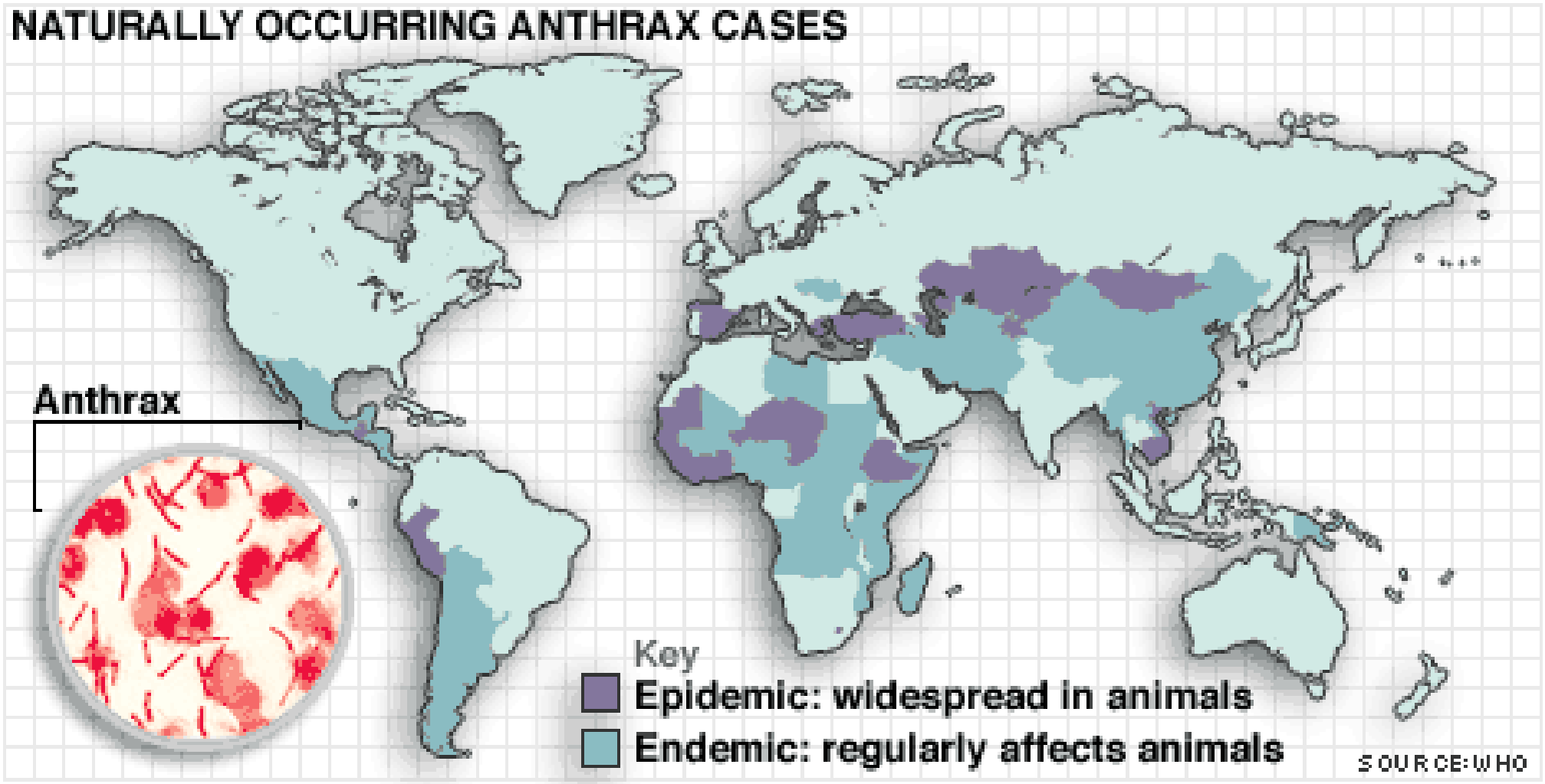
- Closer communication with law enforcement
- Chain of custody of specimens necessary
- Potential for prolonged/serial outbreaks
- Keep an open mind for the unexpected

Clues to an Intentional Biological Event

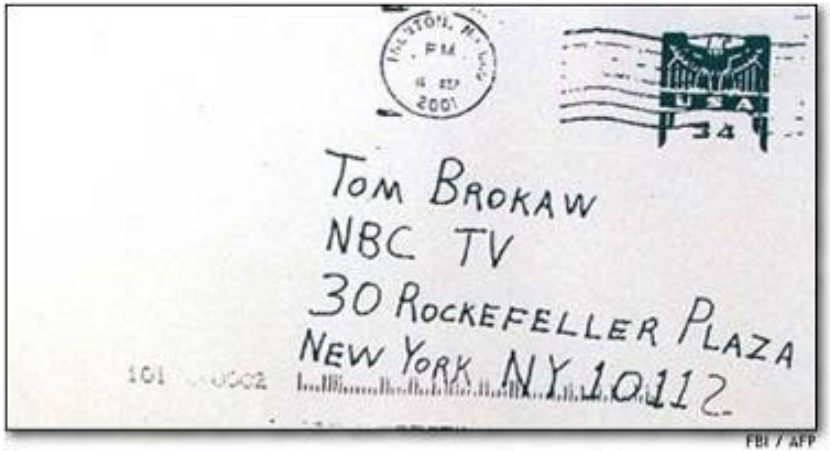
Potential Clues to a BT Event

CLUE 1

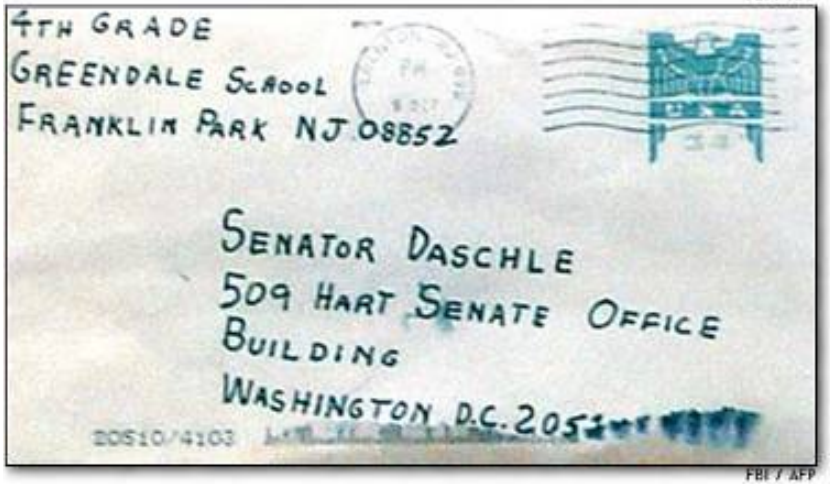
- CLUE 1 - Unusual disease, or one that does not typically occur in a given geographic area
  - Particularly if no competent vector
  - One case of smallpox is intentional until proven otherwise


NATURALLY OCCURRING ANTHRAX CASES
Anthrax
Key
Epidemic: widespread in animals
Endemic: regularly affects animals
SOURCE: WHO


Recent Outbreaks
1990-95 Outbreaks

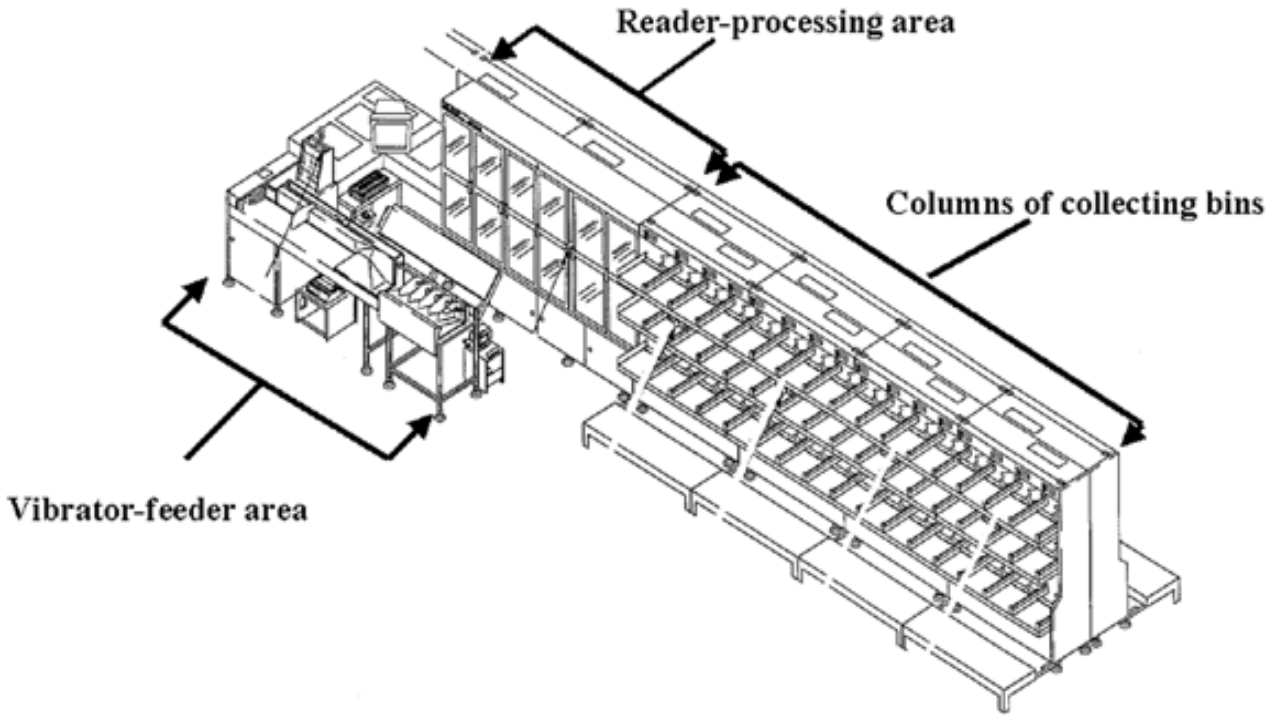


FBI / AFP

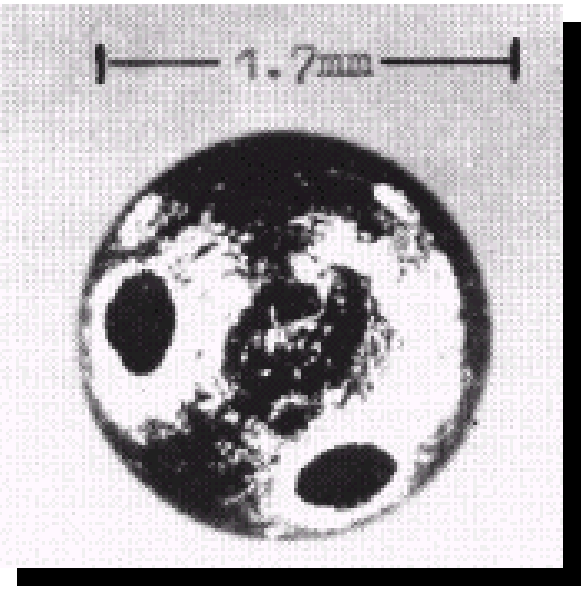


FBI / AFP

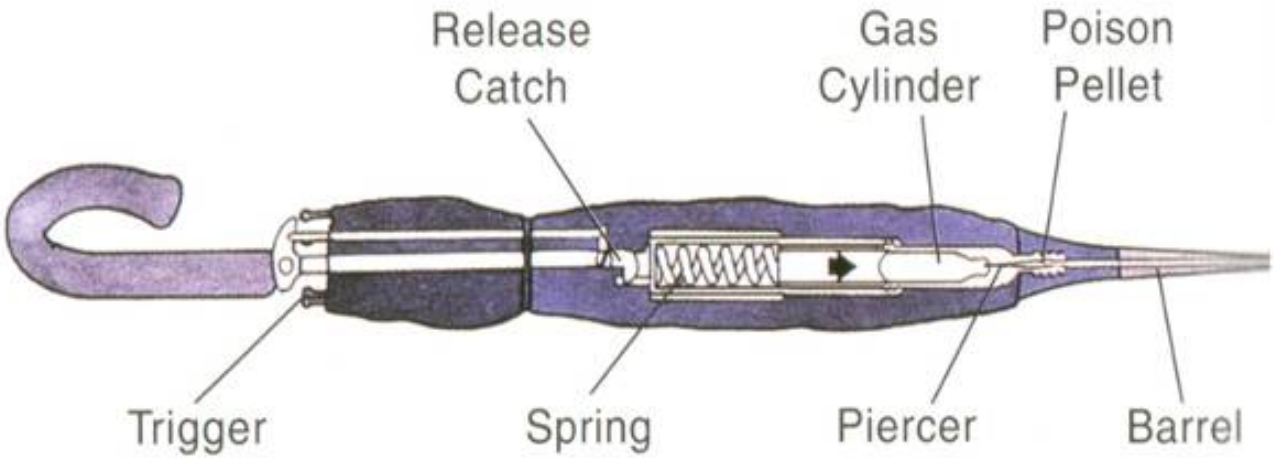
Reuters file


Reader-processing area
Columns of collecting bins
Vibrator-feeder area

Markov Assasination



- London, 1978
- Developed by Soviet KGB
- Ricin (castor bean toxin)
- Used in at least 6 other assasinations



Knight. BMJ 1979. 1:350-1.

Not all botox is Botox®[1]

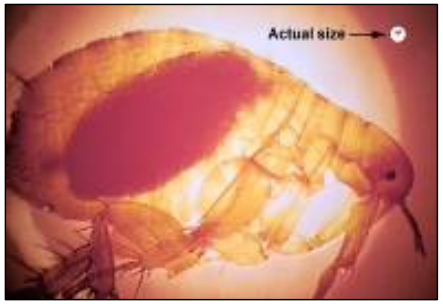
- Bach McComb, D.O.

  (oculofacial plastic surgeon, license suspended)
- 4 people paralyzed
- Sentenced to 3 years in prison
  - Wire fraud
  - Mail fraud
  - Mislabeling of a drug

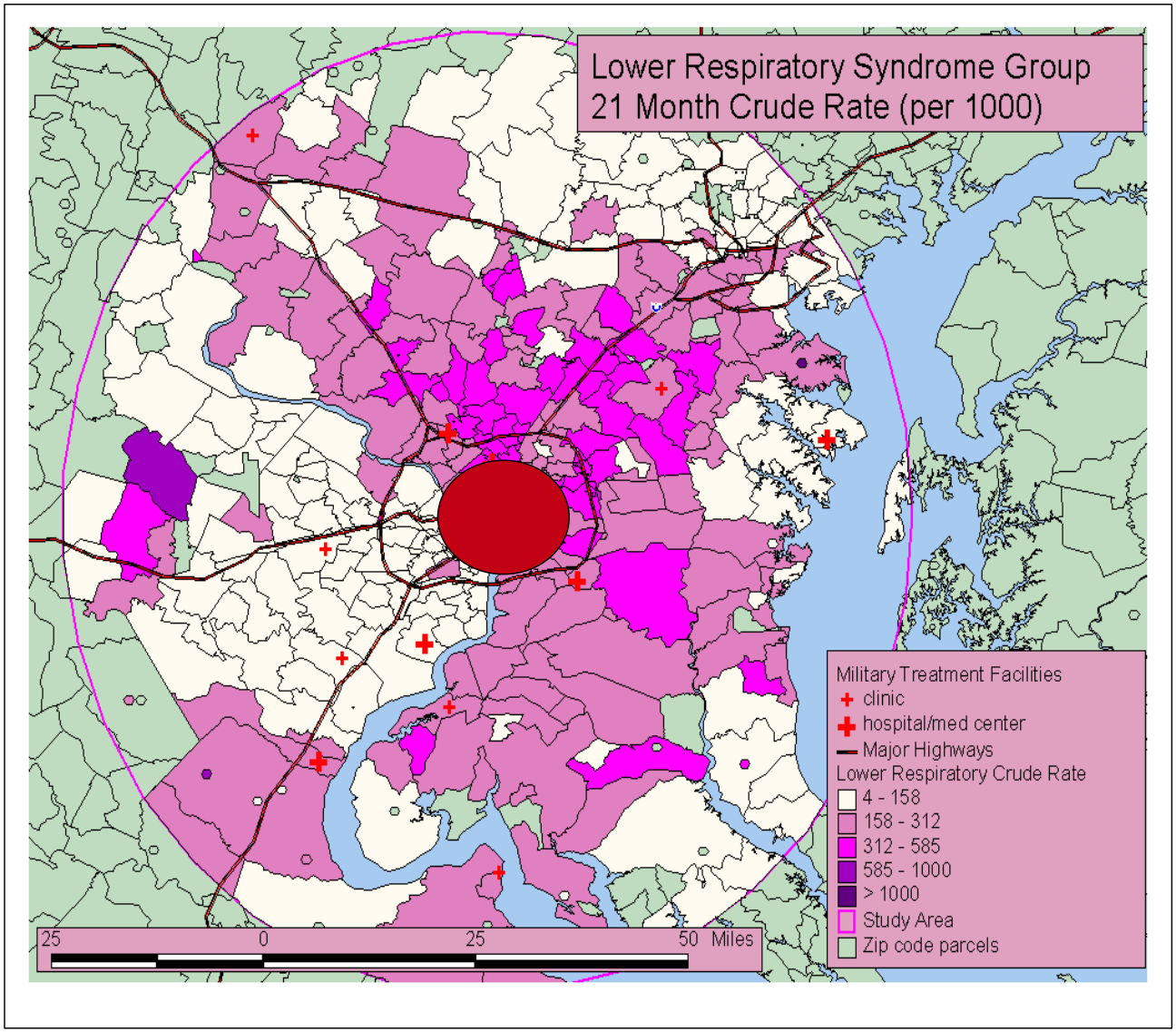
[1]With thanks to Ted Cetaruk, MD

CLUE 1 (and others…)

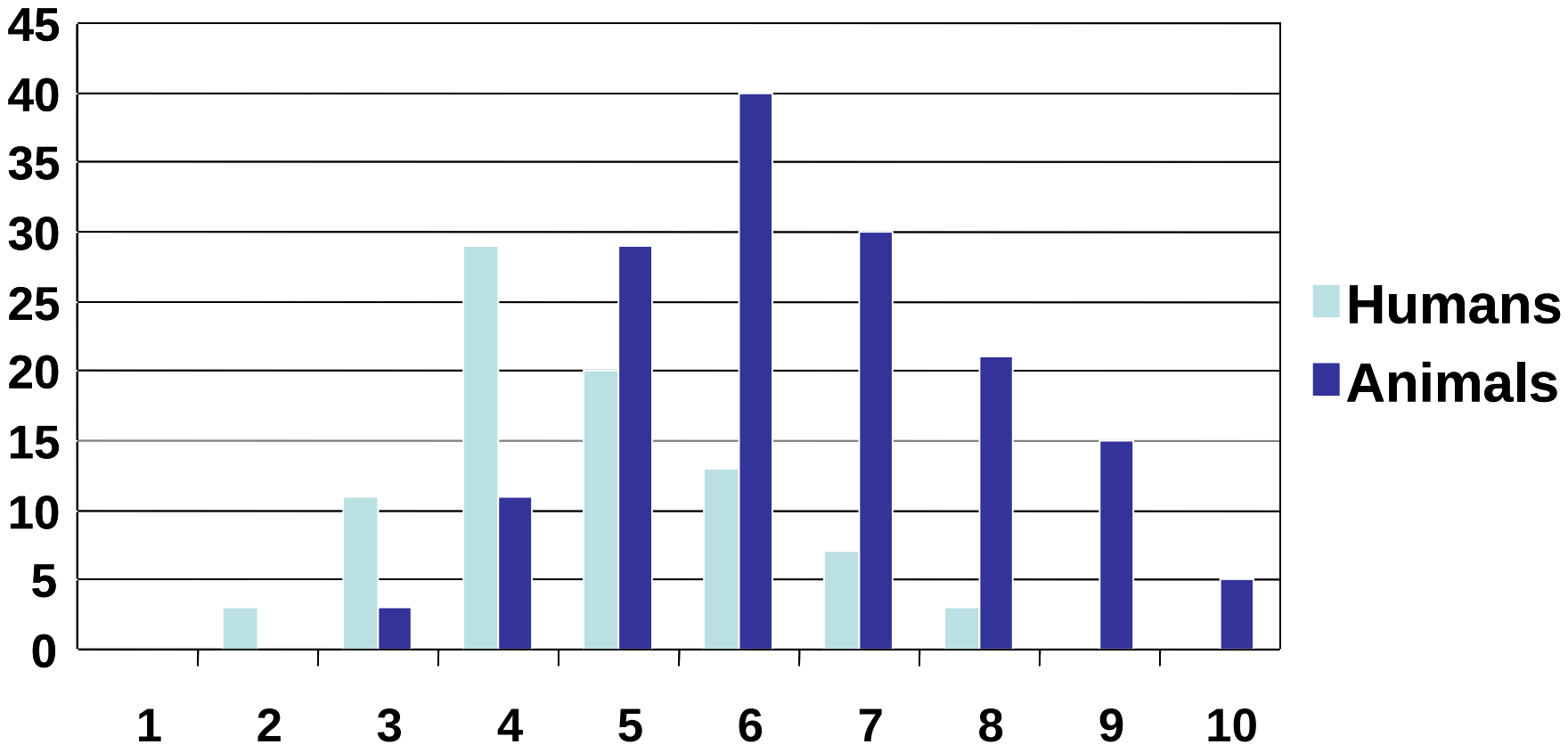
- Changteh (Chengdu), China - Nov 1941
  - City part of shipping trade industry
  - Plague outbreak
  - Japanese plane seen two weeks before dumping mixture of wheat and rice, paper, cotton wadding



CLUE 2: Illness limited to local geographical area



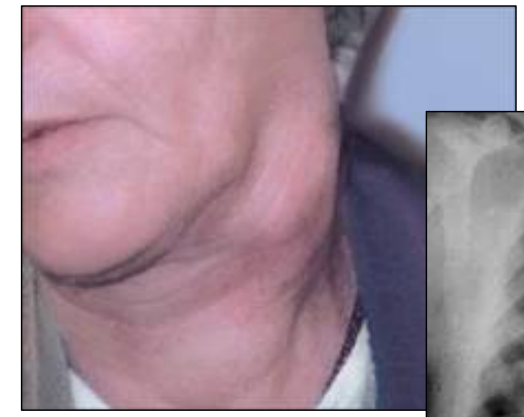
CLUE 3:
Reverse or Simultaneous Spread

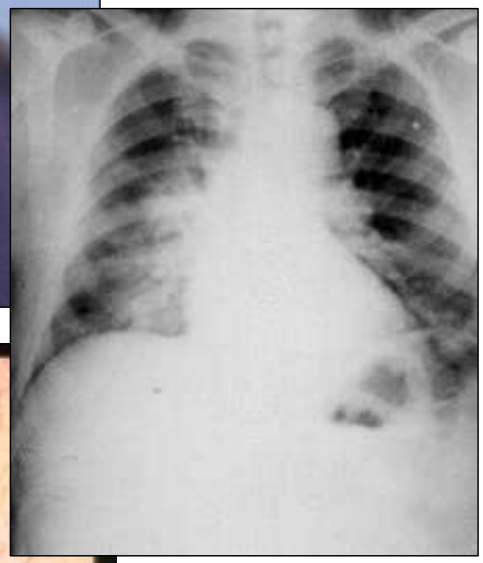


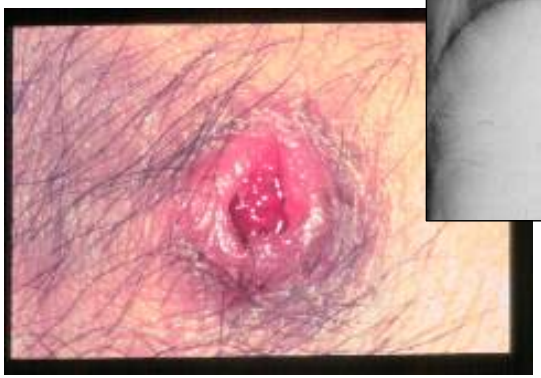
CLUE 4: Multiple Diseases

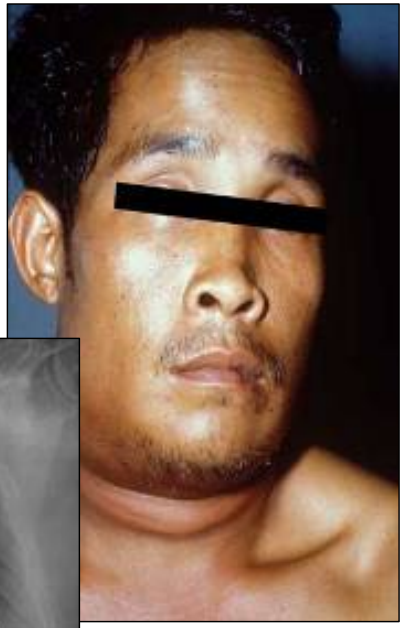
- CLUE 4 – Combinations of unusual disease entities in the same population, or multiple disease entities in the same patients
  - Think mixed agents (Soviet program)

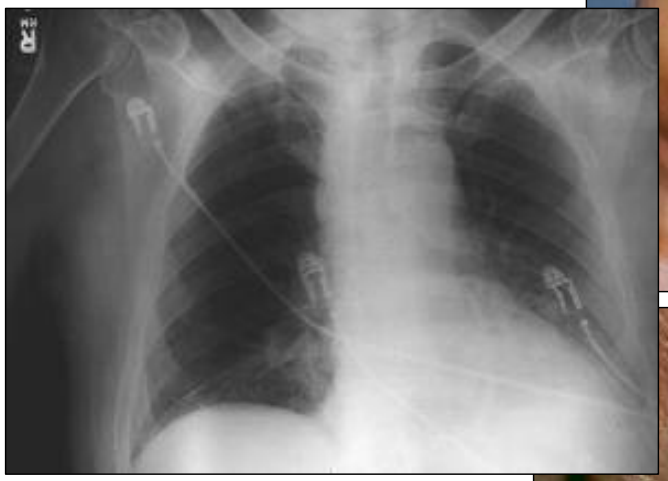
CLUE 4

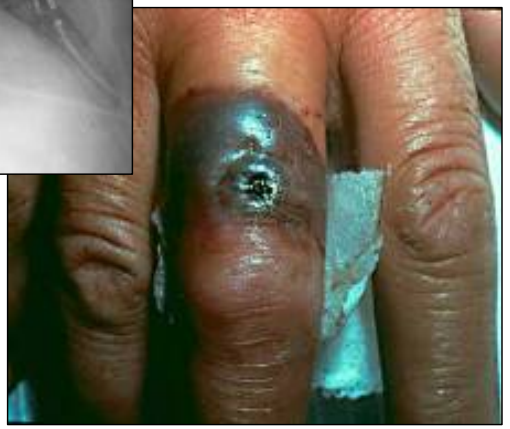










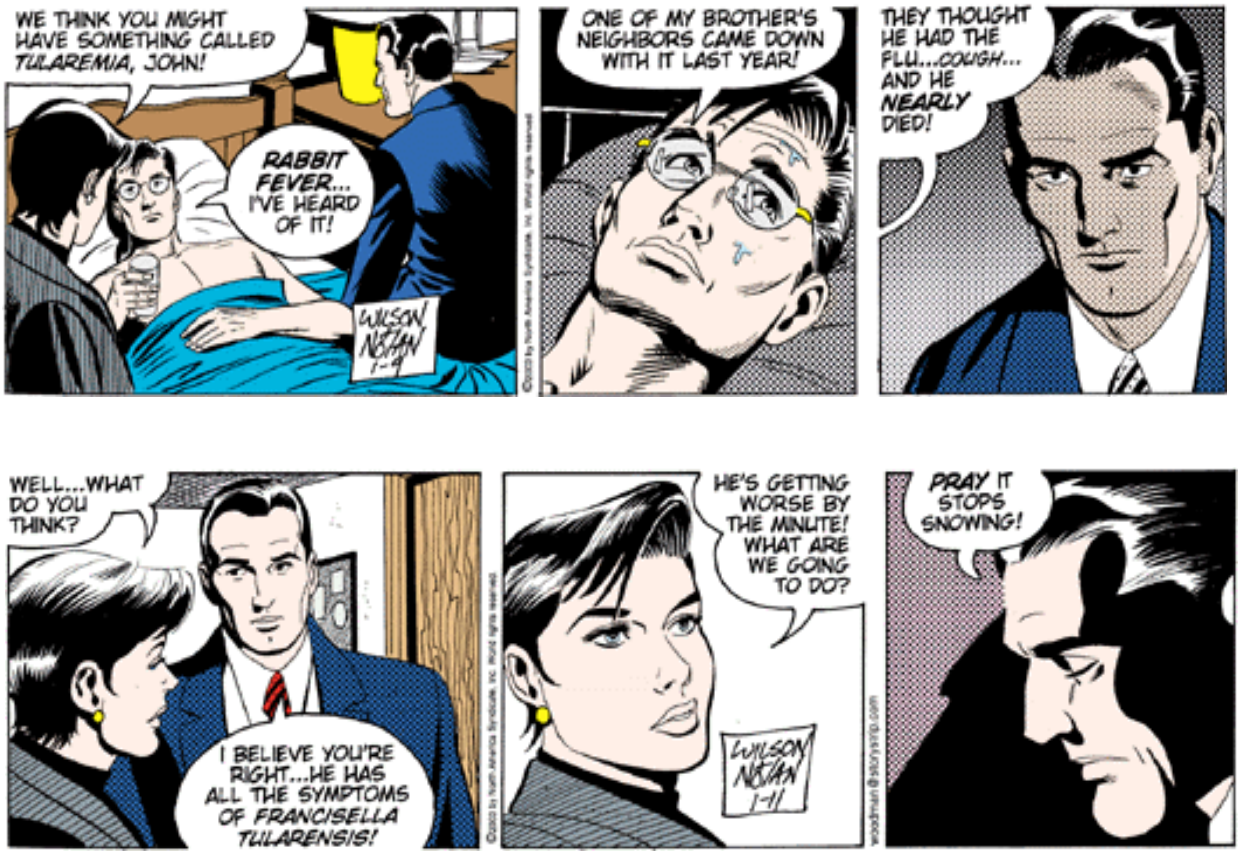


Potential Clues to a BT Event, continued

CLUES 5 & 6

- 5 - Unusually high morbidity & mortality relative to number of personnel at risk
- 6 - Disease in civilian and military personnel occupying the same area

Tularemia Transmission



Tularemia Transmission

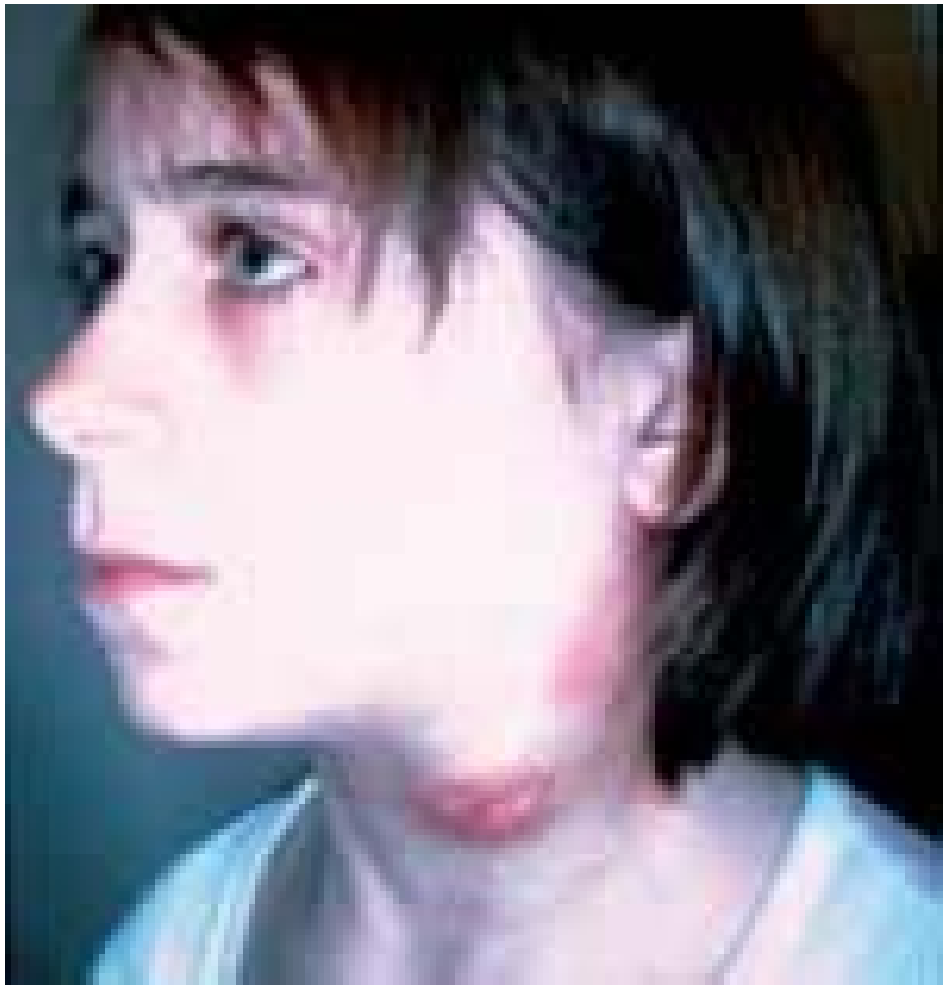
- Arthropod bites (ticks, deer fly, mosquitoes)
- Inoculation of skin, eyes or mucosa
  - Contaminated water
  - Blood or tissue from infected carcasses
- Handling / ingesting insufficiently cooked meat of infected animals
- Drinking contaminated water
- Inhalation of dust from contaminated soil, grain or hay

BW or Natural Pathogen?: Important Principles

- Disease outbreaks during war or in crisis-afflicted regions may be suspect
- Risk assumed in regions where BW agents are developed, produced, stored, or could be released
- Many BW agents are zoonotic pathogens

Kosovo Tularemia Outbreak: History

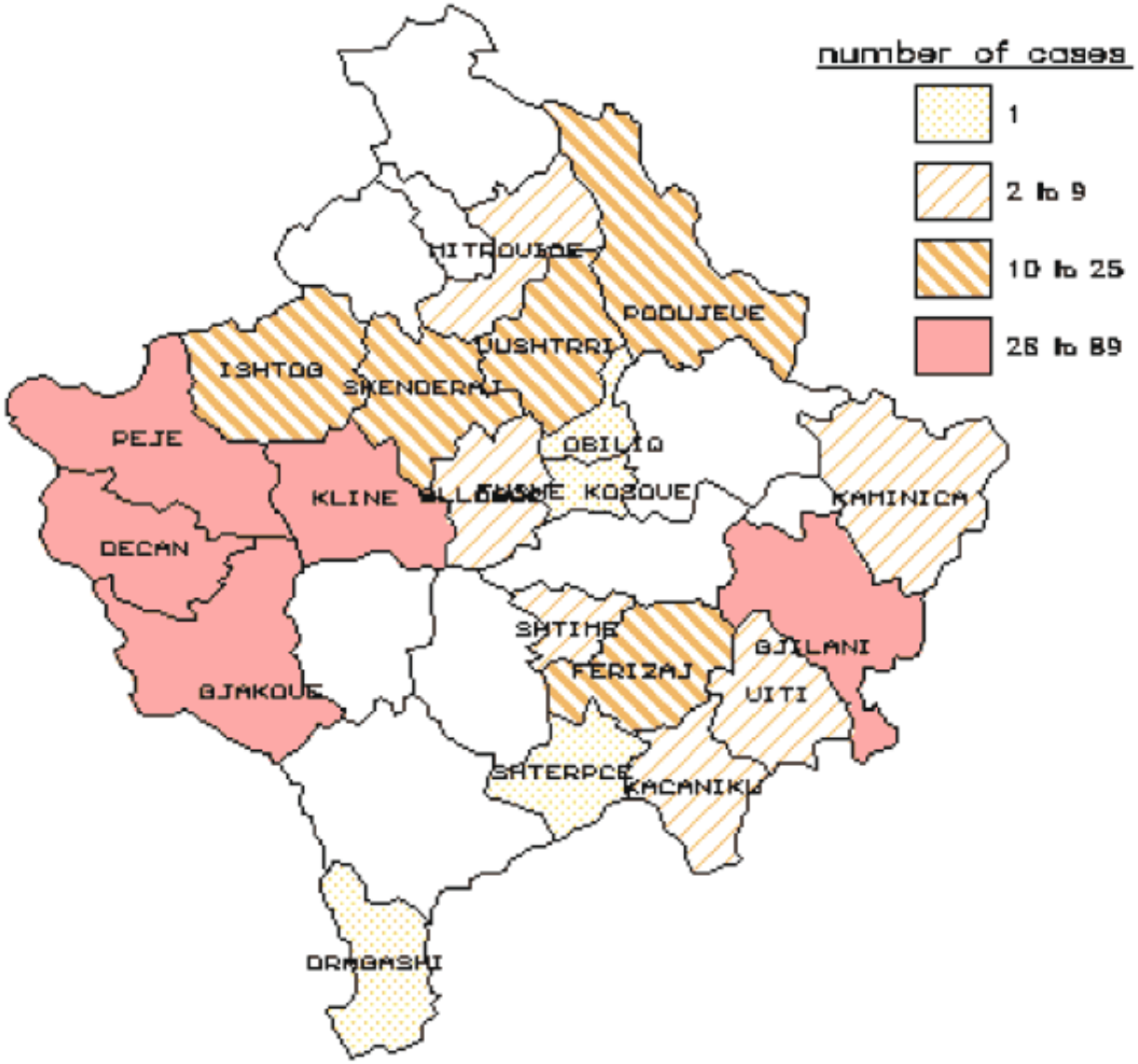
- 1995 - Tularemia reappeared in northern Bosnia
  - Balkan combatant factions accuse each other of using tularemia as a BW
- 1999 - Head epidemiologist at the Institute of Public Health claims that unidentifiable ampoules and “white powdery substances” were found in and around wells
  - Could not be verified



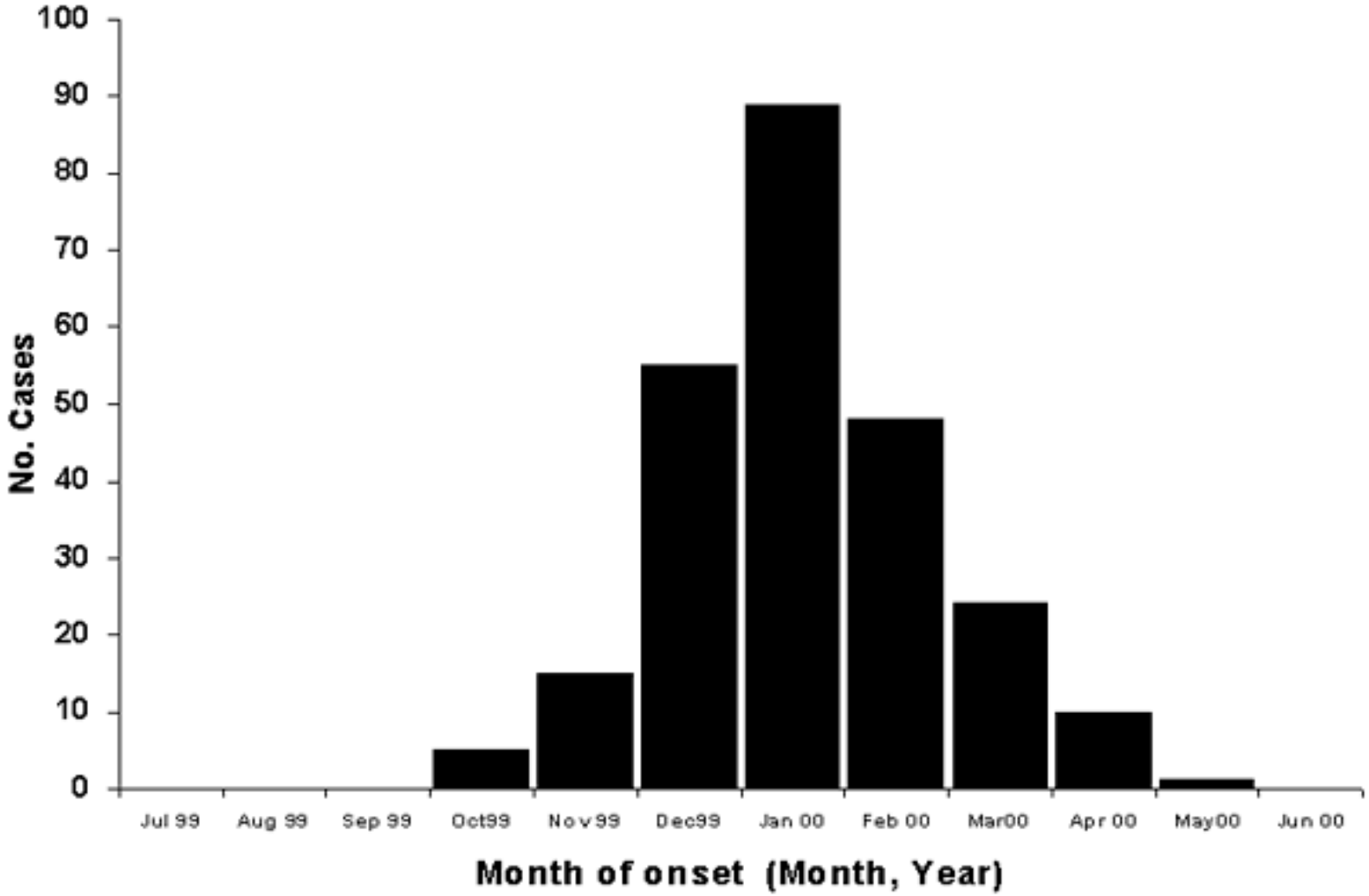
Girl with ulcerating lymphadenitis due to tularemia, Kosovo, April 2000

Kosovo Tularemia Outbreak: History

- By June, 2000, >900 suspected tularemia cases identified in Kosovo
- 327 confirmed positive
- Confirmed cases in 21 of 29 Kosovo municipalities
- References:
  - Clin Microbiol Infect 2002; 8:510-21.
  - EID 2002; 8:69-73.



Laboratory-confirmed tularemia cases in Kosovo by municipality, July 1999- May 2000



Laboratory-confirmed tularemia cases, Kosovo, October 1999- May 2000

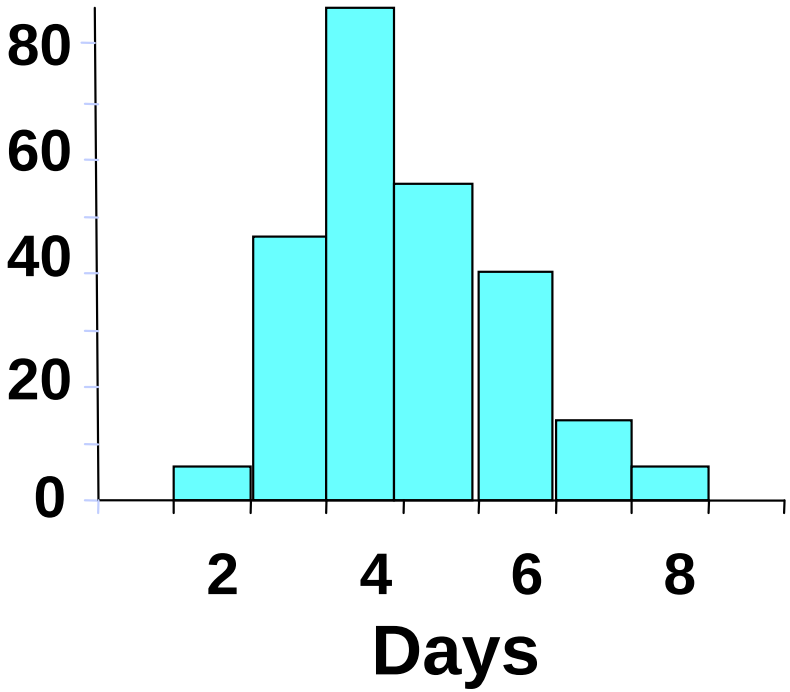
Kosovo Tularemia Outbreak: History

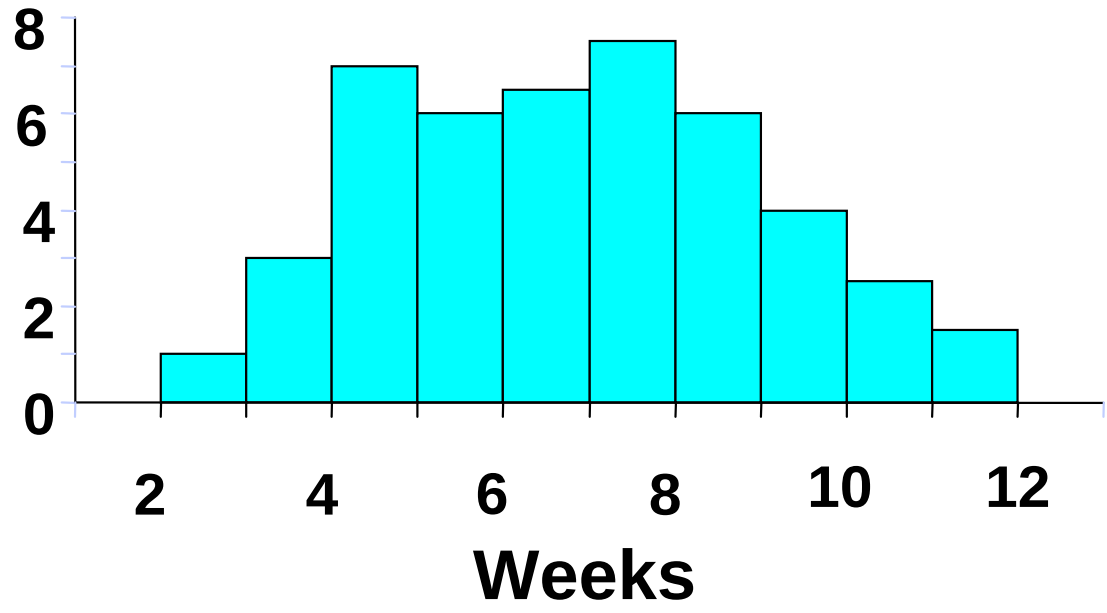
- Almost all tularemia cases were ethnic Kosovo Albanians – mostly rural with poor hygienic conditions
- Tularemia vaccination status in Serbian population unknown
- No evidence that the Serbian regions of Kosovo were deliberately spared the tularemia epidemic

Cause of Kosovo Tularemia Outbreak

- Natural causes for epidemic:
  - Fall of 1999, unusually rapid growth of field mice and rat populations: tested + for tularemia
  - Caused by the surplus of food in unharvested fields and in vacant or damaged buildings
    - Majority of cases in western Kosovo where damage was the greatest
  - Rodents settle in human dwellings during winter
    - Infected animals, their feces and carcasses likely contaminated drinking water, grain and food supplies
  - Ignorance of risk of infection and resulting lack of hygienic measures led to food-borne infections in humans

CLUE 7: Epidemic Time Curve:
Massive Point Source

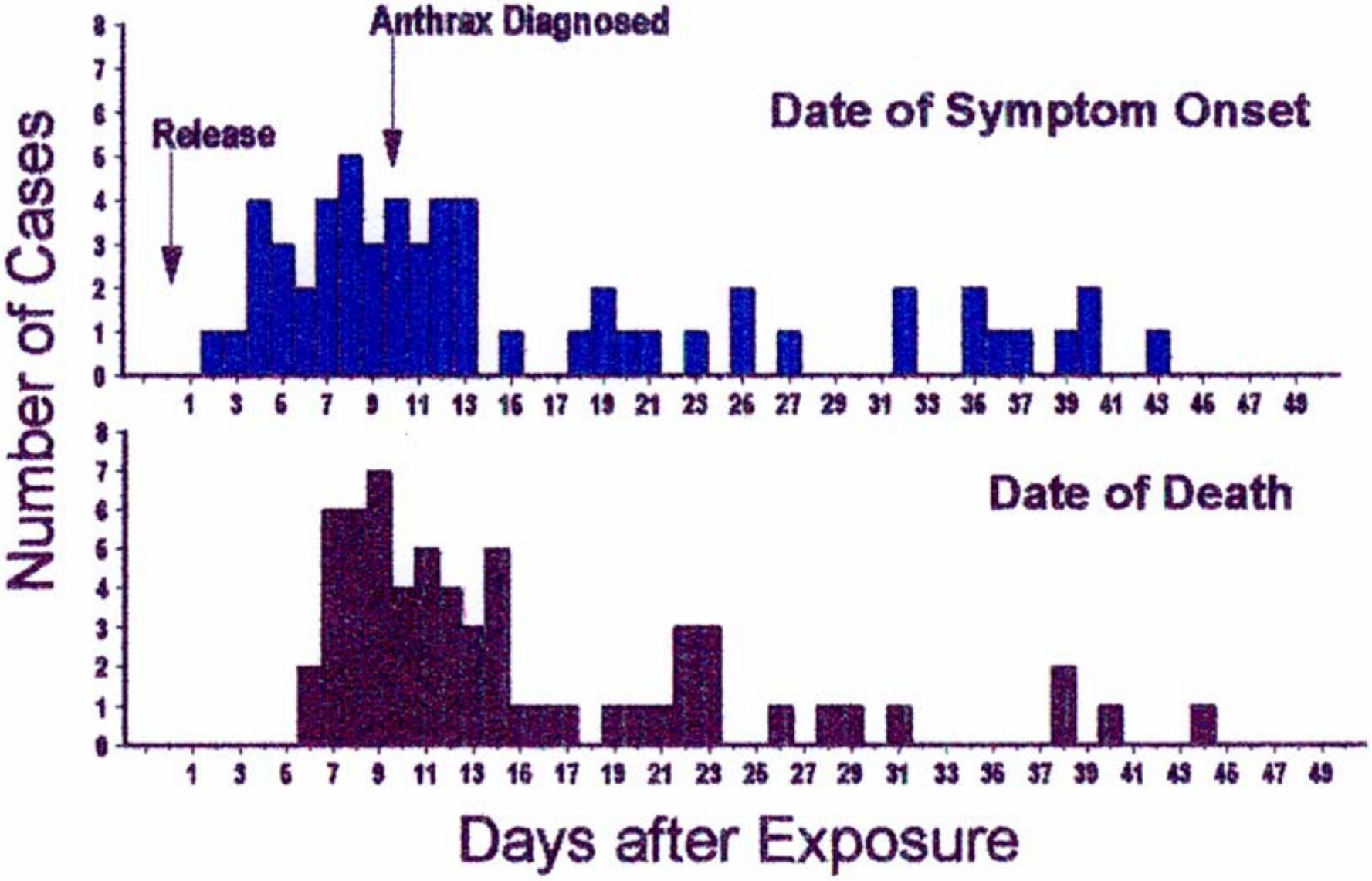




Massive Point Source

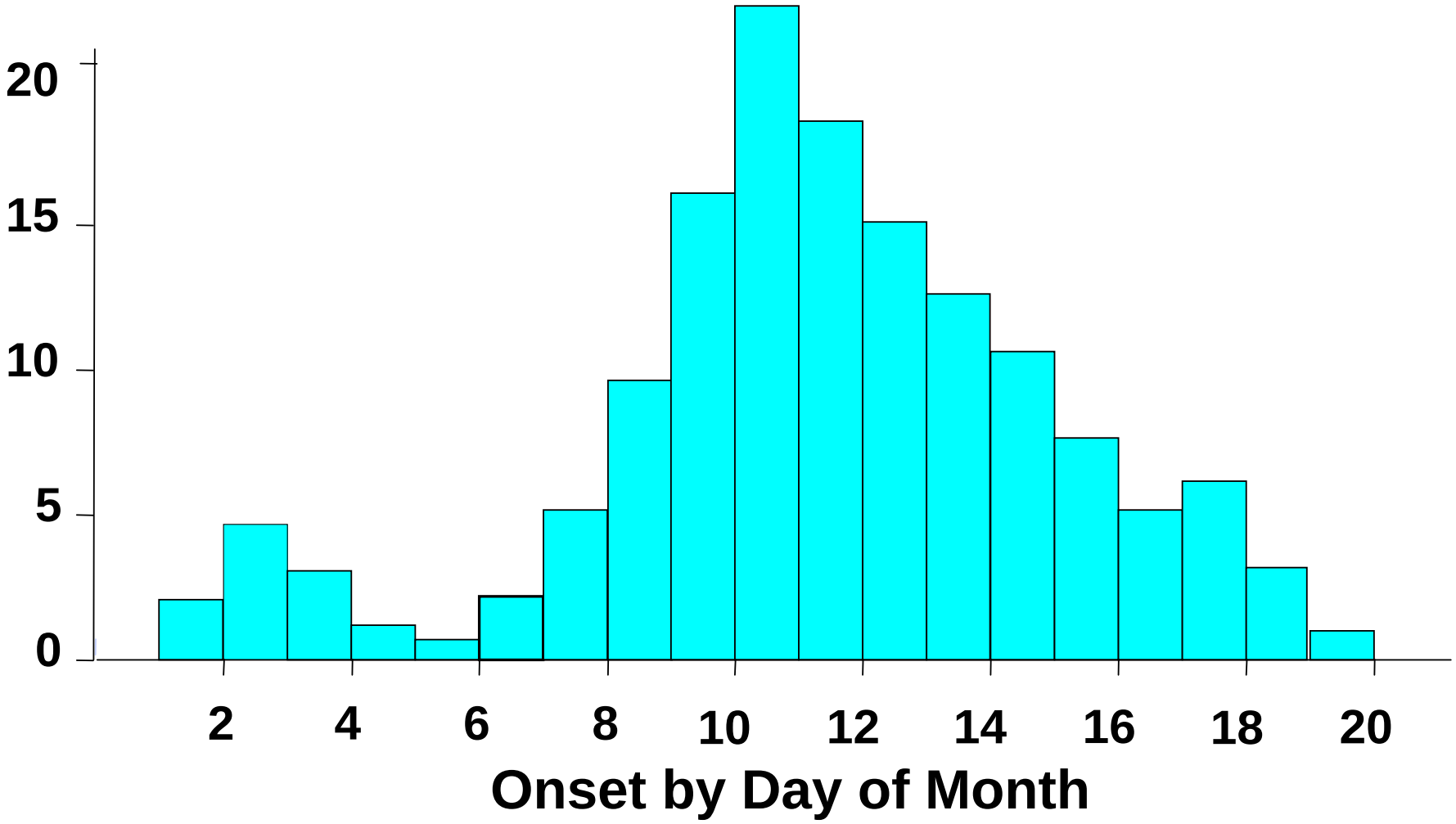
Common Point Source

Inhalational Anthrax--Sverdlovsk, 1979



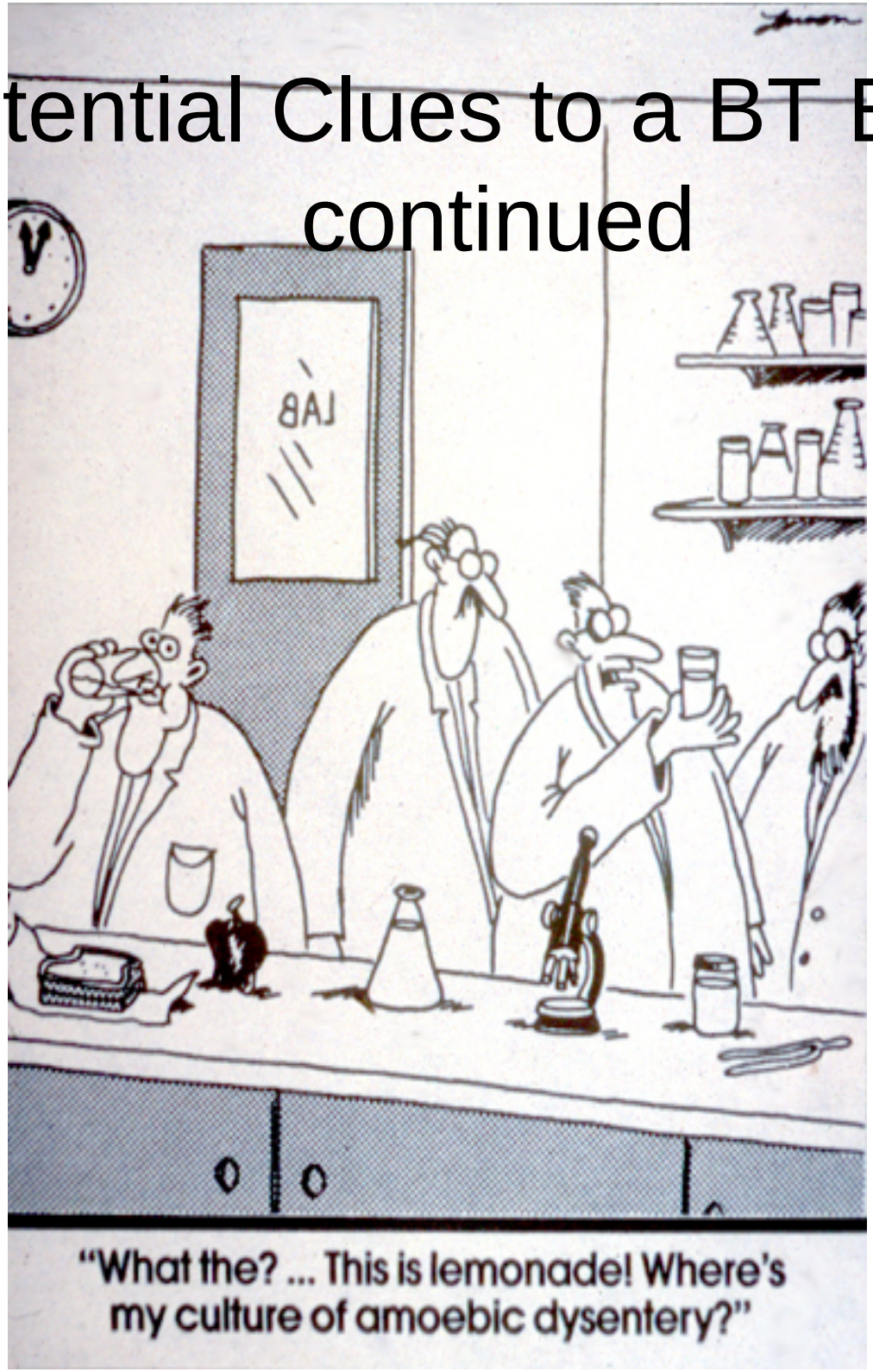
Reference: Science 1994; 266:1202-8.

CLUE 8: Serial Epidemics



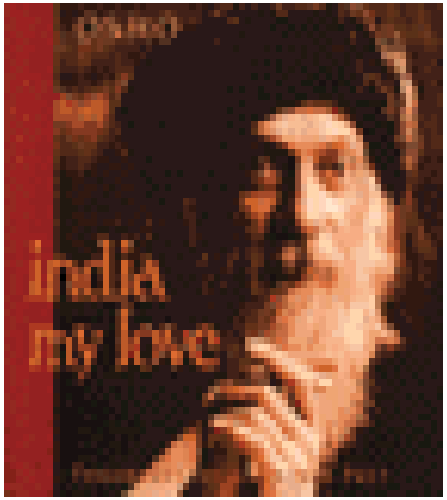
Differentiate from secondary transmission

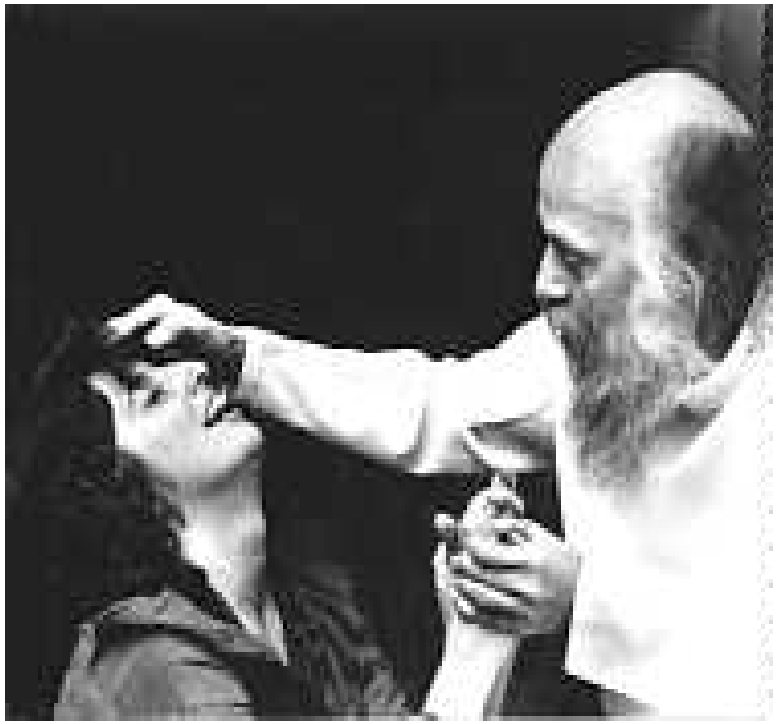
Potential Clues to a BT Event, continued



CLUE 8

- Oregon, 1984
- Contaminated salad bars - *S. typhimurium*
- 751 cases of enteritis





Epidemiological Features

- Most cases associated with 10 restaurants
  - Implicated restaurants had salad bars (RR=7.5, CI 2.4-22.7)
- Implicated foods differed
- Other errors may have facilitated spread, but didn't cause outbreak
  - Errors in food rotation, inadequate refrigeration, and infected employees
- *S Typhimurium* strain from commune lab indistinguishable from outbreak strain
- Reference: JAMA 1997; 278:389-95.

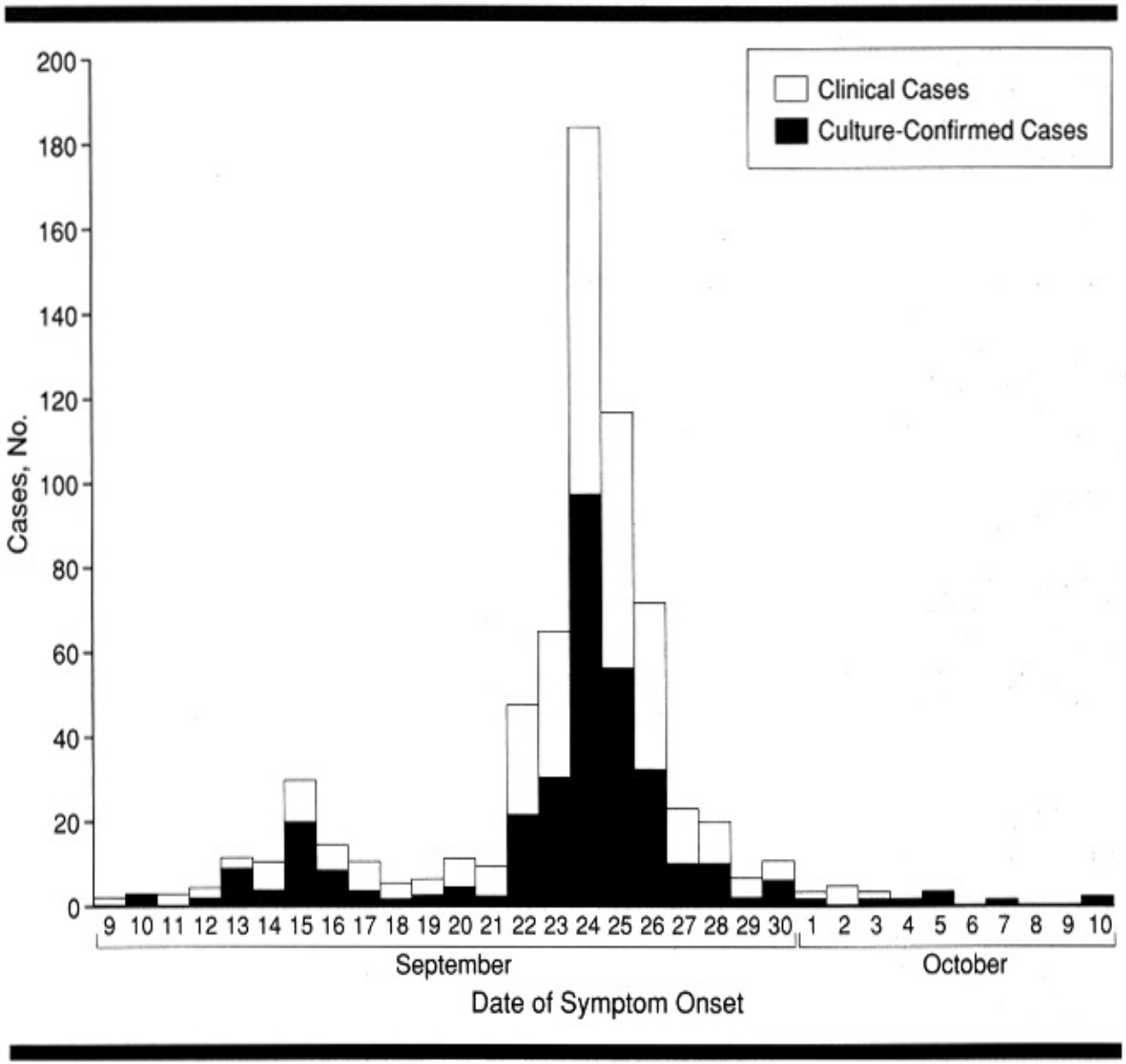


Figure 1.—Reported cases of *Salmonella* Typhimurium gastroenteritis by date of symptom onset for 674 cases (89.8%) with known date of onset, The Dalles, Ore, 1984.

Diane Thompson *Shigella dysenteriae* Food Contamination - 1996

- October - November 1996
- Large medical center in Texas
- 12 of 45 lab staff ill
- Muffins and doughnuts
- *Shigella dysenteriae* type 2
- Laboratory stock culture source
- Unknown motive

Transmission Vehicles: Shigellosis, Dallas, Texas, 12 hospital employees (1996)

Epidemiological Features

- All with shigellosis reported eating pastries
- *S. dysenteriae* type 2 isolates uncommon in the U.S.
- No other documented local outbreaks (i.e. Unlikely secondary to commercial preparation)
- Stock cx not commercially prepared (ie. Unique)
- Gross lab error unlikely
  - No cases dx'ed by the hospital lab >5 yrs
  - no research with pathogen
- Stock Cx = stool isolates = food sample
- Reference: JAMA 1997; 278:396-8.

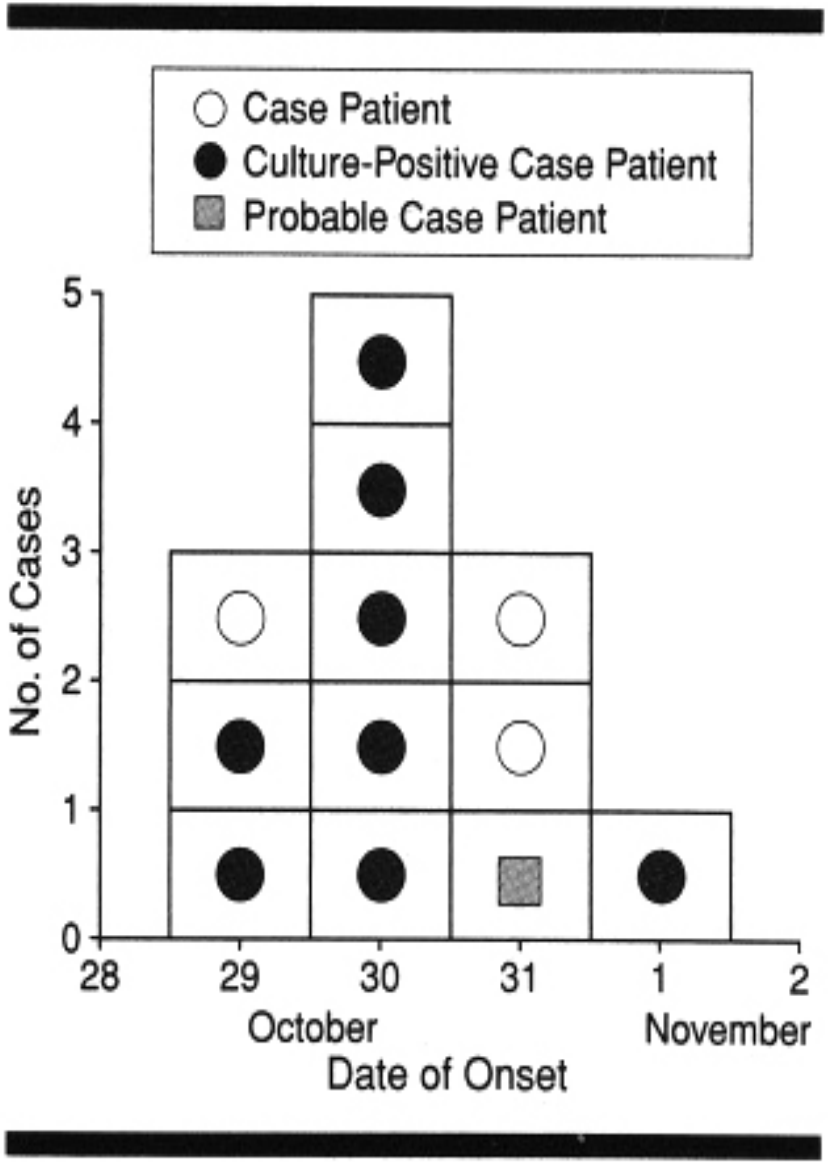


Figure 1.—Cases of shigellosis by onset of diarrhea among laboratory workers in a Texas medical center, 1996.

Common Denominators

- Match of lab samples with clinical samples
- Agents don’t have to be classic warfare agents
- Medical personnel have an advantage
  - Access to identifiable virulent cultures
  - Strain selection
  - Possess an understanding of “cause and effect”
- Look closely at disgruntled medical employees.

CLUE 9: Lower Attack Rates in Protected Personnel

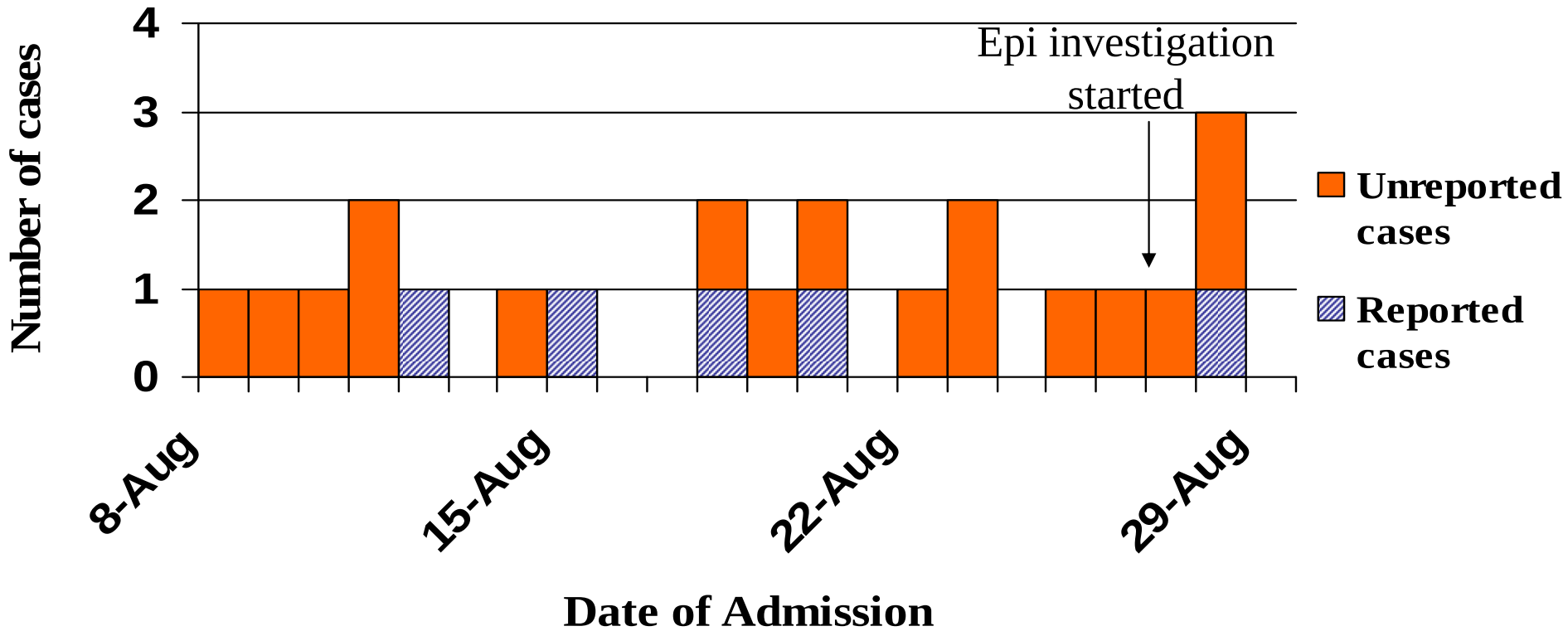
- Those expected to be protected
  - wearing MOPP/JSLIST suit with protective mask
  - Working in environments with filtered air, closed ventilation systems

CLUE 9

CLUE 10: Dead Animals of Multiple Species

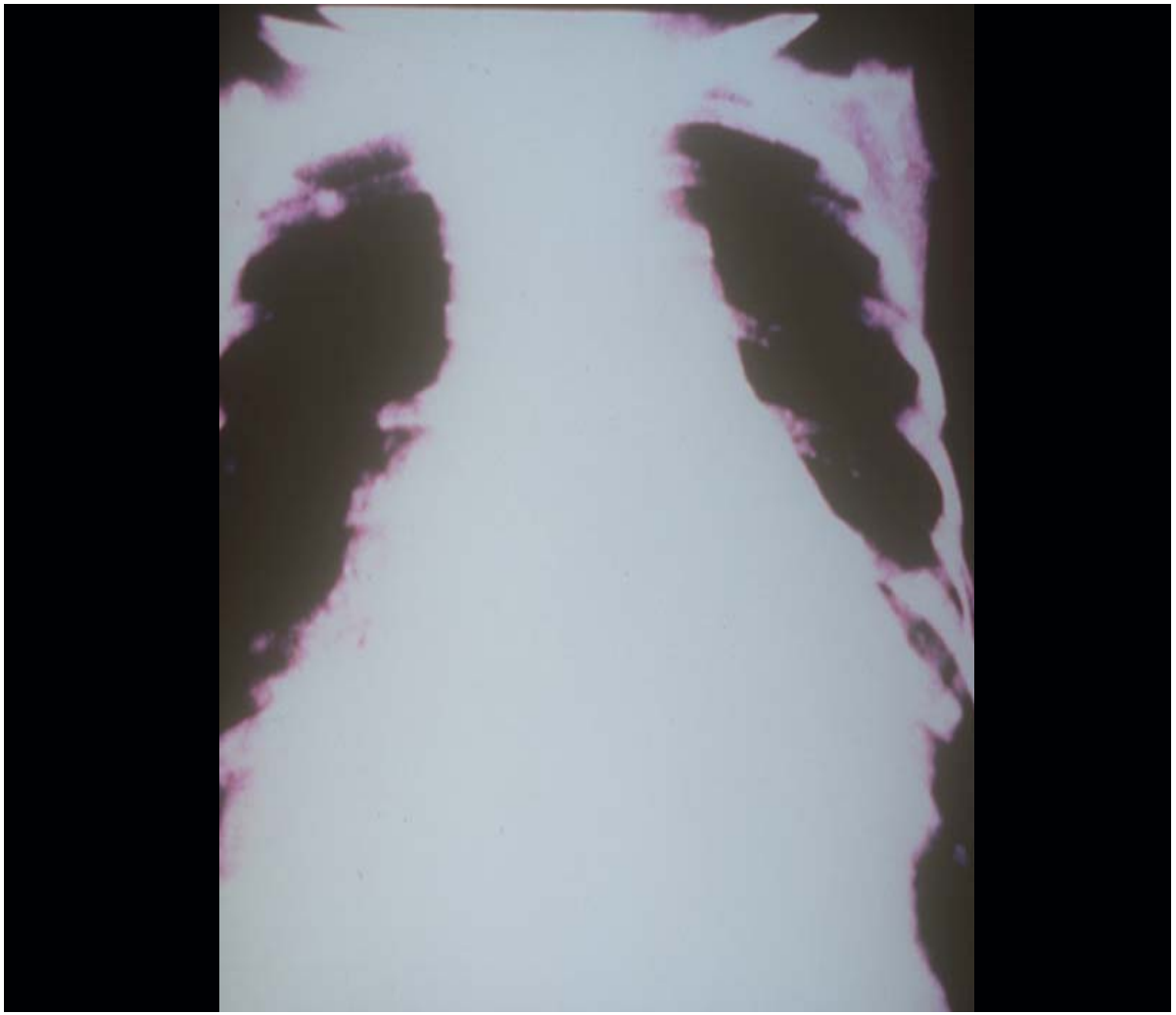
West Nile Encephalitis Outbreak New York, 1999

Unreported WNV Encephalitis Cases



Shieh, et al. EID 2000. 6:370-2.
Fine and Layton. CID 2001. 32:277-82.

CLUE 11: Apparent aerosol route of infection



Tularemia in Martha's Vineyard 2000

- 5 cases pneumonic tularemia between May 30 – June 22
- 15 tularemia cases subsequently identified, 11 pneumonic
- 14 male, average 43 y.o., 1 death
- Naturally occurring?
- References:
  - NEJM 2001; 345:1601-6.
  - J Am Board Fam Prac 2003; 16:339-42.

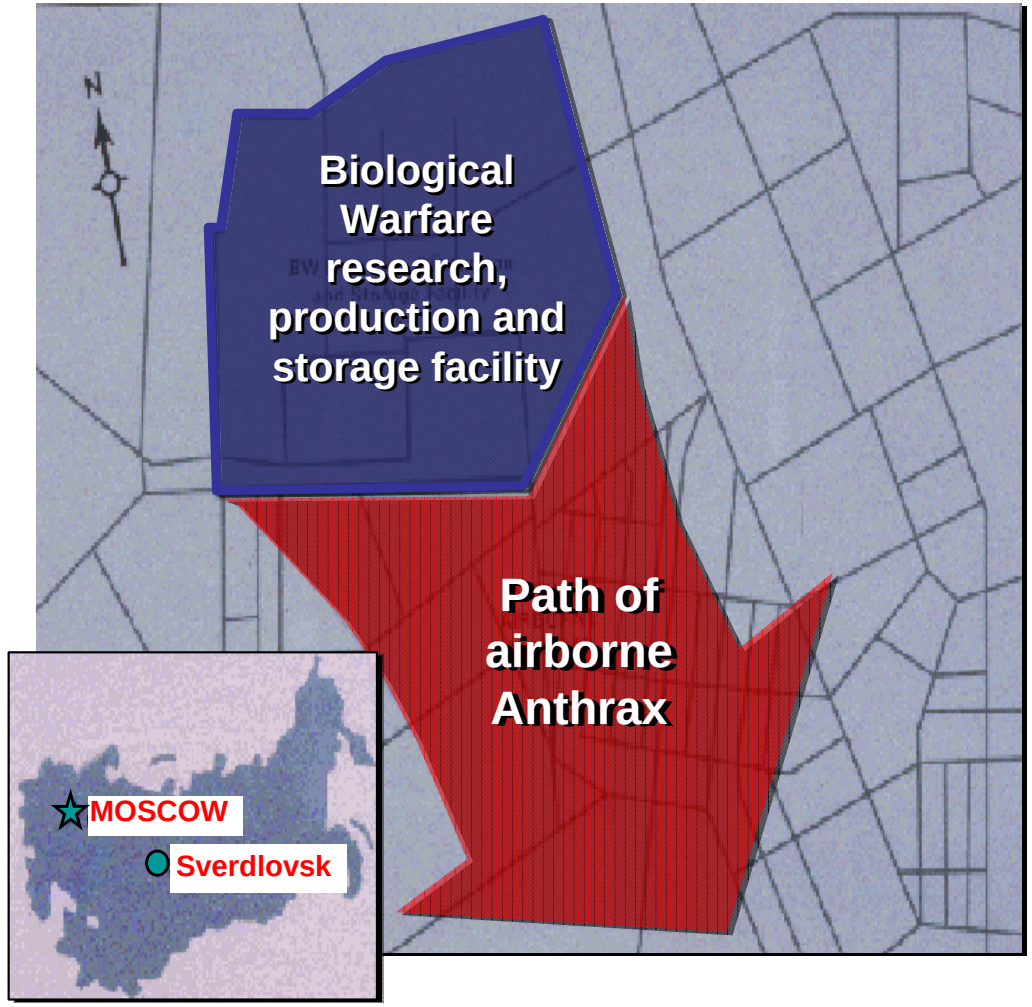
CLUE 12
Distinctive Downwind Plume

- Unusual for natural outbreaks to follow weather pattern
- Downwind plume pattern of infection
  - Indicates aerosol transmission
  - May point to the source
- Inversion cloud
  - More likely at dawn and dusk
  - Rule out normal sources

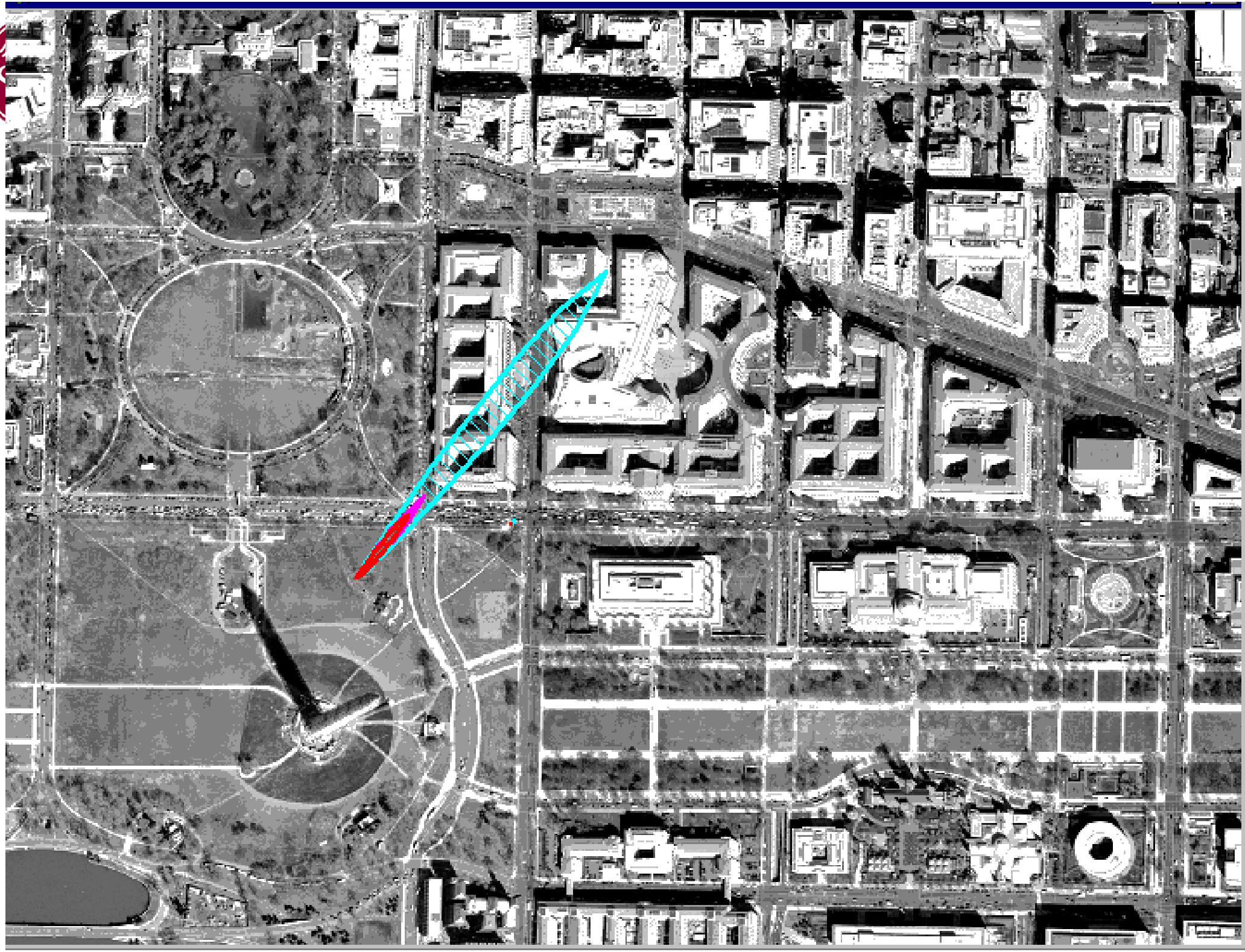
CLUE 12

Anthrax:
Sverdlovsk Incident

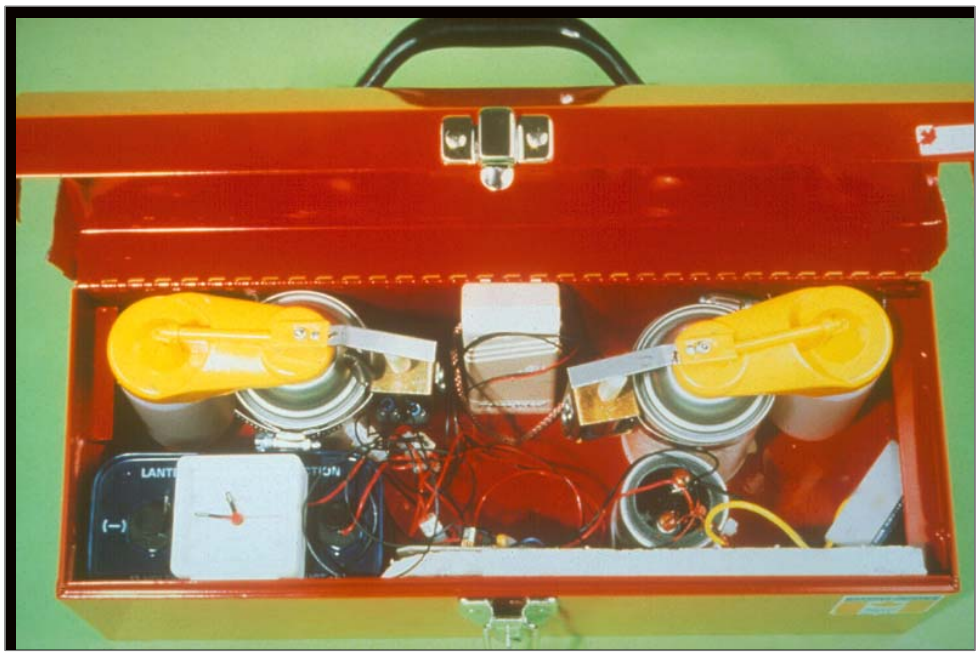
- April 1979
- Accidental release of <1 gram of anthrax spores from a Soviet military compound
- Resulted in ≥ 66 deaths downwind



Meselson et al. Science 1994. 266:1202-8.



CLUE 13: Direct Evidence



Meanwhile, back in Tokyo……

Keim et al., 2001. J. Clin Microbiol. 39:4566-7.

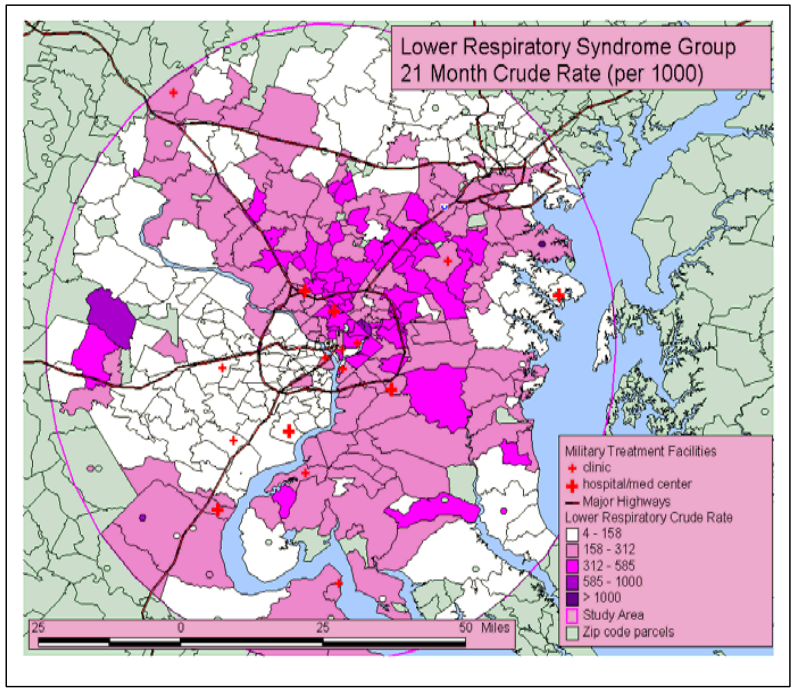
Current State of Surveillance

- Civilian community may rely on passive reporting - transitioning to electronic reporting
  - People don’t report
  - People don’t know/how/where to report
- Relies on meeting a case definition
  - Usually must be culture proven
  - Epidemiological links typically need to be made before increasing concern
- When not automated
  - Delay in reporting - especially if done by paper

Syndrome Based Triggers

- NYC, Harvard Pilgrim HC, various cities, counties and States
- ESSENCE / DoD GEIS
- Others: Laboratory monitoring, OTC Drug sales, EMS visits
- Many systems based on diagnoses of illness in one of these categories:
  - Respiratory, Gastrointestinal, Neurological, Dermatological (infectious or hematologic), Fever/Malaise/Sepsis (FUO), Coma/Sudden Death
- Risks > < Benefits
  - Risk: Intervention too early
  - Benefits: Faster, broader, more meaningful

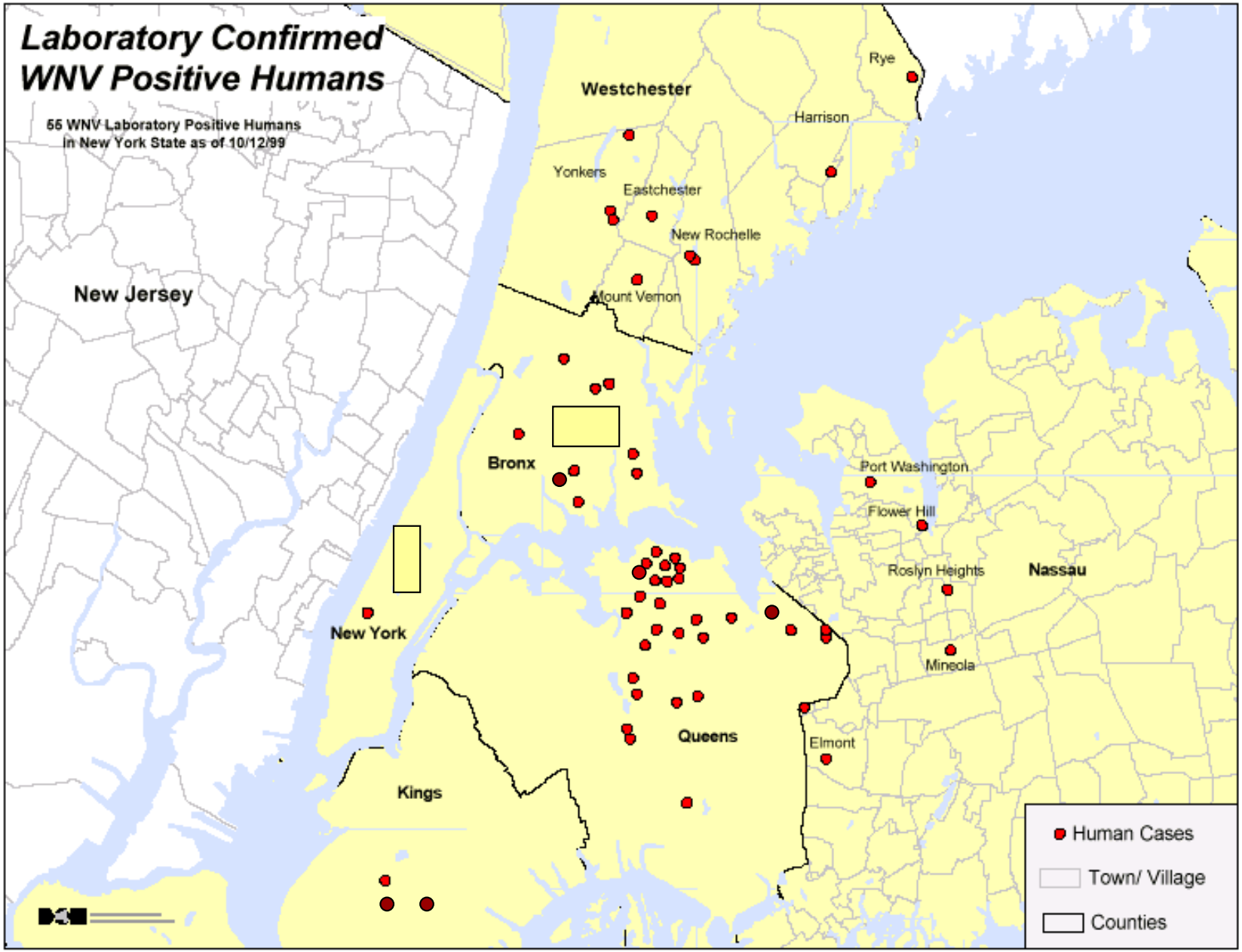
GIS Component

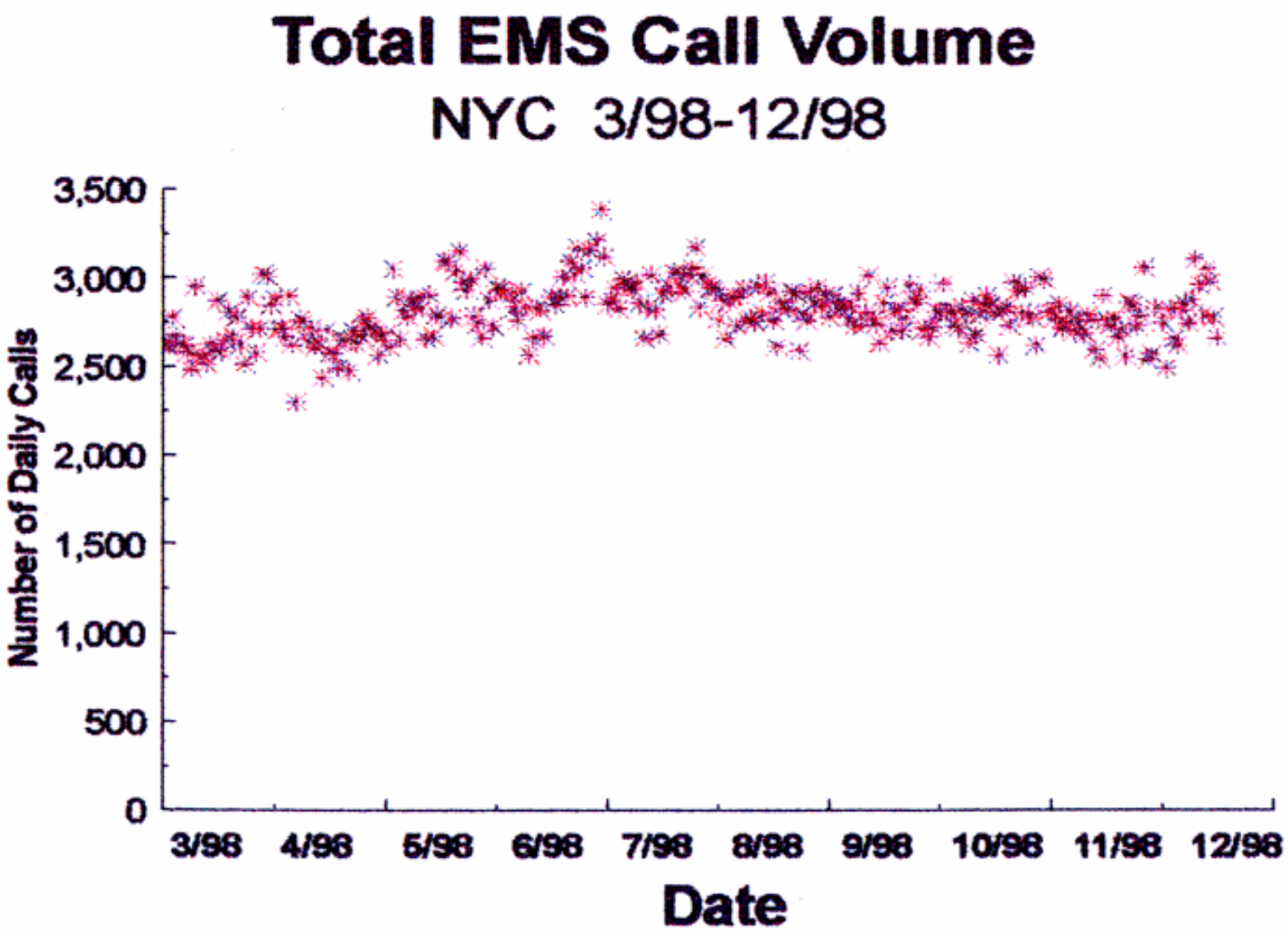


- Cases plotted by spatial identifier such as zip code, long-lat, map grid
- Crude rates based on population within space
- GIS customized for web-based:
  - near-real-time display of cases
  - Interactive playback of occurrences (chronologic)


Map
OF
CENTRAL
LONDON
c. 1854
0
50
100
150
Yards
= Deaths from Cholera
Pumps
John Snow's Map
The Xs mark the water pumps; black rectangles represent cholera deaths.
Oxford Street
Broad Street
Warwick Street
Regent Street
King Street
Golden Square
Brewer Street
Savile Row
Regents Quadrant
Piccadilly

WNV Human Cases




Total EMS Call Volume
NYC 3/98-12/98
Number of Daily Calls
3,500
3,000
2,500
2,000
1,500
1,000
500
0
3/98
4/98
5/98
6/98
7/98
8/98
9/98
10/98
11/98
12/98
Date

Goals for Future Surveillance

- Set appropriate alert levels
- Define proper channels
  - Data flow bottom up
  - Intervention decisions top down
- Appropriate incorporation of:
  - automation
  - use of internet
  - data sources
- Resources:
  - www.syndromic.org
  - http://www.cdc.gov/epo/dphsi/syndromic/index.htm
  - http://www.geis.fhp.osd.mil/GEIS/SurveillanceActivities/ESSENSE/ESSENCE.asp

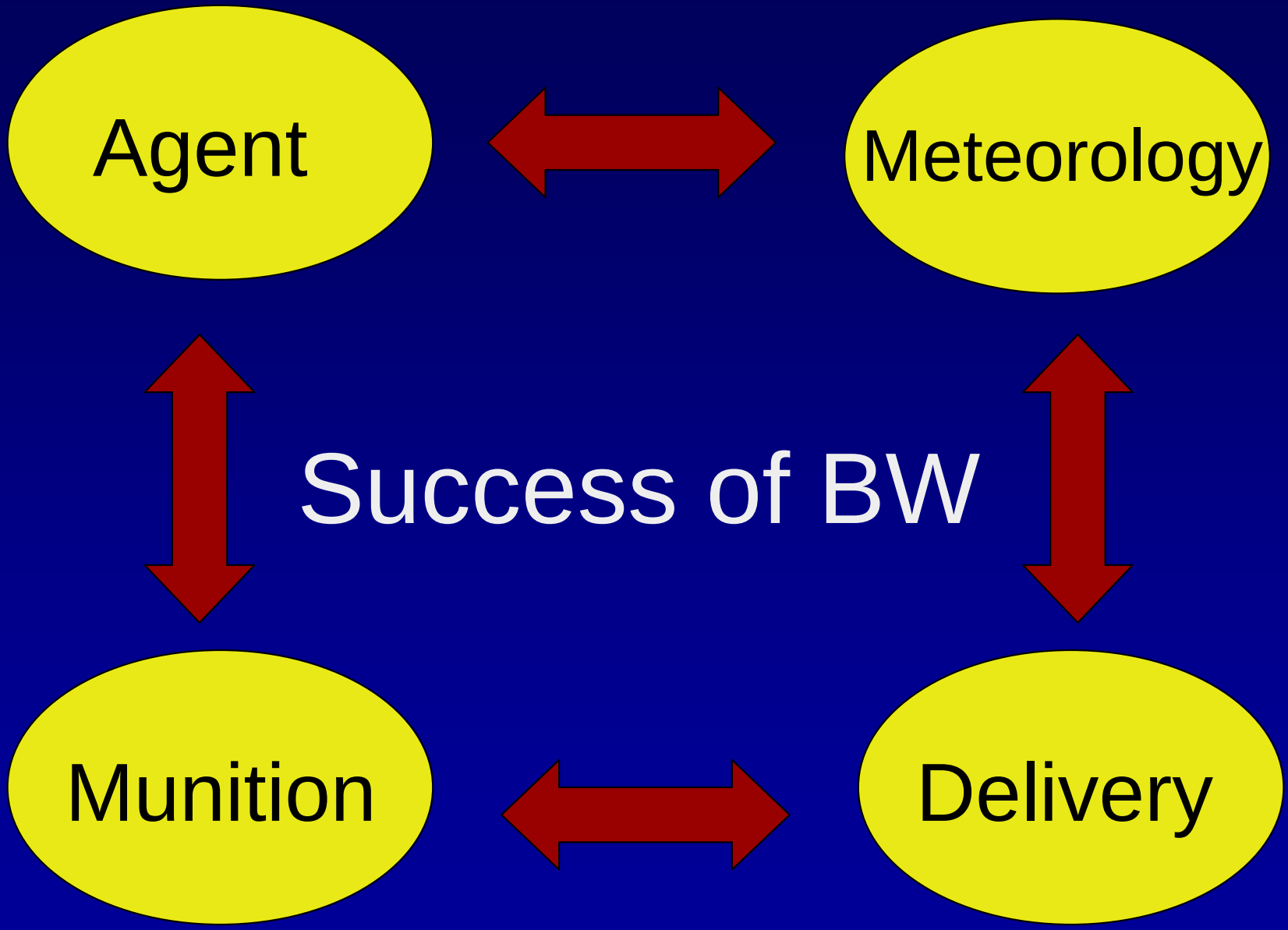
Summary

- Epidemiology is crucial to identify and stop outbreaks – especially intentional ones!
- Epidemiological clues should raise index of suspicion
- Disease surveillance supports public health response
- Communicate with law enforcement
- **You can make the difference!**

Questions?

BioThreats Assessment

William C. Patrick III


Agent
Meteorology
Success of BW
Munition
Delivery

Atmospheric Conditions

Aerosol disseminated in bright sunlight will not remain at ground level

- Rises immediately into atmosphere

Atmospheric Conditions

(continued)

BW attacks usually pre-dawn, sunset, or night

- Temperature inversions more likely

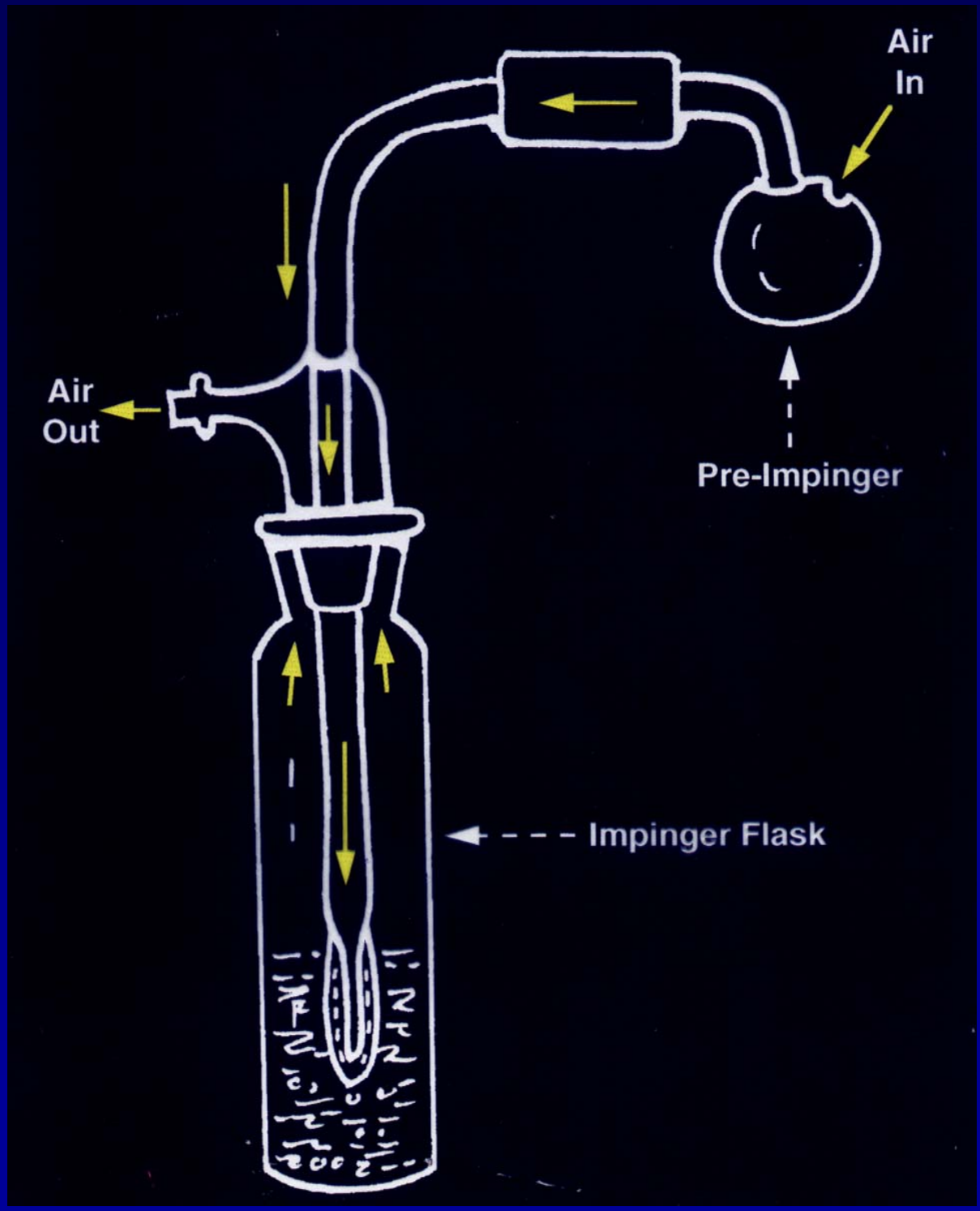
Wind

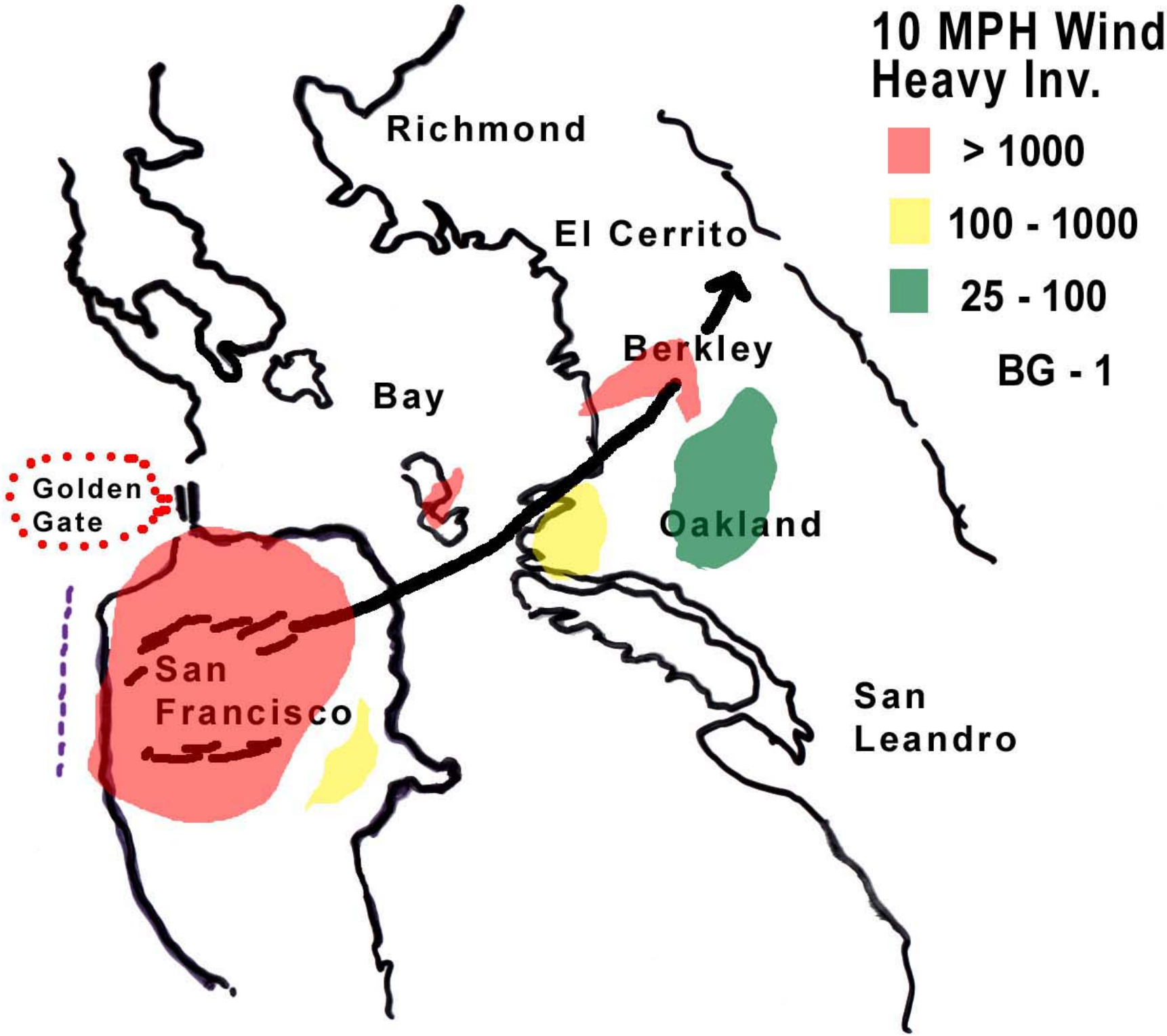
Important factor in preplanning BW attack

- If less than 5 mph, aerosol will be limited in coverage
- If more than 30 mph, aerosol disintegrates and loses integrity
    - Results on target unpredictable

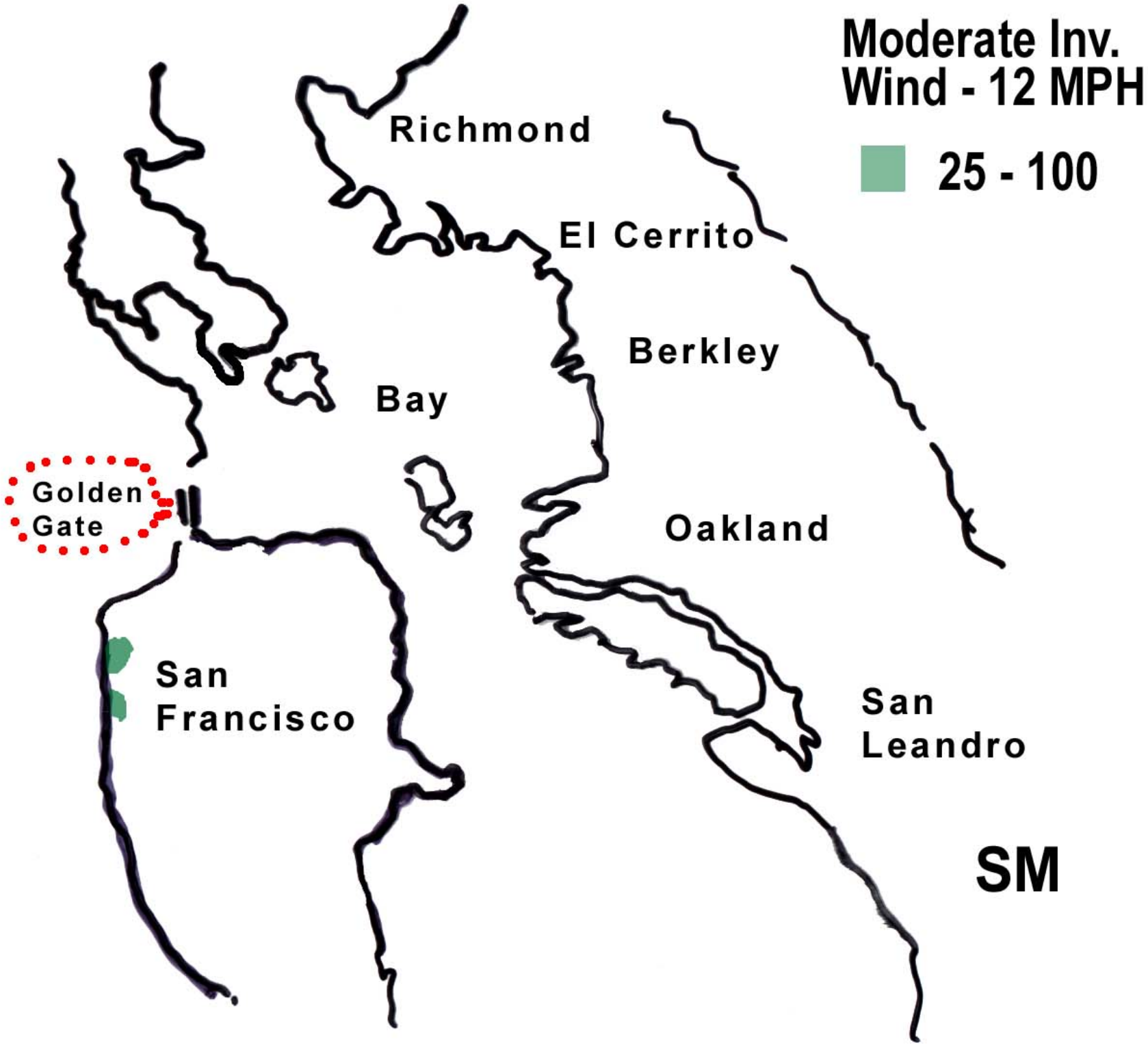

Richmond
El Cerrito
Berkley
Bay
Golden
Gate
Oakland
San
Francisco
San
Leandro

All Glass Impinger With Pre-Impinger




10 MPH Wind
Heavy Inv.
> 1000
100 - 1000
25 - 100
BG - 1
Richmond
El Cerrito
Berkley
Bay
Golden
Gate
Oakland
San
Francisco
San
Leandro


Richmond
El Cerrito
Berkley
Bay
Golden
Gate
Oakland
San
Francisco
San
Leandro


Moderate Inv.
Wind - 12 MPH
25 - 100
Richmond
El Cerrito
Berkley
Bay
Golden
Gate
Oakland
San
Francisco
San
Leandro
SM

Scenario: Attack on New York Subway System

- One of the most important vulnerability studies conducted by the former U.S. Offensive Program concerned the N.Y. subway system.

- A unique simulant BG powder was prepared that had good *secondary aerosol properties*

- Light bulbs were filled with the special powder. Three light bulbs (filled with a small amount of powder) were thrown onto the tracks from the rear car during passage through each subway tunnel.

- A total of 3 North/South tunnels were attacked.
- The BG quickly spread through each tunnel by passage of the trains over the powder.
- BG penetrated all test trains and remained in high concentration for 1.5 hours. Thereafter, the spore concentration in the subway cars dropped markedly and was not a factor after 2 hours.
- Risk of infection and exposure levels were shown to have been highest for personnel using the subway near the site of the powder dissemination and within the first hour after dissemination.

- Studies showed that the average time on the train during rush hour in AM and PM was 8 minutes.
- Studies also showed that in 1965 approximately one million workers used the subways daily in the mid-Manhattan business district to reach their work during rush hours.
- Less than one kilogram of dried anthrax would produce 50% casualties throughout the entire NY subway system.
- If ridership today of 1,000,000 passengers per day during AM and PM rush hours, it seems logical to conclude that 500,000 infections would occur.

- Since the window for initiating treatment for pulmonary anthrax is quite short, perhaps as many as 90% of infected patients would die.

- This level of deaths simply cannot be conceived…all deaths occurring in 3 to 5 days post attack.

Physics of Aerosol

Lou Dixon

And

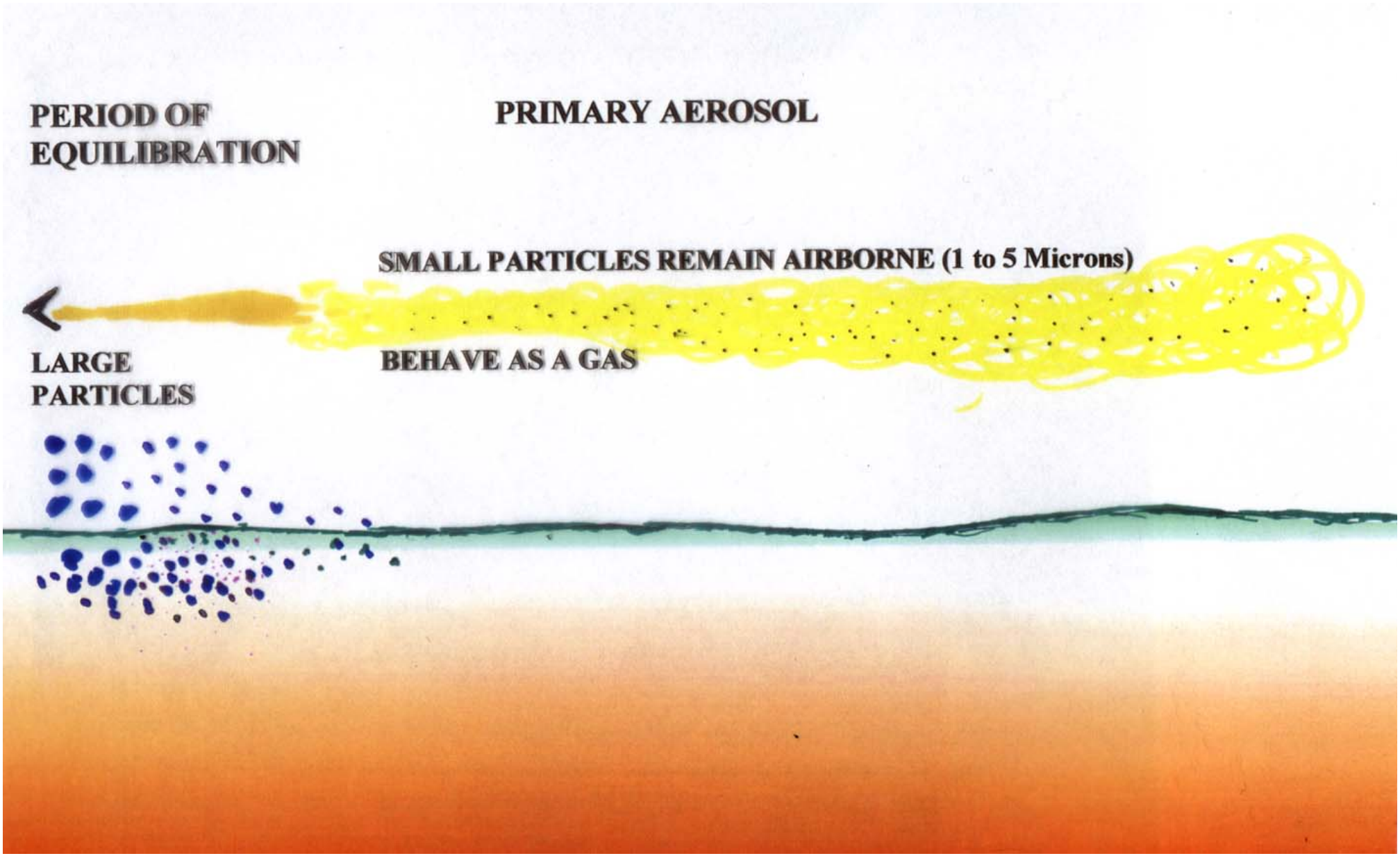
The Gas Mask

Mask Protection for Individuals

Type of Protection	Filter Efficiency (%)**
HEPA	**99.99**
Dust/Mist	**99.7**
Sub-Micron Surgical Mask	**96**
Handkerchief - 5 folds	**94**
Toilet Paper - 3 layers	**91**
Bath Towel - 2 layers	**85**
Cotton Shirt - 2 layers	**65**

NOISH estimates that leakage around the seals is the dominant factor. **0.3 micron particle

Physics Of Primary Aerosol



Man - Monkey - Guinea Pig: Influence of Particle Size on Tularemia Infectivity

Number of Tularemia Cells

Aerosol Particle Diameter (microns)	Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50}
1	2.5	14	10-52
6.5	4700	178	14-162

BG Simulant Tests: Interim Report 113*

When HRS-2 helicopters land in area previously contaminated by BG fallout from primary aerosol, there will be little or no contamination and personnel receive little or no respiratory exposure while moving through dust created by rotor movement.

* DTIC Recovery Number AD222-773

Physics of Aerosols

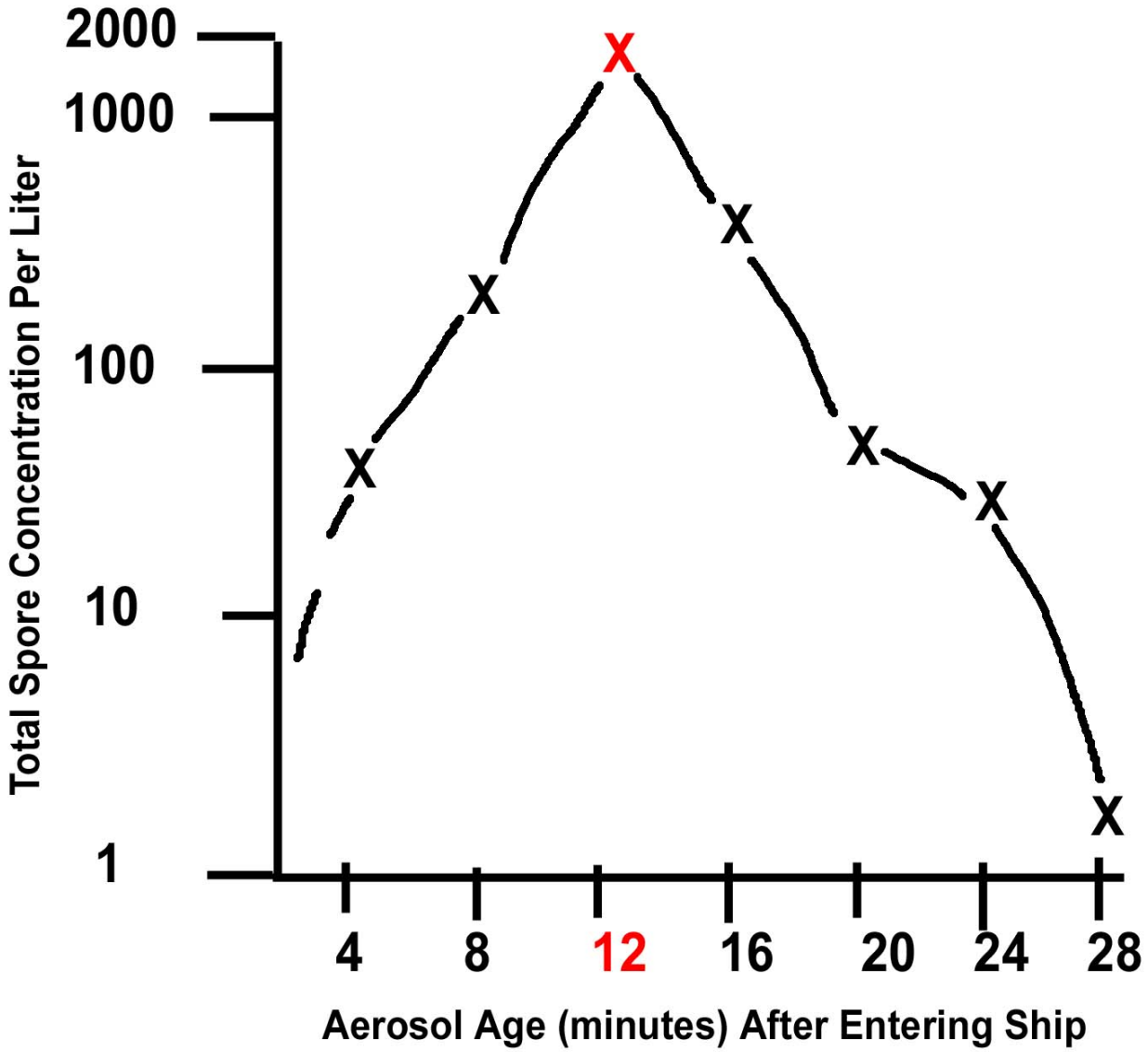
Residual Hazards Ref Primary Aerosols

- Copper Head Test in Arctic: Aero 14 sprayed simulate BG 20 miles upwind of Naval test ships
- Impinger samplers indicated large number of spores per liter of air in interior of ships
- Particularly heavy concentrations present in air circulating in engine rooms where air sucked in to dissipate heat

- Exterior and interior of ship surfaces showed marginal contamination

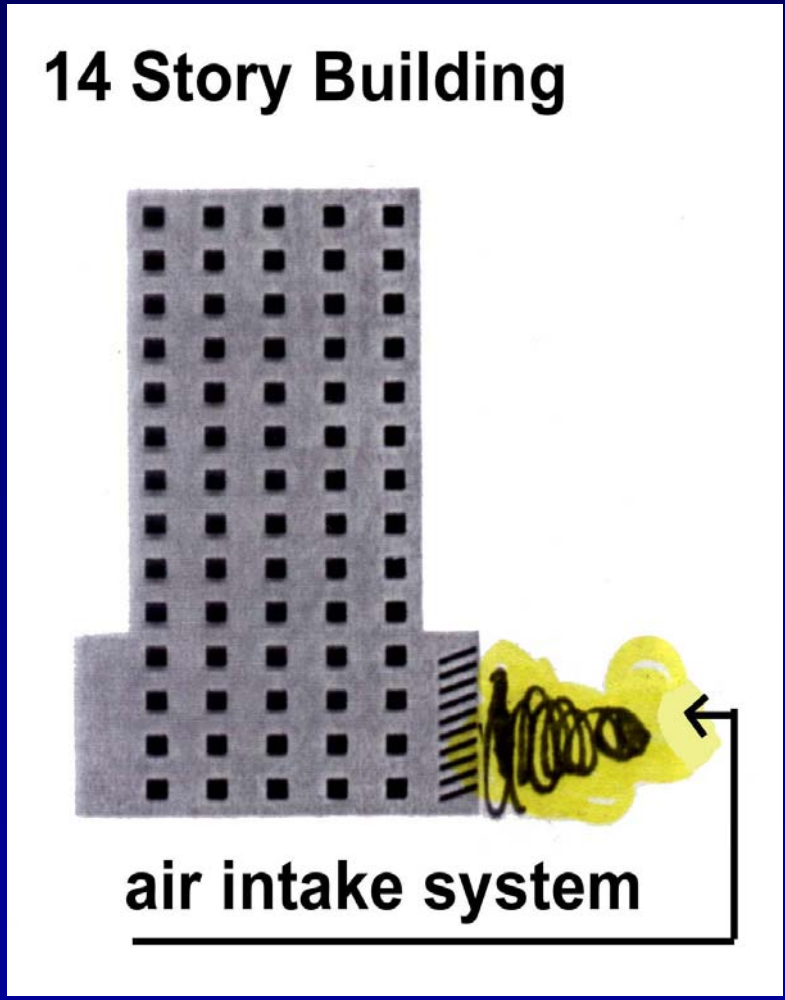
- Sea water wash was effective in removing the light concentration

Penetration of Destroyer by Primary Aerosol of BG Spores Released Up-Wind



Primary Aerosol Behaves As A Gas

- In 1960s the Federal Civil Defense Administration requested Ft. Detrick to assess the vulnerability of buildings to biological attacks
- Impinger samplers distributed throughout all floors
- Building contained 3 million cubic feet of air (84 million liters)



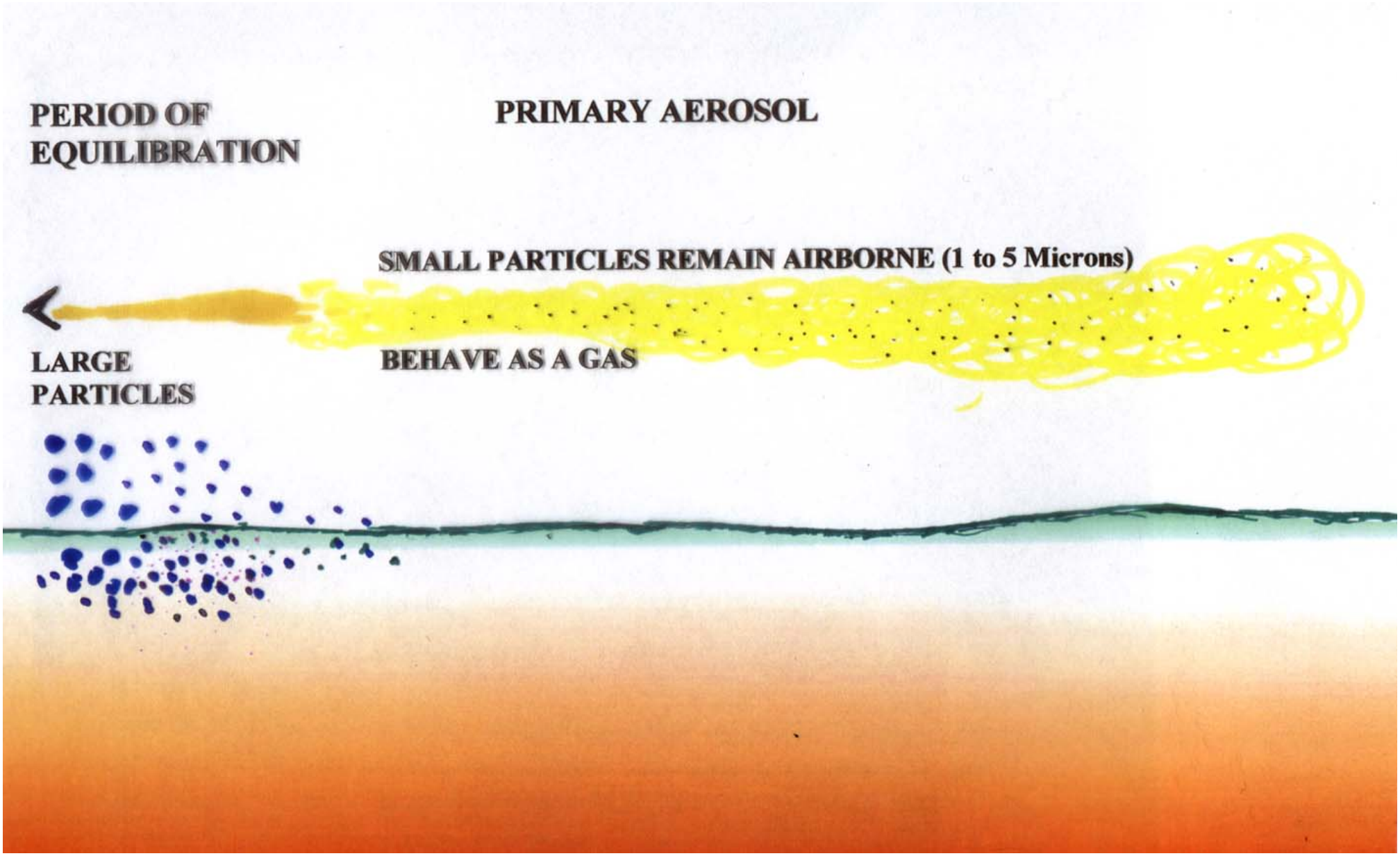
- After 2 hours, spores not detected in building air

- Spore concentration was extremely light on floors, walls and ceilings

Conclusion

Building air system brings primary aerosol into building and then removes it, leaving little or no evidence of its passage.

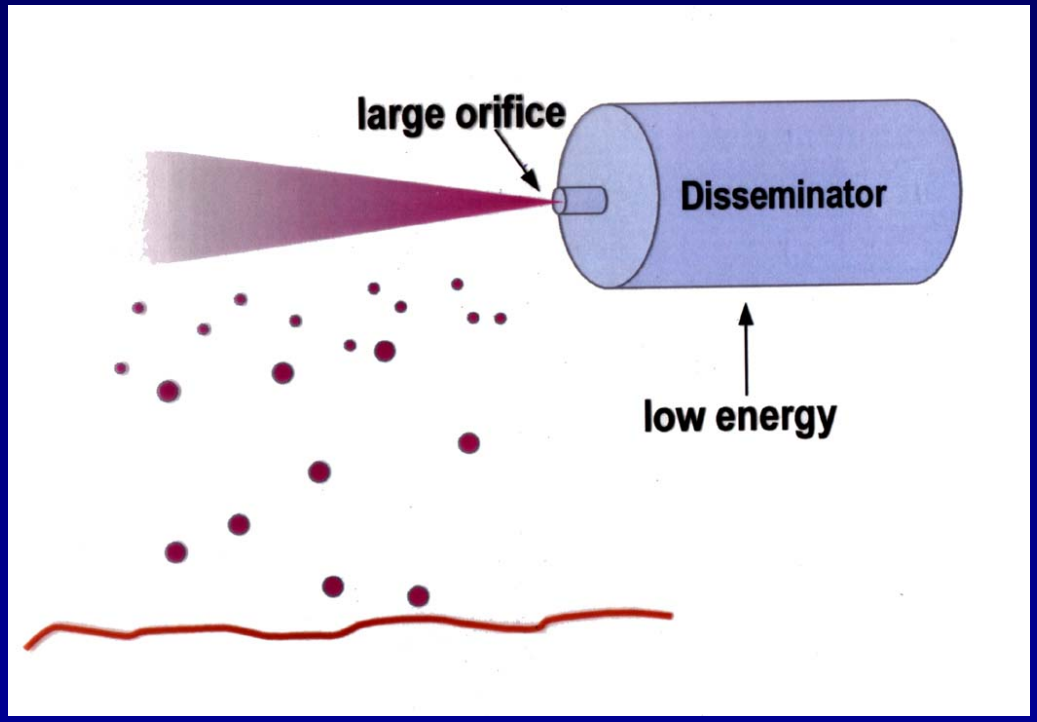
Physics of Primary Aerosol



Fate of Large Particles That Fall Out Of Aerosol During Equilibration

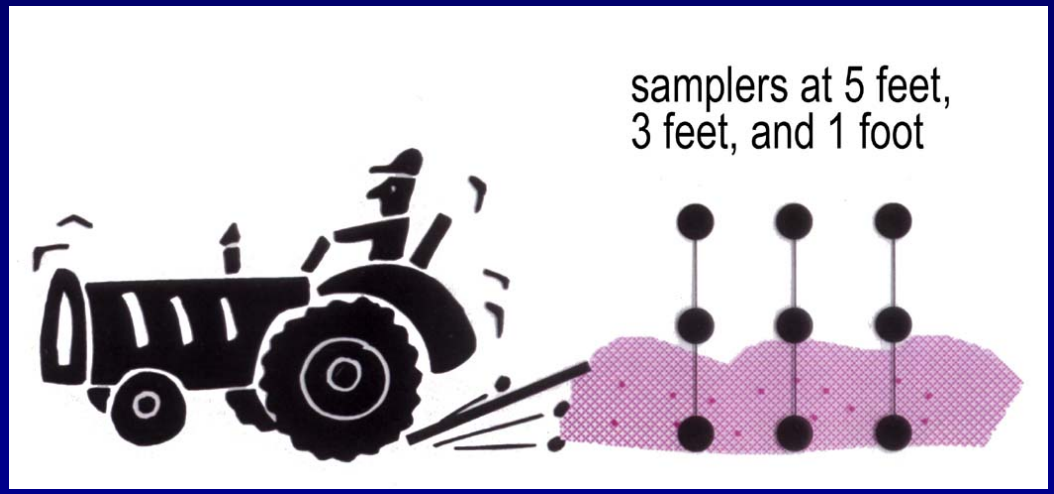
- In the 1950s, Wagner deliberately generated large particle aerosols to study terrain contamination and secondary aerosol relationships

- Disseminated 60 liters of BG slurry over a small grid



Efforts to Create Secondary Aerosol

- Wagner drove a tractor with large sheet of rubber beating the ground over BG-contaminated terrain creating lots of dust



- Sampled dust with impinger samplers located throughout the grid at 1, 3 and 5 feet above the ground, one hour and five hours after BG dissemination

Fallout of BG Spores During Aerosol Equilibrium: Sand

Concentration of Spores on Sand

Spores per Meter^2	Post Hours	1 Foot	3 Feet	5 Feet
$1x10^4$	1	67	2	1
	5	0	0	0
$6x10^7$	1	2150	62	22
	5	58	3	1

Efforts to Create Secondary Aerosol *(continued)*

- Wagner also sprayed BG slurry directly onto the terrain in order to achieve very high levels of contamination
- This method produced concentrations as high as 15 billion spores per square meter

Direct Spray of BG Spores Onto Sand

Concentration of Spores on Sand

Spores per Meter^2	Post Hours	1 Foot	3 Feet	5 Feet
$6x10^9$	1	158,000	3,250	3,180
	5	61,200	2,300	1,610
	12	34,000	3,100	286

Particle Size - Aerosol - Tularemia

Particle Diameter Microns	Number of Cells For Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50}
1	2.5	14	10 - 52
6.5	4700	178	14 - 162
11.5	23,000	672	No data
18	125,000	3447	No data
22	230,000	>8500	No data

Agent Particle Size

Therefore, agents that fall out during aerosol equilibrium are not of primary concern because:

- Many large particles are required to cause respiratory infection
- Strong adhesive forces between agent and terrain

Secondary Aerosols *(continued)*

- **Special BW agent powders can be prepared**
  - **Overcome adhesive forces and form good secondary aerosols**
    - Require special processing
    - Much more difficult to handle safely than ordinary dry agent

Show film that demonstrates

Primary and Secondary Aerosols

from Dry Powders and Liquid Agents

Estimate Human Anthrax Doses Airborne

Based on 20 grams *Bacillus Globigii* powder: Disseminated from smashed Christmas tree ball

Conc. Of Simulant (per gram)	x	Volume Airborne (grams)	x	% Volume in 1 to 5 (microns)	÷	Human LD_{50} Dose (spores)	x	% Lung Retention of Particles	=	Total Doses Available (time 0)
$800x10^9$		**13.8**		**50**		**8000**		**40**		**$2.76x10^8$**

- 276,000,000 infectious doses airborne represent a catastrophic level of contamination
- Based on this concept, $2.76x10^8$ doses would infect a building roughly the size of the former World Trade Center

Estimate Human Anthrax Doses Airborne

By Fanning Pool of Powder Following Smash of Christmas Tree Ball

Conc. Of Simulant (per gram)	x	Volume Airborne (grams)	x	% Volume in 1 to 5 (microns)	÷	Human LD_{50} Dose (spores)	x	% Lung Retention of Particles	=	Total Doses Available (time 0)
800×10^9		**5.1**		**35**		**8000**		**40**		**14×10^6**

- 14,000,000 infectious doses rendered airborne as a secondary aerosol; very serious level of contamination
- 33 HVAC systems would require closure to seal and isolate contamination, based on one HVAC per 150,000 cubic feet

The two previous experiments just shown were the types of studies we performed in 1965 that provided the basis of the New York Subway Trials in 1966.

AGENT SELECTION

Criteria for Potential BW Agents

- Pathogenic for humans (animals or plants)
- Cause a severe disability or lethality
- Highly infectious but generally not contagious
- Prophylactic and/or treatment measures generally available
- Infectious by the aerosol route
- Stable as a small particle aerosol
- Stable during logistical operations
- Readily and rapidly produced
- Weaponized in munitions and delivery systems
- Produce desired effects on the target

What Constitutes An Effective BW Agent?

- Many organisms that appear on "BW Lists" would be very difficult to weaponize.
- The properties of "The Disease" desired on the target do not necessarily reflect the inherent problems of weaponizing the agent.
- Two diseases can be illustrated:
  - Influenza virus, until recently (?) could not be stabilized with respect to virulence
  - *Yersina pestis*, frequently used in today's scenarios, is an extremely difficult organism to grow. It is difficult to stabilize virulence and decays rapidly in both logistics storage and as an aerosol.
- Both of these organisms require sophisticated programs and money to meet target requirements

What Constitutes An Effective BW Agent?

- In modern times (2004), a panel of BW experts was convened to discuss new potential BW agents.
- Hanta virus was one agent under consideration.
- The problem of growing this virus was discussed.
- Some of these experts concluded that growing this virus was not a problem.
  - The virus could be consistently grown to titers of $1x10^7$ infectious units per ml.
- This level of growth places a tremendous burden on the purification - concentration aspects of the process:
  - The process should increase concentration from 100 to 500 times over growth.
  - If agent stability is a factor, this increase in concentration becomes a significant problem.

What Constitutes An Effective BW Agent?

$$\left(\begin{matrix}\text{Product conc.}\\ \text{Per ml/gm}\end{matrix}\right)\left(\begin{matrix}\text{Vol. Of}\\ \text{1 ml/gm}\end{matrix}\right)\left(\begin{matrix}\text{\% Dissemination}\\ \text{efficiency}\end{matrix}\right) \div \left(\begin{matrix}\text{Human}\\ \text{RLD}_{50}\end{matrix}\right) \text{ To achieve } 1\text{x}10^{7} \text{ doses/meter}$$

- Agent disseminated under unfavorable conditions: URBAN TARGET, poor meteorological conditions, average decay rate (2.5% per minute)

Downwind Distance (km)	Line Source Strength: LD_{50} doses per meter				
	10^5	10^6	10^7	10^8	10^9
0.5	1.6*	15.2*	80.7*	100*	100*
1.0	0.5	5.2	41.2	99.5	100
2.0	0.1	1.4	13.1	75.5	100
4.0	0.0	0.3	1.2	11.7	71.3
8.0	0.0	0.0	0.2	2.0	18.3
16.0	0.0	0.0	0.0	0.0	0.3

*** % Infections at points downwind**

Botulinum Toxin: A Potential BW Agent Via Aerosol?

- Grows to concentration of ± $1x10^6$ $MIPLD_{50}$ per ml
- Purify and concentrate: alternate precipitation-reconstitution to yield 50% purity
- Spray Dry: Powder contains on average $4x10^9$ $MIPLD_{50}$/gm
- Disseminate one kilo over one kilometer as line source, good met conditions; no biodecay, Urban target
- Total Doses = $(5x10^9)$ (1000) (25) ÷ 14,000 = $9x10^7$
- Doses per meter = $9x10^7$ ÷ 1000 = $\underline{9x10^4}$

Distance Downwind	% Infections
500 meters	0.33
1000 meters	0.15
1500 meters	0.1

Why Did Bot Toxin Fail?

- Toxin is highly effective when injected into the gut or by the oral route
- Significantly less effective by the aerosol route
    - i.e. 1500 Mouse Gut Doses required for 1 (one) Mouse Aerosol Dose

U.S. vs. USSR: Dry Agent Production
(metric tons per year)

Agent	U.S.	USSR
SEB	1.9	0
Tularemia	1.6	1500
Q Fever	1.1	-
Anthrax	0.9	4500
VEE	0.8	150
Botulinum	0.2	0
Plague	0	1500
Smallpox	0	100
Glanders	0	2000
Marburg	0	250

A Final Word About Agents:

U.S. vs. USSR Agent Production Capabilities

U.S. vs. USSR
Dry Agent Production

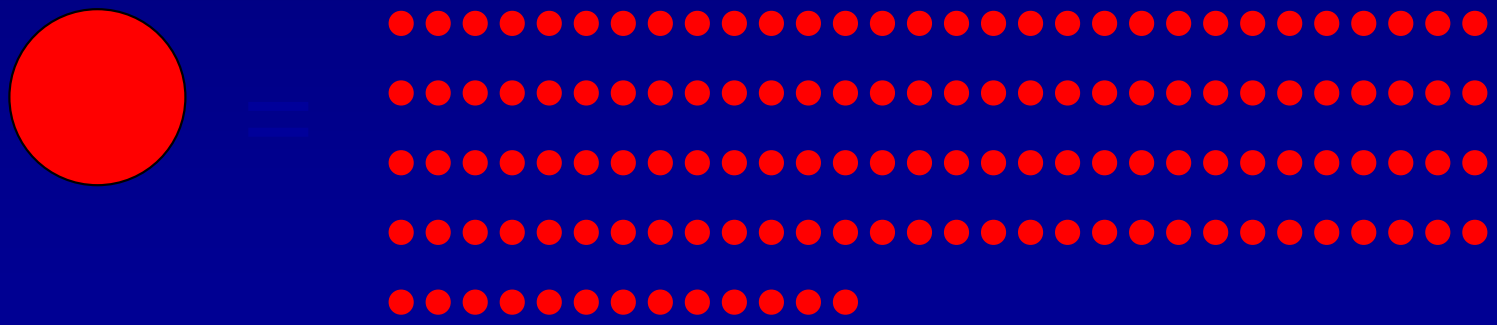
Agents

William C. Patrick III

Particle Size: Microns, Mass Median Diameter

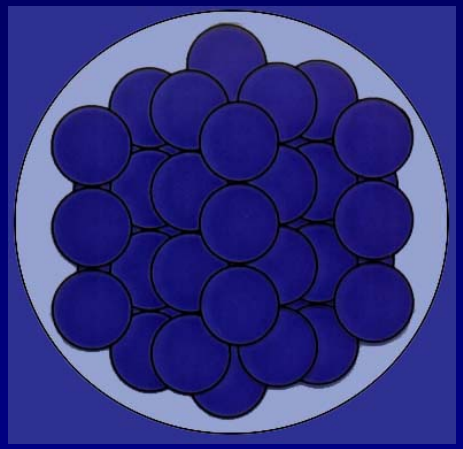
5μ

1μ



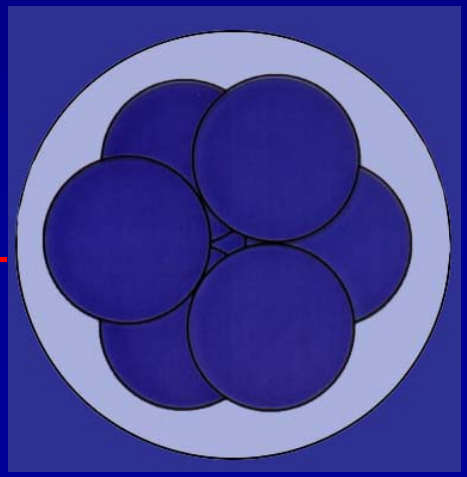
Size *DOES* Matter

- For successful weaponization, agent that can be disseminated into small particle aerosol must be developed
  - More efficient to place 53 one-micron particles in a 5 micron aerosol particulate than 15 two-micron particles in the five micron particulate



53 one-micron spheres in a five-micron sphere

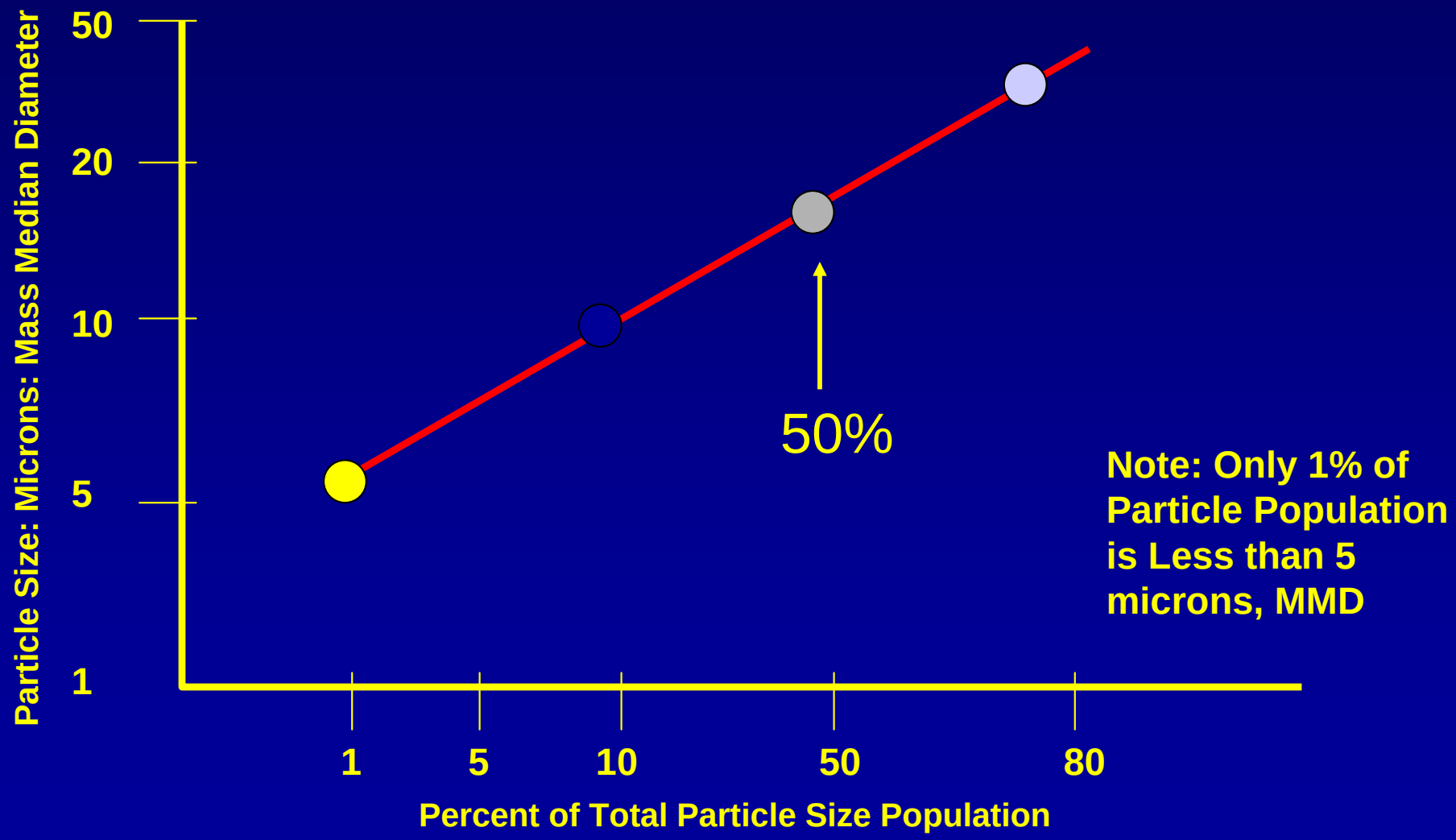
15 two-micron spheres in a five-micron sphere



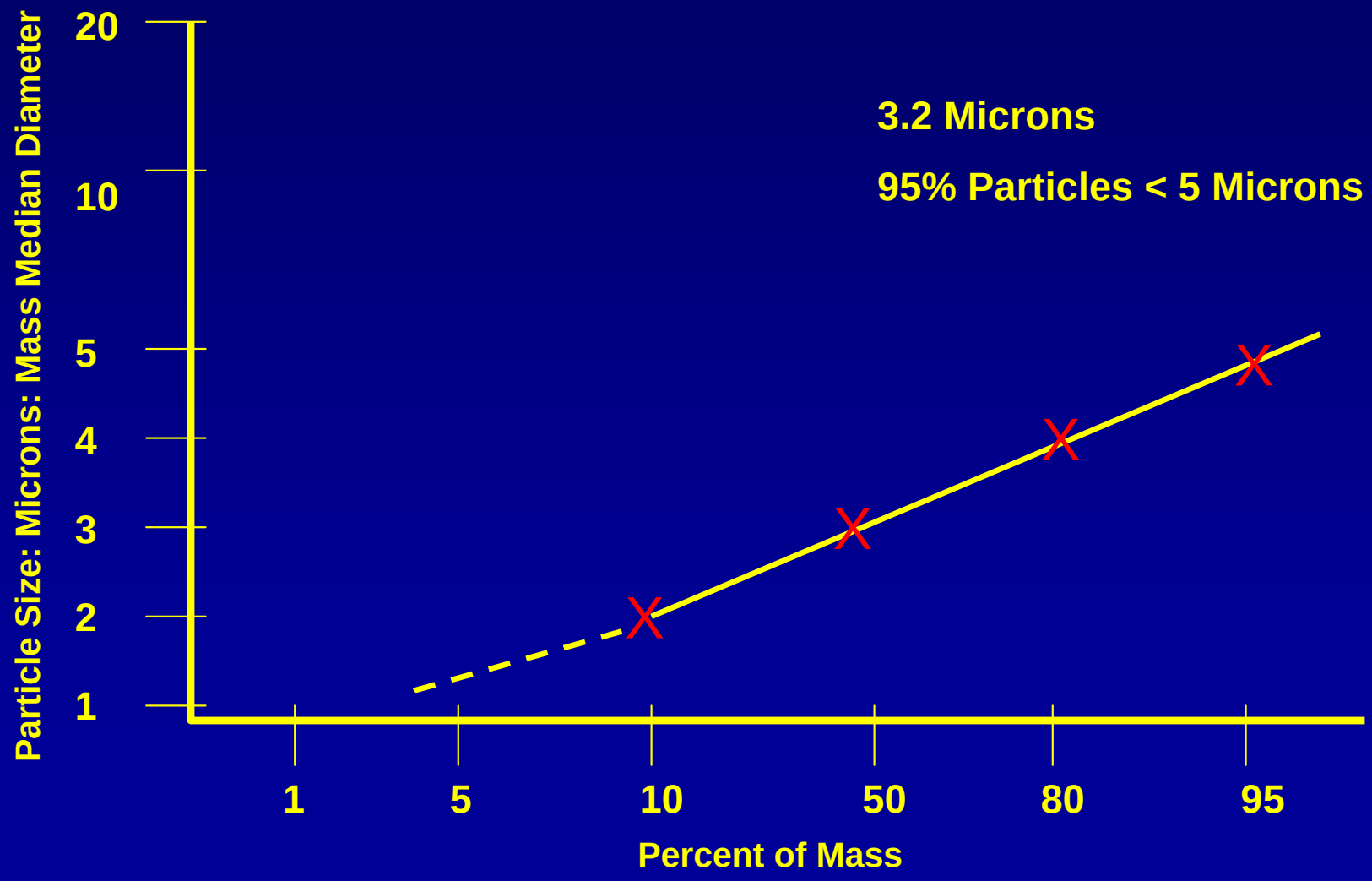
Influence of Particle Size on Respiratory Virulence of 5 Agents to Guinea Pigs (LD_{50})

Aerosol Particle Size (Microns)	*Bacillus anthracis*	*Francisella tularensis*	*Yersinia pestis*	Q Fever	VEE Virus
0.3 - 1.5	23,000	2.5	12,000	10^6	20
4.6 - 6.5	221,000	6,500	250,000	$52x10^6$	19,000
8.5 - 13	700,000	19,500	450,000	$>2x10^6$	280,000

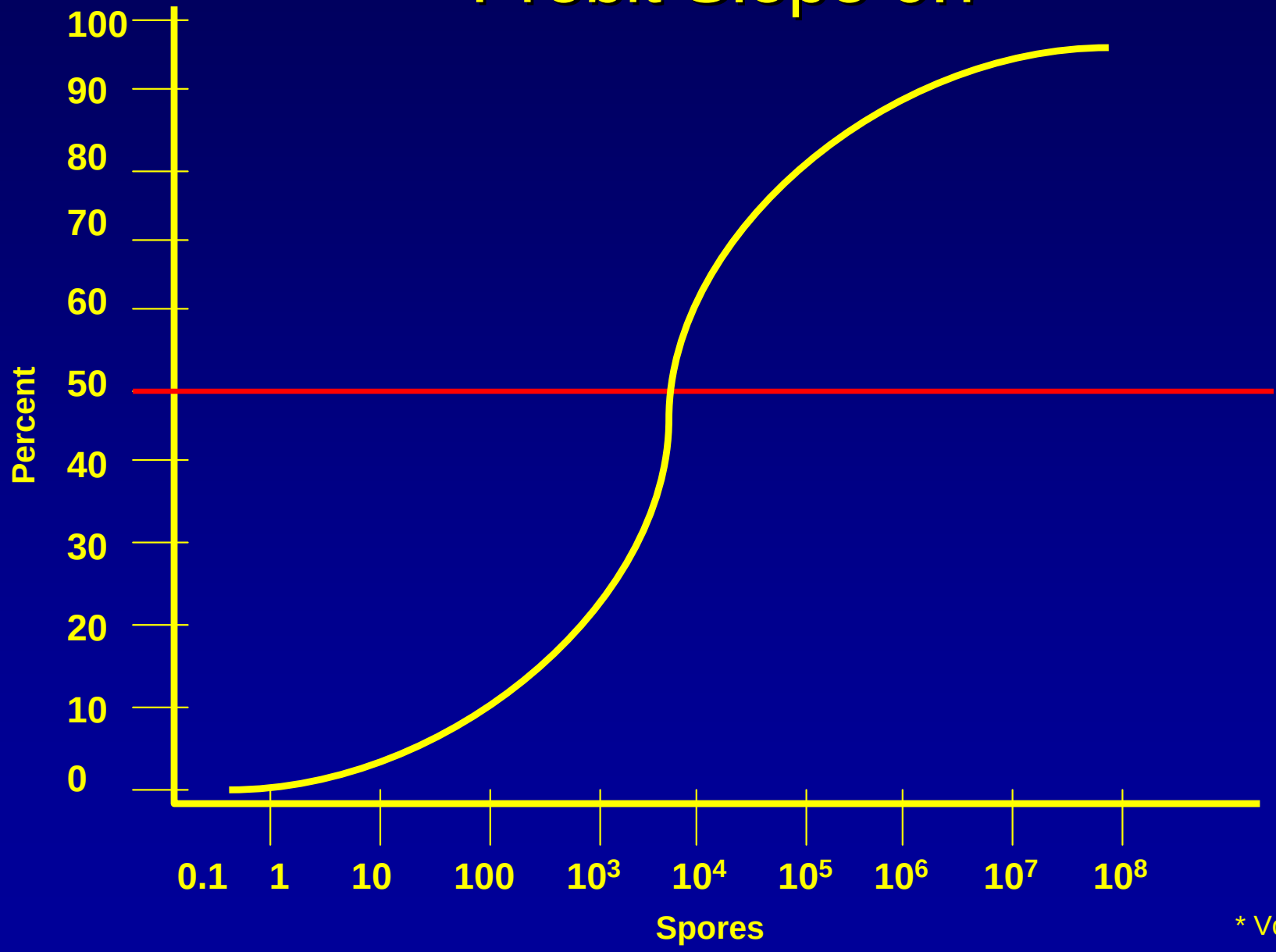
Particle Size Distribution of 18 Micron (MMD) BG Powder Using Whitby Centrifuge Technique



High Grade B.G. Powder



Anthrax*/Lethal Dose/Cyno Monkey
Probit Slope 0.7



* Vollum Strain

Human Dose: Vollum Strain: Cyno Monkey: Probit Slope 0.7

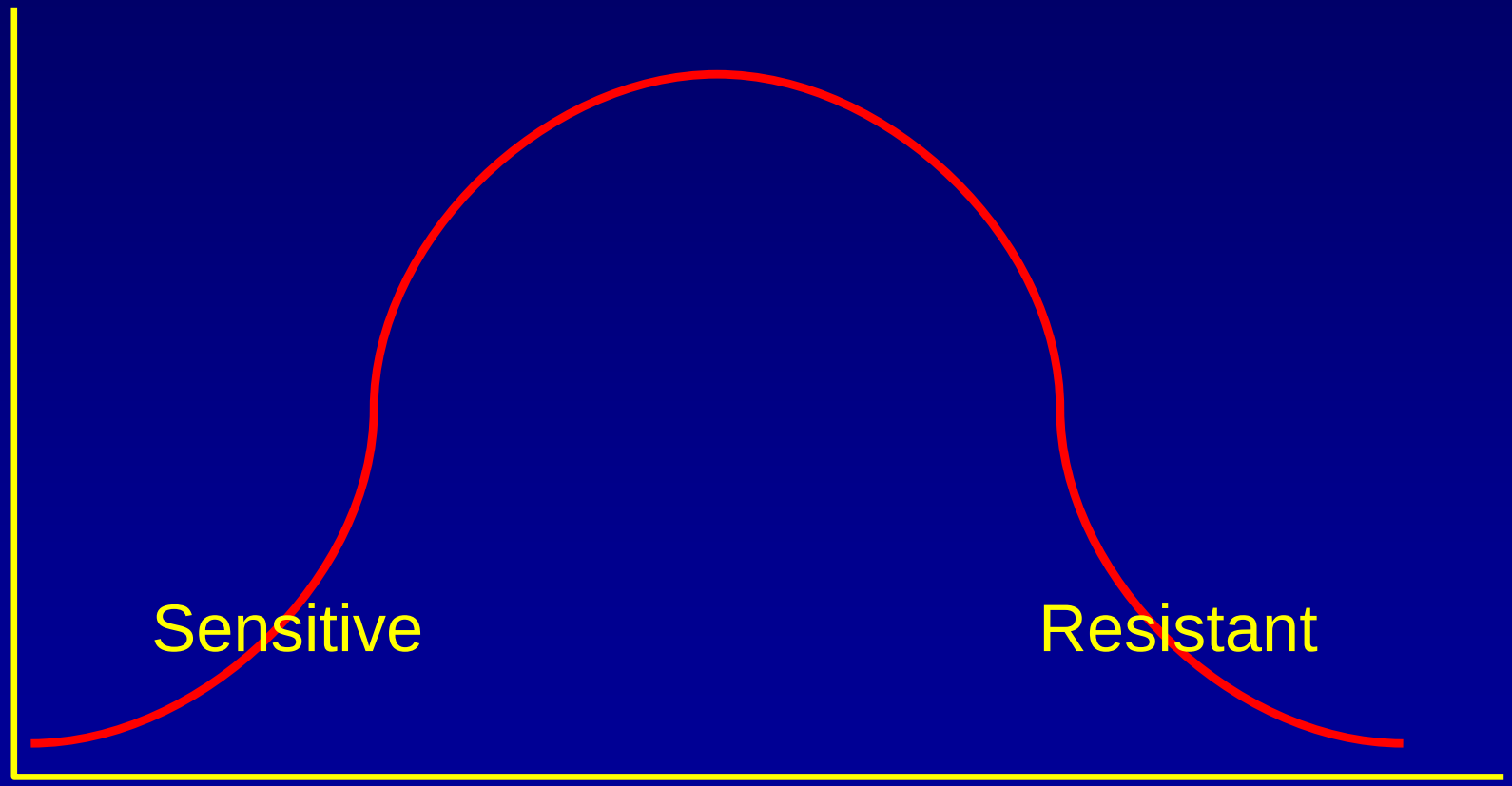
Lethal Dose	Number of Spores (Microns*)
10	120
20	500
30	1,400
40	3,500
50	8,000
60	18,000
70	45,000
80	130,000
90	540,000

*1 to 5 microns

RLD_{50} Anthrax Spores and Particle Size (Microns) For Man

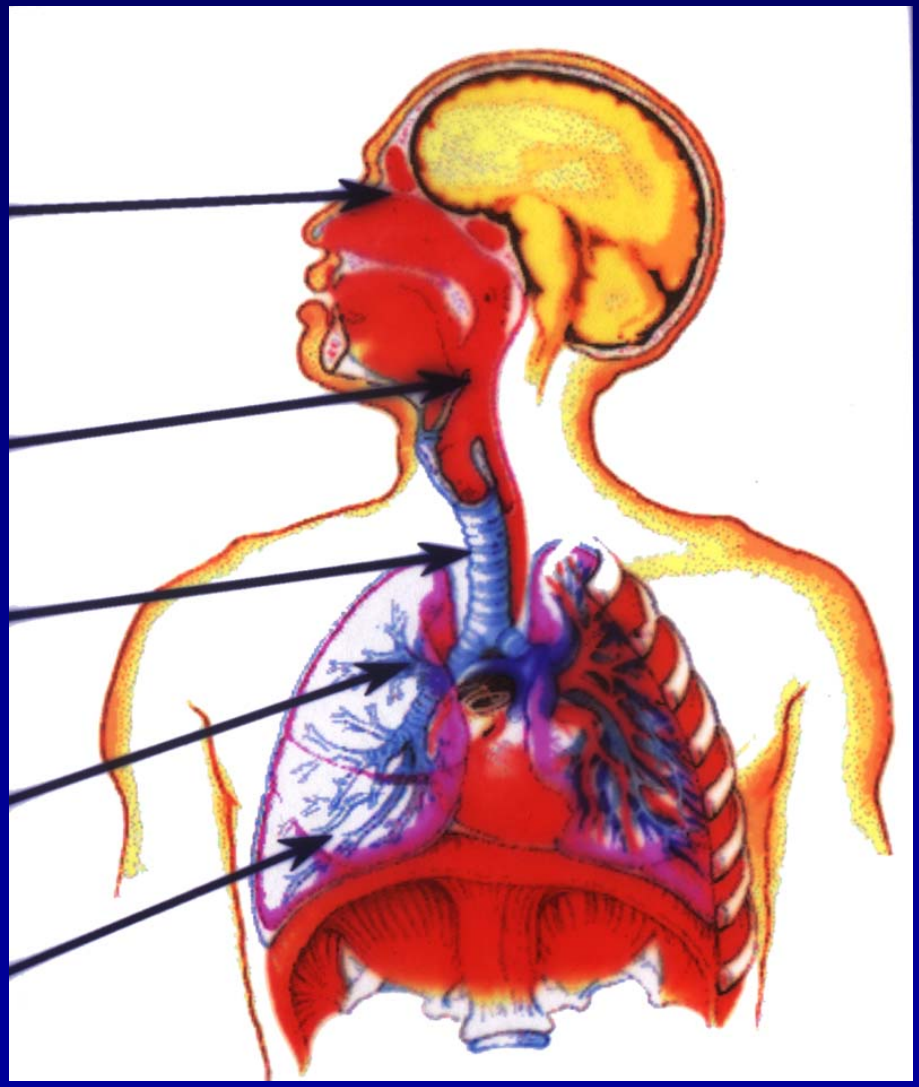
1 - 5	6.5 - 8.0	10 - 13
8,000	24,000	104,000

Bell Curve



Tularemia Aerosol, Particle Size and Type of Infection

	Particle Size (Micron, Mass Median Diameter)
18 - 20 micron particles fall out of aerosol, lodge in eye	**18 - 20**
15 - 18 micron particles lodge in pharynx	**15 - 18**
7 - 12 micron particles lodge in trachea	**7 - 12**
4 - 6 micron particles lodge in bronchiole	**4 - 6** Bronchioles
1 - 3 micron particles lodge in alveolus	**1 - 3** alveoli



Detrick Infections: 1943 - 1969 -- 456

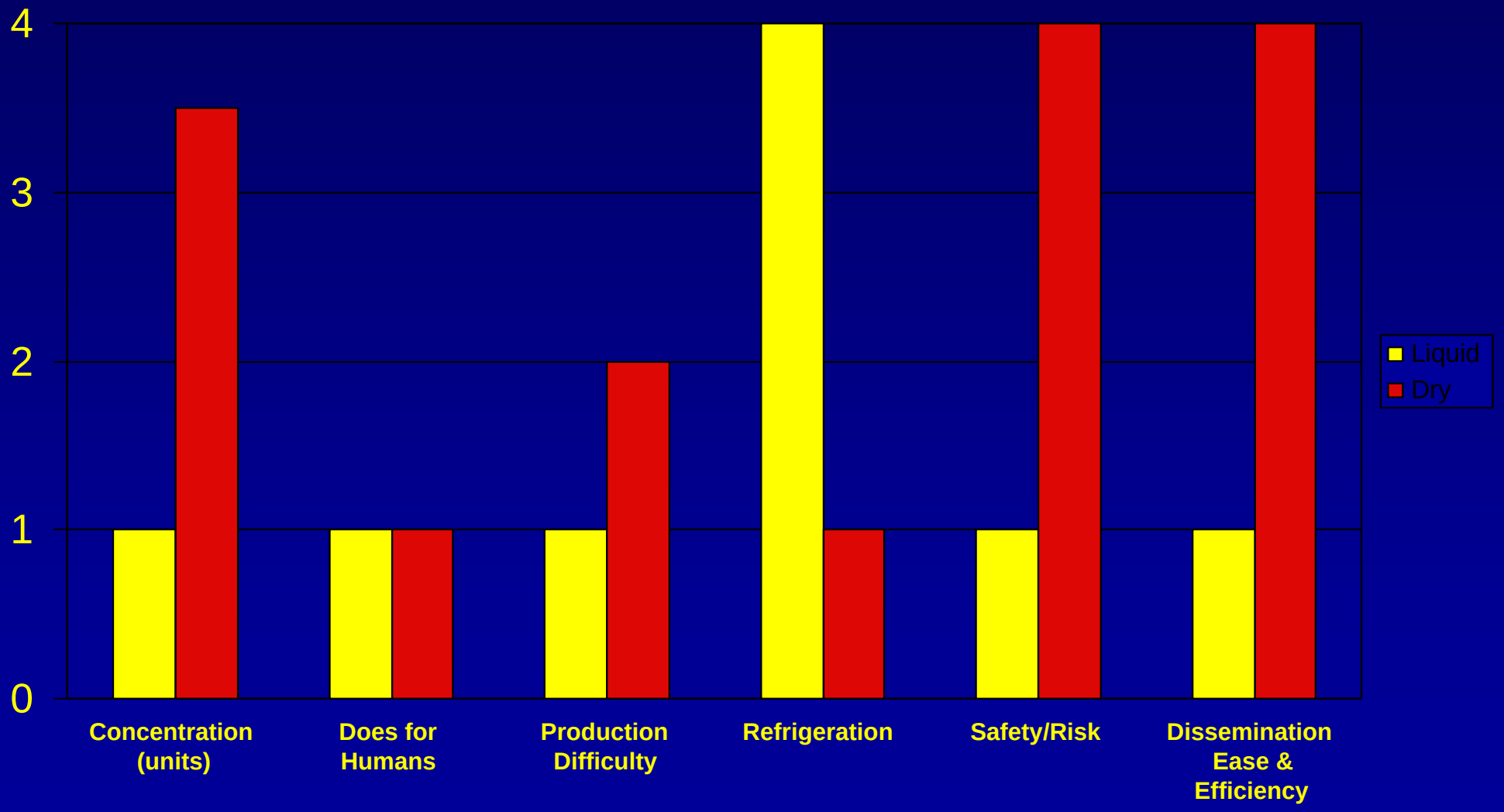
Tularemia*	153	Shigellosis	6
Brucellosis*	94	RMSF	5
Q Fever*	55	Newcastle	3
VEE*	43	BHF	1 **
Psittacosis*	32	Chikunguna	1
Anthrax*	31 **	Plague	1
SEB*	12	Salmonella	1
Coccidioidomycosis	9	Tuberculosis	1
Glanders	7	Blastomycosis	1

Bot Toxin* - 0

* Major Effort

**Lethal

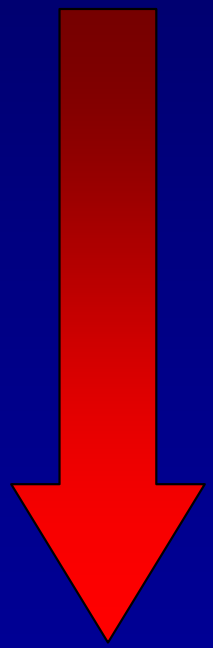
Liquid/Dry Agent Formulation Comparisons and Characteristics



Relative Aerosol Potency for Agents with BW Potential

Agent	Respiratory Dose For Man (micrograms)
Q Fever	0.000002
Tularemia	0.0001
VEE	0.0004
Anthrax	0.008
SEB	0.025
Botulinum A	4.8
Nerve Agent VX	8,000.00

Less weight = better infectivity



More weight = worse infectivity

U.S. vs USSR: Comparison of Agent Products (kilo per one km^2)

Dry Agent	U.S.	USSR
Anthrax	4	5
Tularemia	3	4.5
Q Fever	2	-
Brucellosis	6	8 - 10
VEE	4	6
Botulinum Toxin	85	>100
Plague	-	3
Smallpox	-	3
Glanders	-	5
Marburg	-	0.2 to 0.8

U.S. vs USSR: Dry Agent Production
(metric tons per year)

Agent	U.S.	USSR
SEB	1.9	0
Tularemia	1.6	1500
Q Fever	1.1	-
Anthrax	0.9	4500
VEE	0.8	150
Botulinum	0.2	0
Plague	0	1500
Smallpox	0	100
Glanders	0	2000
Marburg	0	250

Crude Liquid Slurry/Not Stabilized

5 ml Disseminated from Single Fluid Nozzle at 75°F, 50% RH: In Darkness*

	Conc. Per mil ($\times 10^9$)	Organisms Per Liter of Aerosol			
		4 Min	60 Min	120 Min	180 Min
Fresh Slurry at 0 Day	10	40,000**	2,000**	100**	6**
Monkey RLD_{50} (cells)		3	55	264	1370
Monkey doses per Liter		3333	127	-	-
Stored Slurry 4°C at 30 days	1	40	-	-	-
Monkey RLD_{50} (cells)		45	-	-	-
Monkey doses per Liter		0.88	-	-	-

* On overcast day - not bright sunshine, biological decay of tularemia is 20 to 30 percent per minute

**Biological decay for non-stabilized liquid tularemia in darkness is ±5% per minute

Tularemia Field Test in Marine Environment: Line Source Dissemination of Stabilized Liquid*

Aerosol Age	Aerosol Traveled	Virulence for Monkey	Biodecay Over 144 Min.
30 min	14.5 kilometers	11 cells	1.7%/min
144 min	67 kilometers	57 cells	

*Sampling station not available beyond ±67 kilometers

- Liquid Tularemia, when properly cultivated, processed and stabilized was shown to be an *outstanding* agent in Field Tests in Pacific (1964).

- Line Source dissemination, from high performance aircraft indicated 180 gallons could produce 50% infections over 9,000 miles^2

Realistic BW Agents & Common Misconceptions

	Bot A	Plague	Anthrax	Tularemia
Growth Conc. ($x10^9$)	0.001	35	1	35
Purification of Conc. ($x10^9$)	0.02	350	50	350
Dose for Human	14,000	3,000	8,000	50
Respiratory Dose (per ml)	143	1.2B	6.2M	7B
Logistical Stability	Fair	Poor	Outstanding	Good
Aerosol Stability	Fair	Poor	Outstanding	Good
Target (kilometers)	1	5	100,000	100,000

Botulinum Field Test: Horn Island; 10/28/1943

- 54 MK1 four-pound bombs filled with slurry
- Test grid contained stands of boxed Guinea Pigs
- Bombs were fired singly, then in combinations of 2, 3 and 4 bombs simultaneously
- None of the bombs, even in combination, killed a single Guinea Pig by inhalation

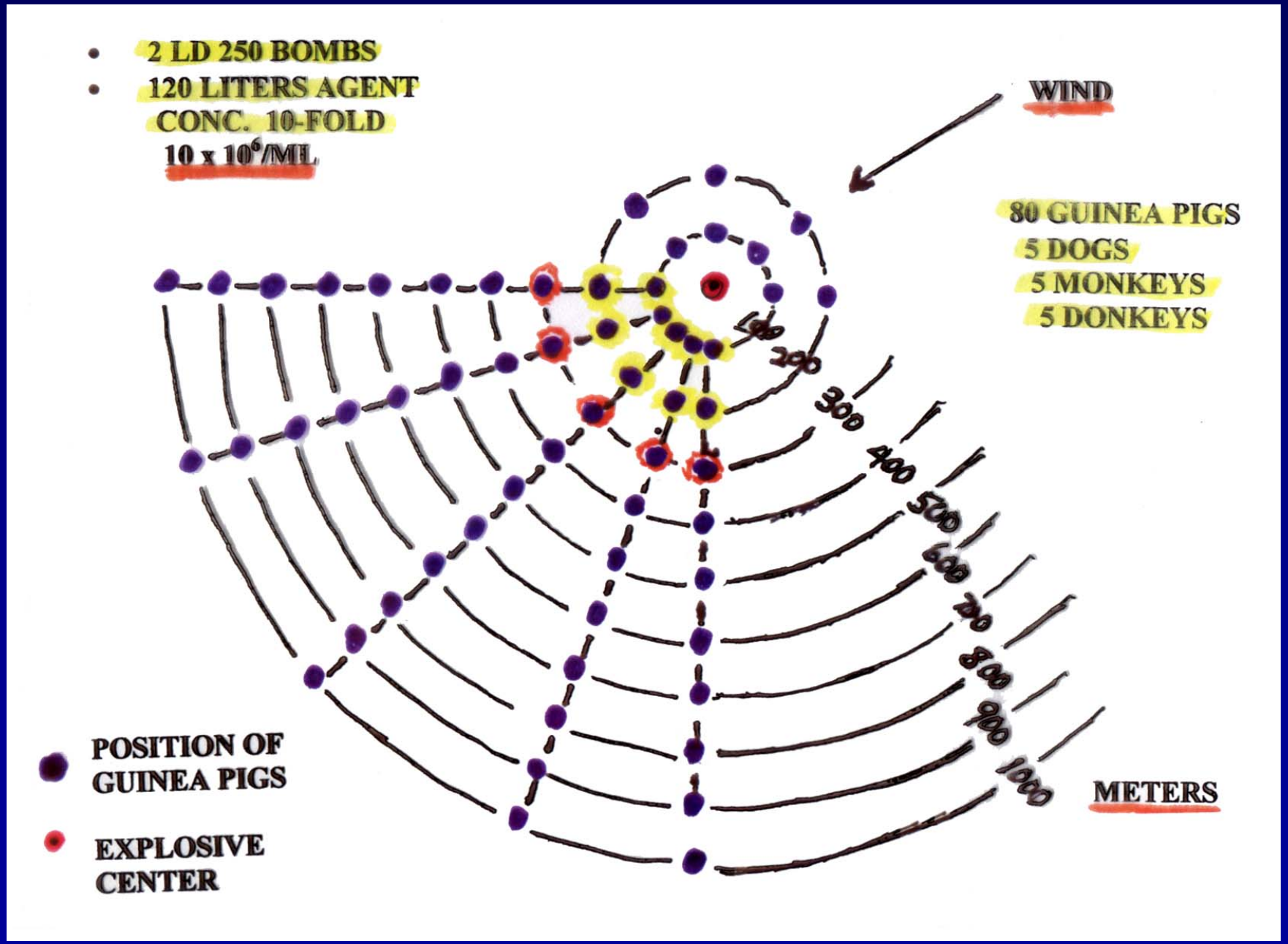
Botulinum Field Test: Horn Island; 10/28/1943 (continued)

- No trace of the toxin could be detected in the lungs during postmortem
- Only Guinea Pigs that died of Botulinum were those that licked the toxin off their own fur

CONCLUSION...

NOT AN EFFECTIVE LETHAL WEAPON

Iraqi Aerosol Test: Liquid Botulinum



Results of Iraqi Botulinum Aerosol Test

- **Guinea Pigs stationed at 100 and 200 meters downwind died TWO days post exposure**
- **Guinea Pigs stationed at 300 meters downwind became SICK but DID NOT DIE**
- **Guinea Pigs upwind of dissemination DID NOT BECOME SICK**

Results of Iraqi Botulinum Aerosol Test
(continued)

- **Monkeys, Donkeys and Dogs were not infected**

CONCLUSION...

The failure of 120 Liters to produce casualties only 200 - 300 meters downwind indicates that Botulinum Toxin is not an outdoor agent.

Pestis: LAB vs. PILOT PLANT

	Number of Cells for RLD_{50}	
	LAB	PILOT
Frozen Seed	3000 ±	3000 ±
25 ml Volume	3000 ±	3000 ±
200 ml Volume	3000 ±	3000 ±
12000 ml Volume	-	20,000
Small Seed Tank (15 gal)	-	800,000
Large Seed Tank		Not Done

Partial List of Organisms That Could Be Used in Oral Contamination

Organism	Growth Conc. ($x10^9$)	Effective Oral Dose (ED_{50})	Human Dose per mil
E. Coli -157.1 + >	40	2×10^1	2×10^9
Salmonella Quailes	30	1×10^7	6000
TY2-W	30	1×10^9	30
Meleagridis	50	4×10^7	400
Anatum	40	8×10^6	5000
Pullorum	20	$>1 \times 10^9$	±1
Shigella p.	50	50×10^9	±1
Brucella s.	40	1×10^6	40,000

Oral Dose (ED_{50}) In Volunteers*

Organism	Number of Organisms
Salmonella Anatum	6.5×10^7
Salmonella Newport	1.4×10^6
Salmonella Pullorum	1×10^9
Salmonella Typhosa	1×10^7
SEB	± 2.5 MCG
Shigella	1×10^8
Franciscella Tularensis	1×10^8

On average, these organisms grow to 35×10^9 cells per ml

* DTIC Recovery No. AD723-054

Contamination of Water Supply*

1. Salmonella Pullorum grows to conc. of $35x10^9$
2. Requires $1x10^9$ organisms to produce one ED_{50} (dose)
3. Therefore, 1 ml contains 35 doses or 0.028 ml per dose
4. Target: Reservoir contains $4.78x10^{10}$ gallons

Contamination of Water Supply* (continued)

5. Reservoir requires the addition of following GALLONS

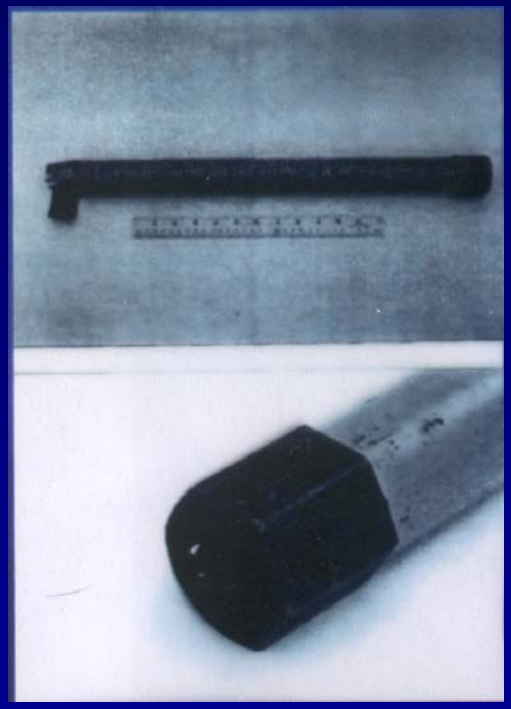
   Salmonella Pullorum 148,444 gallons

*Based on Ft. Collins, CO. City of ±100,000 people.
H_2O reservoir contains 150,000 acre foot of raw water
x 328,000 gallons per acre foot.

Dissemination

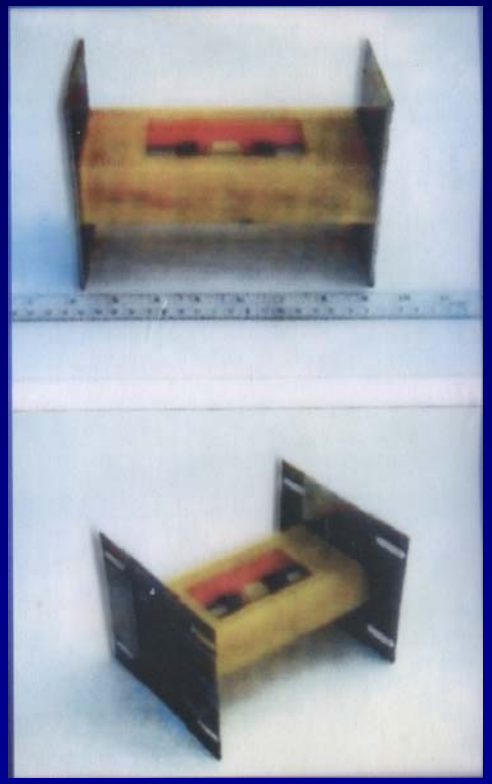
- Several means possible:
  - Aerosols most efficient
    - Droplets from liquid suspensions
    - Small particles from dry powders
- Insect vectors
- Contamination of food and water supplies

Examples of Point Source Bomblets



M114 Pipe Bomb

M143 Spherical Bomblet



Flettner Rotor Bomblet

Munitions (Terrorist)

Paint Sprayer

Trailed Sprayer

DRY

Leaf Blower

2-gallon garden sprayer

Munition Efficiency
(Aerosol Recovery)

- Defined as the number of organisms delivered airborne in the right particle size to cause respiratory infection

- Right particle size is 1 to 5μ, MMD

Munition Efficiency
(Aerosol Recovery) (*continued*)

- **Example: 1,000 organisms available**
  - **Munition efficiency is <u>one</u> percent**
  - **Only 10 organisms in aerosol available to cause infection**
  - **Other 990 organisms killed or in large particles that quickly drop out of aerosol**

Liquid Dissemination

- The generation of a small particle, infectious biological aerosol is a complex relationship between the device and the liquid
- This relationship is more complex for liquids than for powders
- For example, what is the disseminating efficiency of liquid tularemia using the line source tank the Aero 14 B tank?

Liquid Dissemination
(continued)

- **My response before providing an answer:**
  - What is the speed of the delivery vehicle?
  - What are the physical properties of the liquid; ie., viscosity, solids content, surface tension, etc.?
  - Is the agent stabilized?
- **These points will be described with experimental data**

Dissemination Efficiency of Dry Agent Powders

- Particle size and the absence of electrostatic charge are the important parameters that determine disseminating efficiency of the device (munitions)

- Quote from Don Falconer, Director of Munitions Development, former U.S. Offensive Program: "Dry agent (and suffering no loss of viability as a result of aerosolization) can be disseminated with efficiencies limited only by the proportion of the fill in the required particle size range."

Aerosol Particle Size and Infectivity

- Today's presentation will describe two important variables and how they interact to cause infections in primary aerosols
- Particle Size and Agent Concentration
- Much of the data to be presented are derived from the extensive studies of Dr. William C. Day, Experimental Aerobiology Division, Former U.S. Offensive BW Program.
- I had the privilege of working with Bill Day in that he requested my division, Product Development, to supply him with unique liquid and dry agents.

- **Bill Day made an extensive survey of particle size in the scientific literature while he was receiving his many immunizations around 1953.**
- **He found that lots of information was available on particle size in many different environments…office buildings, hospital wards, operating rooms, dental offices and even sewage disposal plants.**
- **These extensive studies indicated that in ambient air, the average particle size that contained viable organisms was 12 to 13 microns, MMD.**

- **Only a small fraction of small particles, less than 5 microns, was found in the ambient air.**
  - **and those particles less than 5 microns contained only a few viable organisms.**
- **From these studies it could be inferred that MOTHER NATURE does not usually create small particle highly infectious aerosols.**
- **If she did, perhaps we would not have survived as a species.**

- It is the artificial manipulation of agents to create small particle infectious aerosols that should cause real concern.

- Mother Nature simply does not effectively address those laboratory procedures and protocols found in the laboratory ...

Blending **Centrifugation**

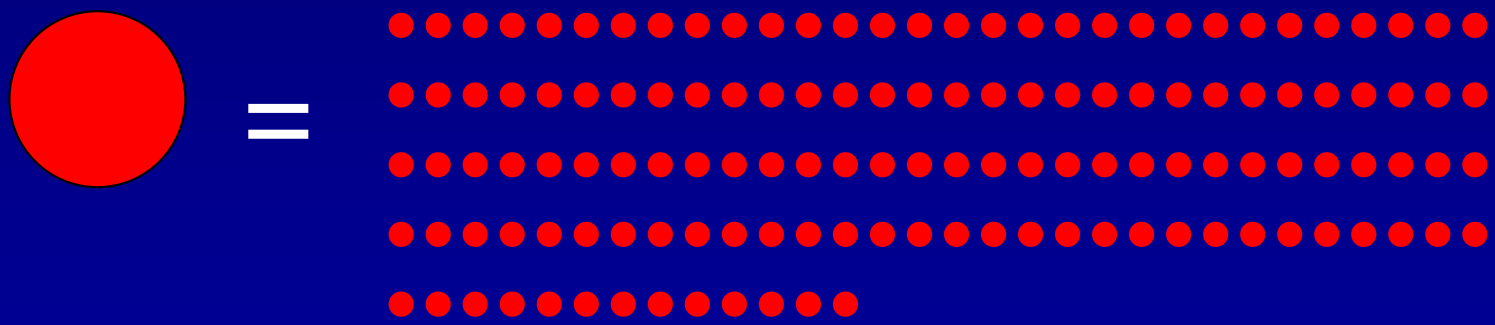
Manipulation of small particle dried agent powders

- It is these types of laboratory operations that produce the majority of infections via the respiratory tract.

Particle Size: Microns, Mass Median Diameter

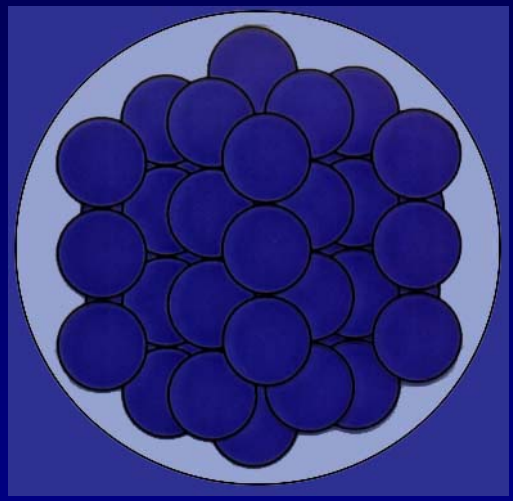
5μ

1μ



Size *DOES* Matter

- For successful weaponization, agent that can be disseminated into small particle aerosol must be developed



53 one-micron spheres in a five-micron sphere

  - More efficient to place 53 one-micron particles in a 5 micron aerosol particulate than 15 two-micron particles in the five micron particulate

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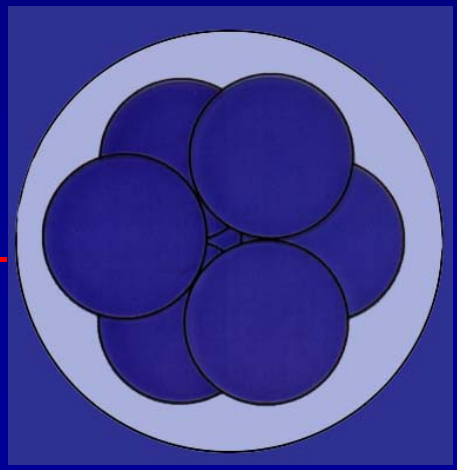
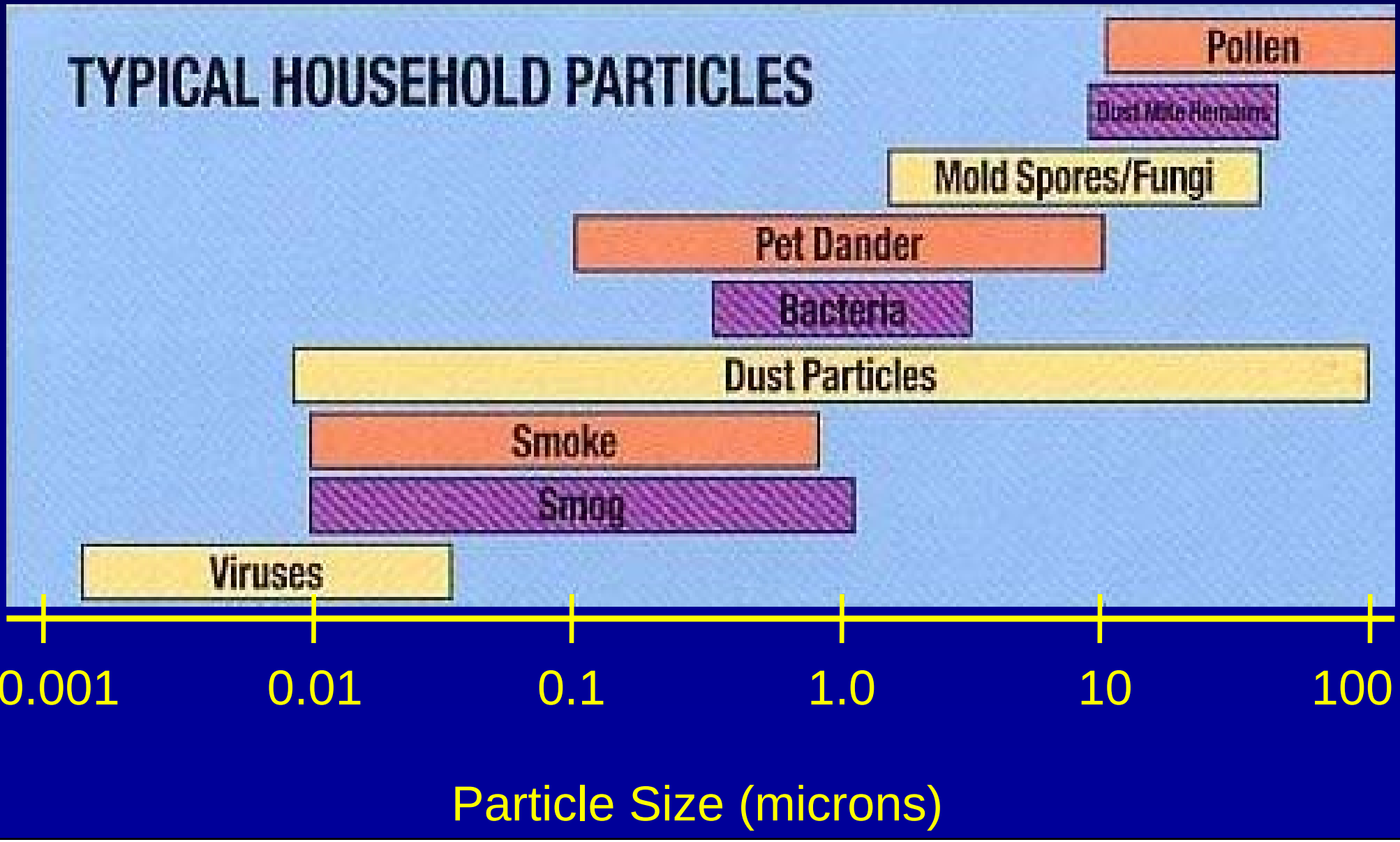


Comparison of Particle Size



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4.6 - 6.5	221,000	6,500	250,000	$52x10^6$	19,000
8.5 - 13	700,000	19,500	450,000	$>2x10^6$	280,000

Particle Size and Infectivity

- Information on how organisms behave during dissemination and as aerosol was sparse or fragmented in early years of U.S. Offensive Program

- Scientists at then Camp Detrick invented science of “aerobiology”

Particle Size and Infectivity

(continued)

- **Early aerosol studies frustrating**
  - **Exposure of animal models to infectious particles produced inconsistent results**
  - **Program did not advance until disseminators with sharp particle-size profiles selected**

Three Disseminators

Particle Size Distribution

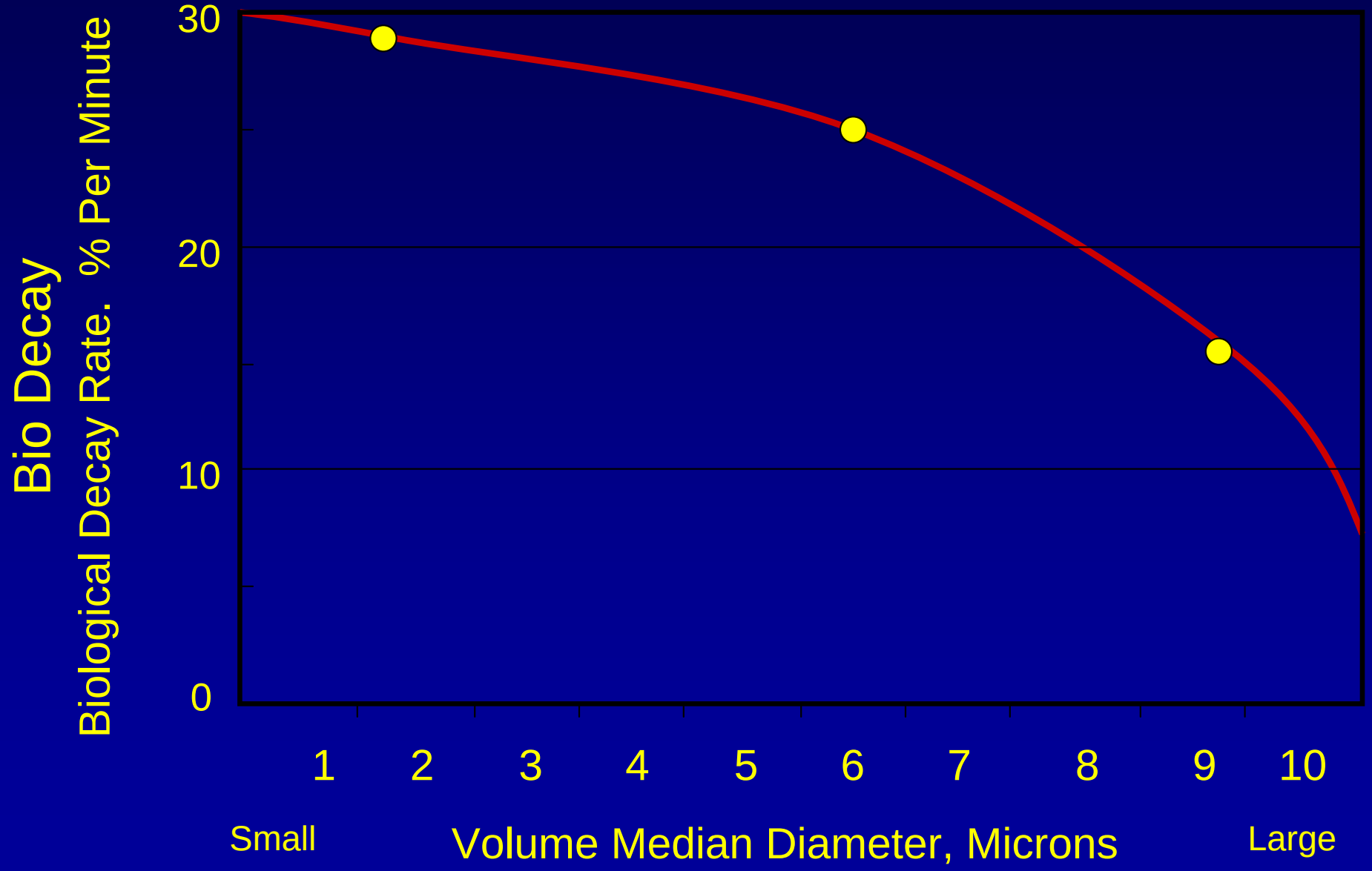
Particle Range (Microns)

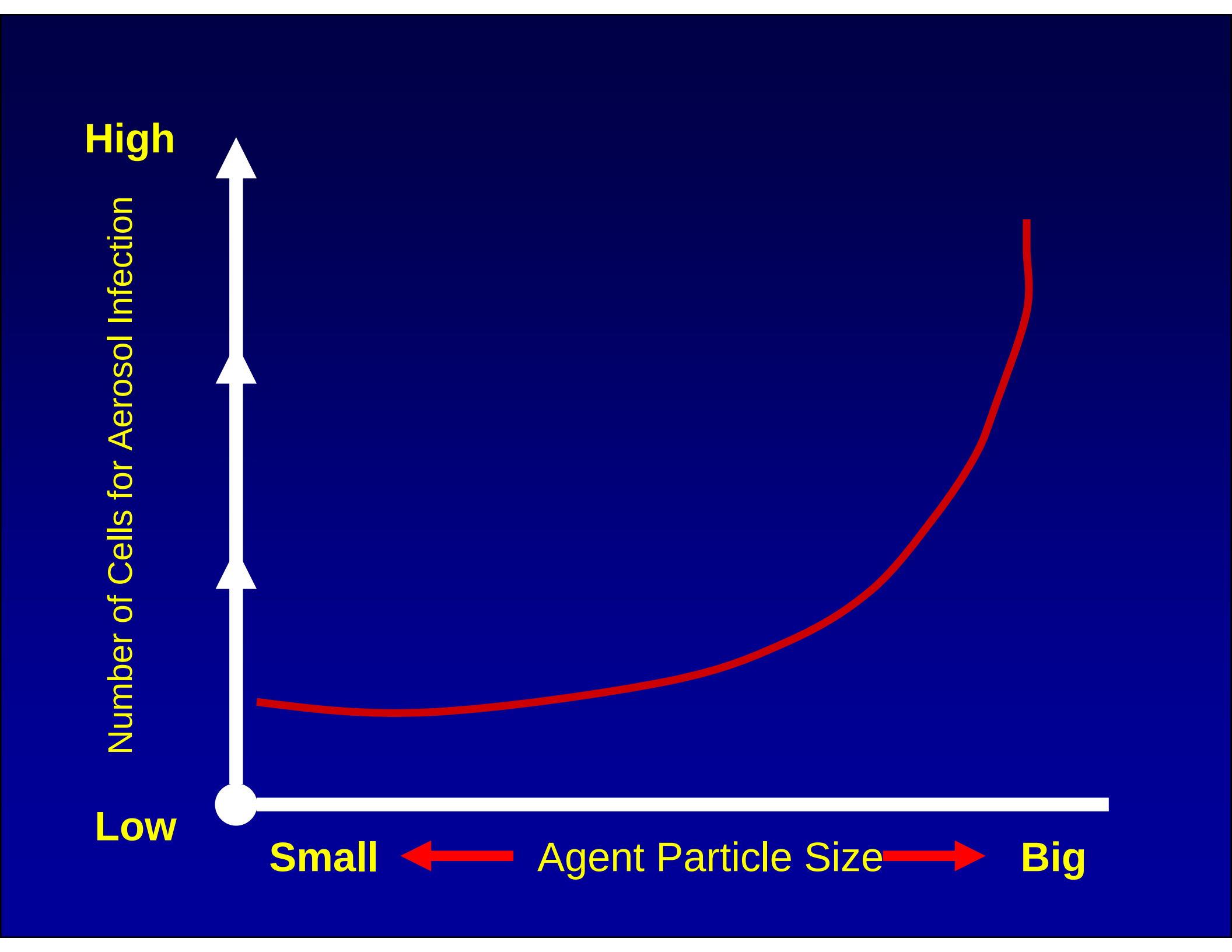
Disseminator	1-1.9	2.0-5.3	5.4-10	10.5-15.0
Vaponefrin Nebulizer	5842	516	0	0
Collison Atomizer	4145	1266	0180	6
Spinning Disc	0	0	3432	180

Table 4: Relationship of Aerosol Particle Size Distribution to Respiratory LD_{50} Values for Rhesus Monkeys Obtained with *P. tularensis*

Aerosol Particle Size (microns)	Aerosol Particle Diameters Defined in Microns											Monkey Respiratory LD_{50} (cells)
	1.4	1.9	2.7	3.8	5.4	7.6	10.8	12.5	17.6	24.9	35.0	
1.0	52.2*	24.9	13.3	6.4	1.4	0.4	0.2	0.0	0.0	0.0	0.0	14
6.5	0.0	0.0	0.0	0.3	4.8	85.4	9.5	0.0	0.0	0.0	0.0	178
11.5	0.0	0.0	0.0	0.0	0.0	0.5	7.8	83.8	7.0	1.0	0.0	672
22.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	3.3	82.6	13.8	3447

Particle Stability




High
Number of Cells for Aerosol Infection
Low
Small
Agent Particle Size
Big

Dry SM: Particle Size, Viable Cells per Particle, Viable Cells per 1000 Particles

NMD μ	Cells per Particle	Viable Cells per Particle	Viable Cell Frequency/1000 Particles
0.8	1.8	0.001	0.5
1.3	4.2	0.01	2.6
3.0	18.0	0.2	15.6
6.5	73	2.5	38
11.5	195	7.7	14
16.0	350	11	6
23.0	670	16	3

Classical Experiment: Man – Monkey – Guinea Pig:

Influence of Particle Size on Tularemia Infectivity

Aerosol Particle Diameter (microns)	*Number of Tularemia Cells for:* Guinea Pig RLD_{50}	Monkey RLD_{50}	Man RID_{50} Mean	Man RID_{50} Range
1	2.5	14	15	10 – 52
6.5	4,700	178	88	14 – 162
11.5	23,000	672	130*	—
18	125,000	3447	10,000*	—
22	230,000	>8500	No Data	

* Data from Dr. Bill Sawyer

Influence of Aerosol Particle Size on Severity of Illness in Monkeys

Aerosol Particle Size (microns)	Number of Cells	Mean Day of Illness (Post Exposure)	Severity of Illness	Fever (°F)	Death
1	14	4	5+	105+	Yes
6.5	178	6	5+	104-105	Yes
11.5	672	9	3+	103-105	Yes/No
18	3447	15	2+	102-103	Maybe
22	>8500	22	1+	101-102	No

Volunteer Study with Tularemia: Severity of Infection

Number of Cells	Days Incubation (Post Exposure)	Fever (°F)	Percent Infected	Numerical Rating
26	4-5	103	86	4+
30	4-5	103	85	4+
38,000	3	105	100	5+
52,000	2	105	100	5+

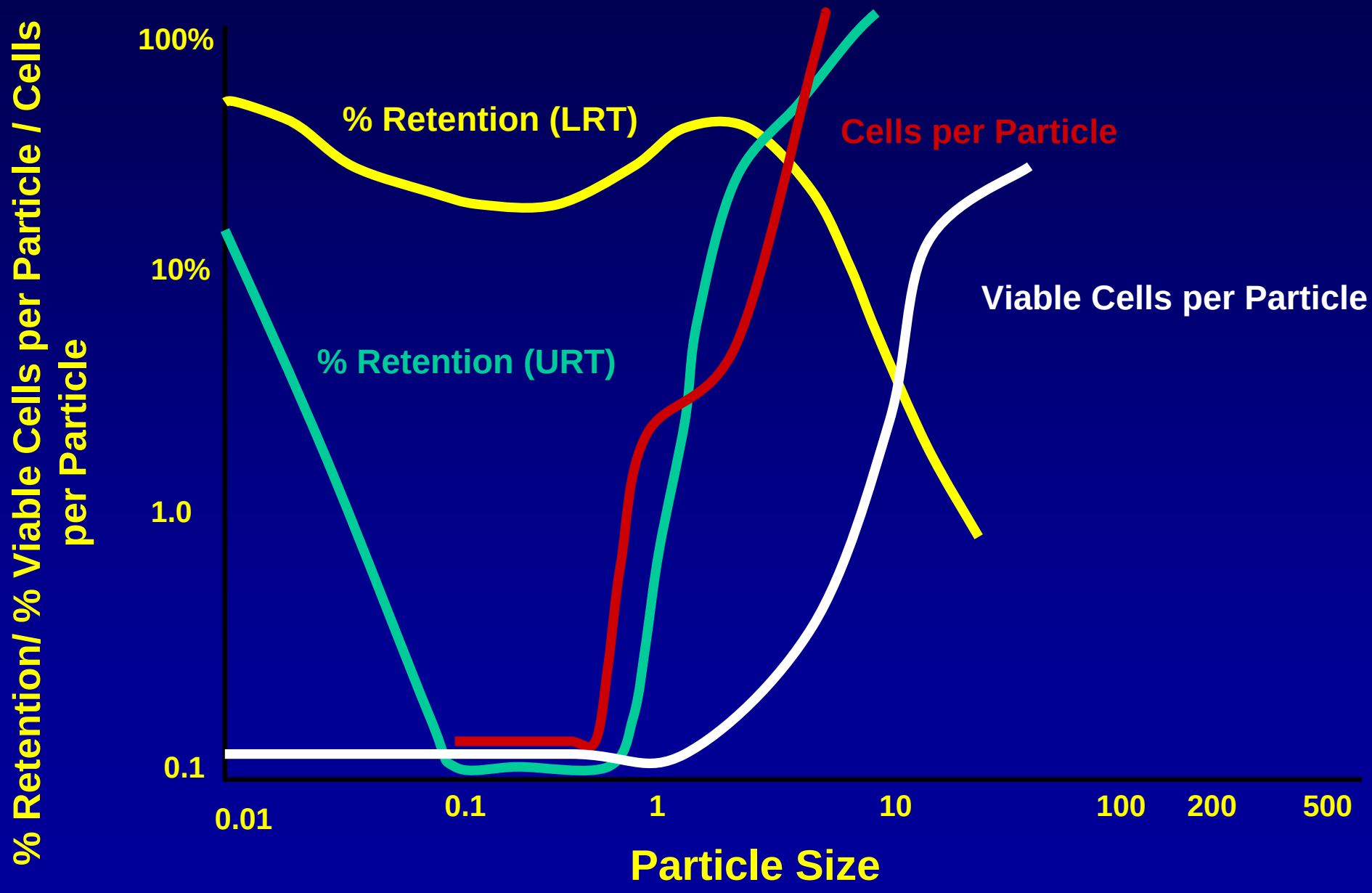
Influence of Aerosol Particle Size on Development of Lung Lesions in Monkeys
(Time Following Exposure)

Particle Size (microns)	Appearance of Lesions on Lungs (hours following exposure)
1	24 hours
8	48 hours
11.5	96 hours

Particle Size, Spore Concentration, Lung Retention: Anthrax / Guinea Pig

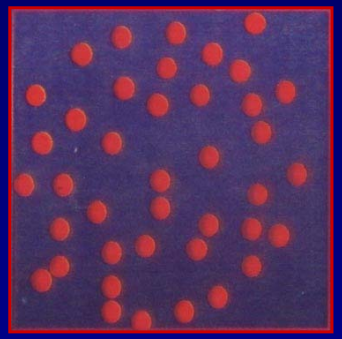
Aerosol Size (m)	Conc./ml $x10^8$	Calculated Inhaled Dose	Viable Spores Retained	Percent Retained
1	5	1×10^4	4×10^2	2.5
1	50	20×10^4	4×10^4	21
1	100	40×10^4	17×10^4	43
5	5	8×10^4	3×10^2	0.4
5	50	91×10^4	5×10^4	6
11	50	89×10^4	5×10^2	0.06
11	500	720×10^4	4×10^4	0.54

Influence of Aerosol Particle Size on: % Retention in Lower and Upper Respiratory Tracts; % Viability of SM; SM Population Density

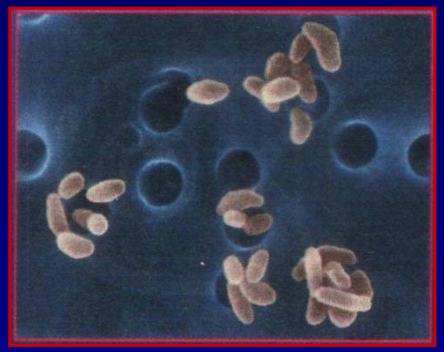


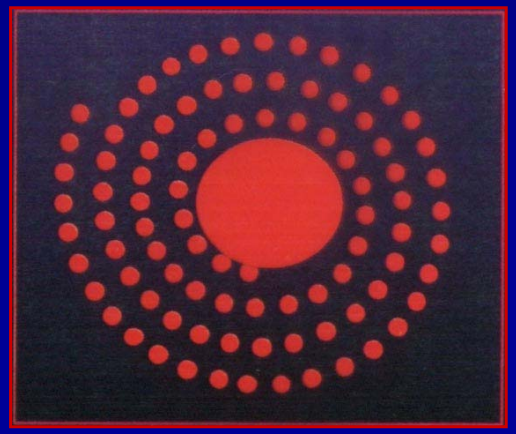
Anthrax Spores vs. Tularemia Cells in Aerosol

SPORES



CELLS





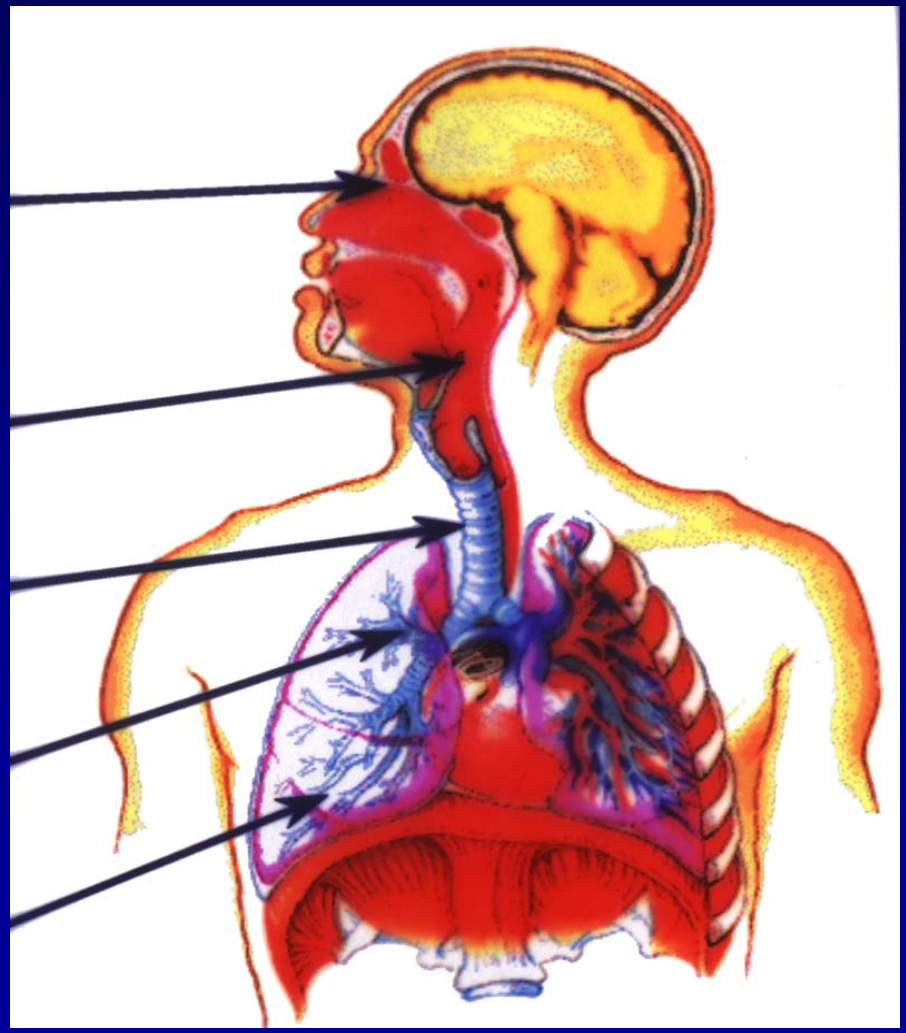
Mean Respiratory Dose for Volunteers as a Function of Aerosol Age (Liquid Tularemia Not Stabilized)

Post Dissemination

4 Min.	120 Min.	180 Min.
15	250	3,000

Tularemia Aerosol, Particle Size and Type of Infection

	Particle Size (Micron, Mass Median Diameter)
18 - 20 micron particles fall out of aerosol, lodge in eye	**18 - 20**
15 - 18 micron particles lodge in pharynx	**15 - 18**
7 - 12 micron particles lodge in trachea	**7 - 12**
4 - 6 micron particles lodge in bronchiole	**4 - 6** **Bronchioles**
1 - 3 micron particles lodge in alveolus	**1 - 3** **alveoli**



Vaccine Protection to Aerosol Challenge

- Killed vaccines do not protect animals or people to virulent aerosol challenge
- This is demonstrated by volunteers from the Seventh Day Adventist Church challenged with killed Tularemia vaccine (Forshay killed)

- **Forshay killed vaccine provided volunteers some protection to intracutaneous challenge***
- **Forshay killed vaccine did NOT protect volunteers from aerosol challenge**

Test	Respiratory dose (cells)	Vaccinated Ill/Challenged	Non-Vaccinated Ill/Challenged
1	15	-	2/2
2	17	1/2	2/2
3	22	1/4	1/2
4	27	3/4	2/2
5	48	3/4	6/8
Means	**26**	**8/14**	**13/16**

*AD285-542: Eigelsback, et al.

- **The live attenuated Tularemia Vaccine (LVS) did protect volunteers to virulent aerosol challenge**

Respiratory Challenge of Volunteers Given Live Attenuated Vaccine (LVS)*

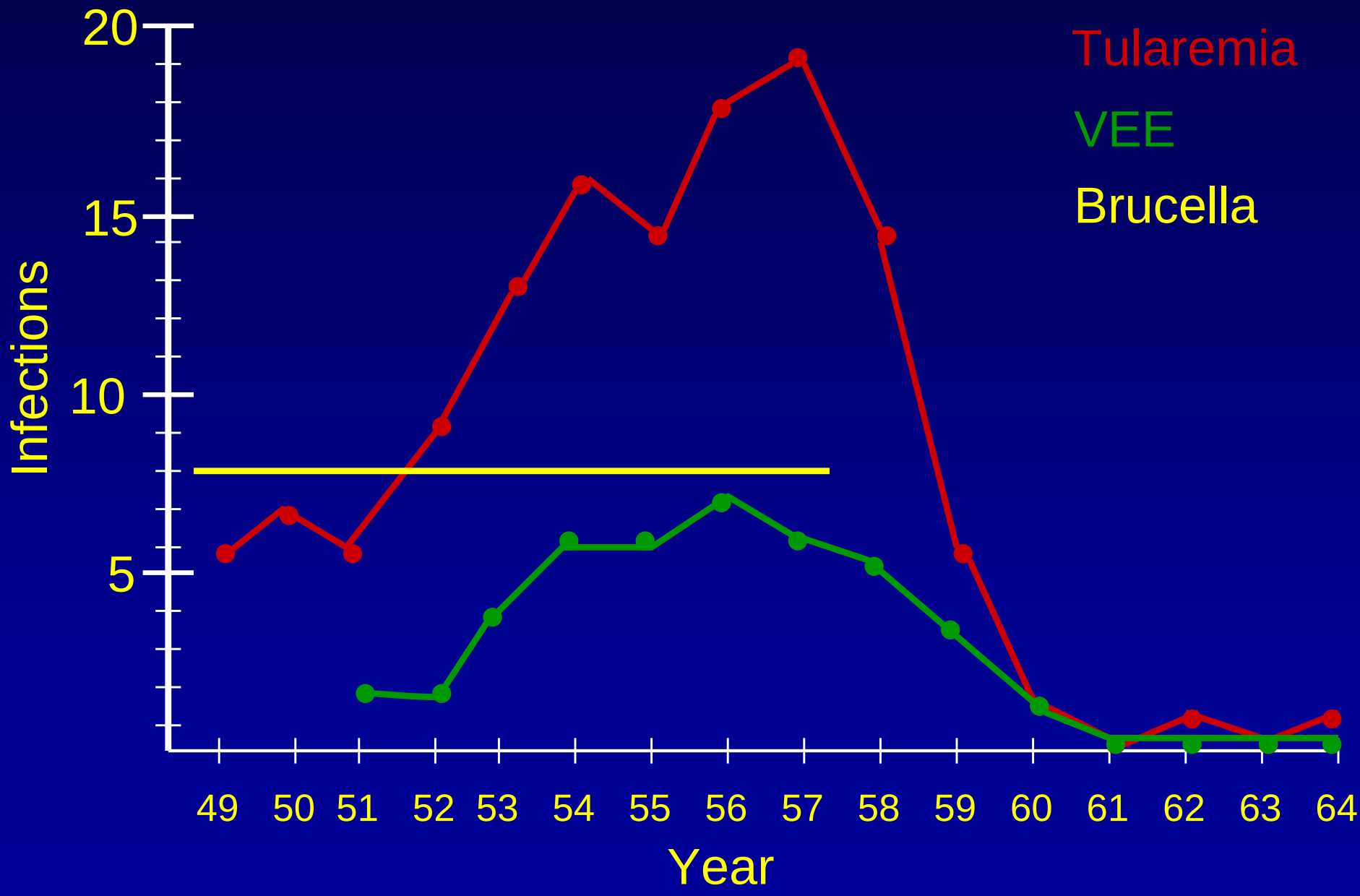
Test	Reparatory dose (cells)	Vaccinated Ill/Challenged	Non-Vaccinated Ill/Challenged
1	12	0/2	1/2
2	48	1/4	2/2
3	25	1/4	2/2
4	11	0/4	1/2
5	47	1/4	2/2
Means	**29**	**3/18**	**8/10**

*AD285-542: Eigelsback, et al.

- **There was a significant drop in the infection rate among “at risk” workers when the old killed vaccine were replaced with live attenuated vaccine.**

- **The next slide shows the infection rate for Tularemia and VEE infection before and after live vaccines replaced killed vaccines.**

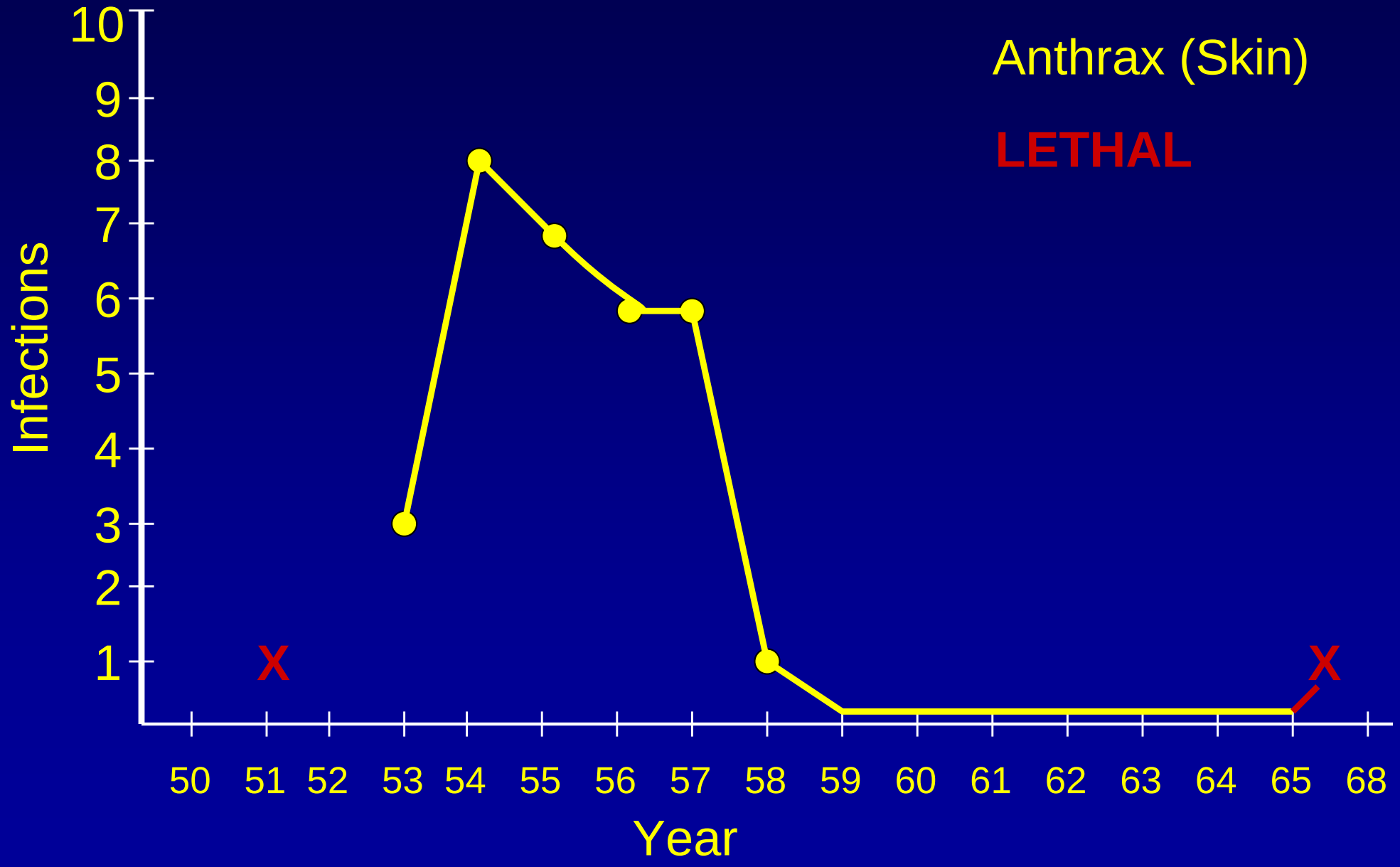
Influence of Vaccine on Infections



- **An effective vaccine was never developed for *Brucella suis***
- **The infection rate remained constant as long as this organism underwent R&D**
- **The data comparing infection rates for Tularemia and VEE, while dramatic, are not entirely clean**
- **The number of man hours devoted to the agent, safety protocols and the number of effective safety hood systems are a part of the information presented**

- The anthrax skin infections follow the same pattern observed for the aerosol challenge of Tularemia and VEE
- Note, however, there are two respiratory anthrax infections that led to death
- The next slide shows the anthrax infections

Influence of Vaccine on Infections



- **The significant impact of a good vaccine on aerosol protection is demonstrated in a large-scale field test of Tularemia.**

- **Non-immunized and LVS immunized Rhesus monkeys were stationed 5 kilometers downwind from the line of dissemination**

- **The Respiratory LD_{50} was:**

Non Immunized	LVS Immunized
34 Cells	14,600 Cells

± 429 fold difference

Conclusions:

- **The appropriate vaccine significantly alters the impact of a biological warfare or bioterrorist attack**
- **Live vaccines, while providing good immunity, have serious limitations, particularly in females of child-bearing age**
- **The current anthrax vaccine, not a killed or attenuated agent, provides good protection because it is a chemical vaccine…neither live nor killed**

Three equations can be used to calculate the success of an enclosed operation, i.e. building

- **Equation 1: Calculate the total number of infectious units available.**
- **Equation 2: Calculate the number of liters of air available in the building.**
- **Equation 3: Divide total number of infectious doses by liters of building air. This provides the number of infectious doses per liter of building air.**

Equation 1: Total Infectious Doses Available (TIDA)

$$TIDA = \frac{\left(\text{Product Conc. per ml or gm}\right)\left(\text{Total Amount of Agent ml/gm}\right)\left(\text{\% Dissemination Efficiency of Device}\right)}{\text{Human Infectious Dose}} \times 40\%$$

Example

A. Product Conc. = 1×10^9
B. 2000 ml of Agent
C. 5% Dissemination Efficiency
D. Human Dose is 8,000 Cells

$$TIDA = \left[(1 \times 10^9/ml)\ (2000\ ml)\ (5\%) \div 8000\ cells\right] \times 40\%$$

$$TIDA - 1 \times 10^8$$

Medical Management of a Biological Attack:

Ten Principles

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USAMRIID, Operational Medicine

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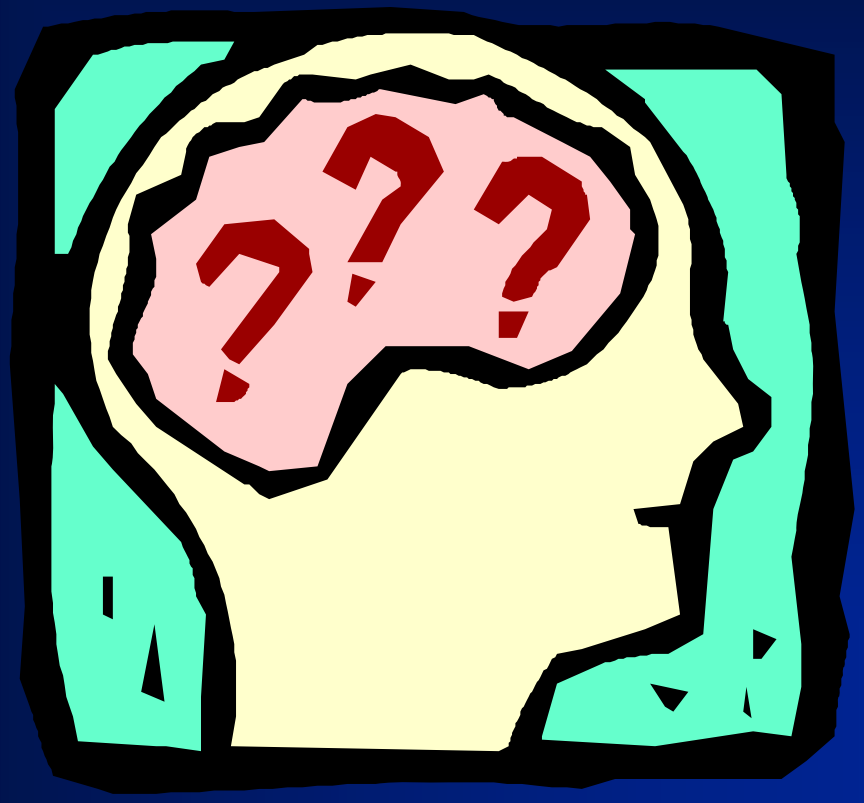
Objectives

- Understand the principles of medical management in a biological attack
- Identify appropriate prevention and control measures to mitigate biological agent hazards
- Identify information sources for reference and further training on biological agents

I.
Maintain an Index of Suspicion



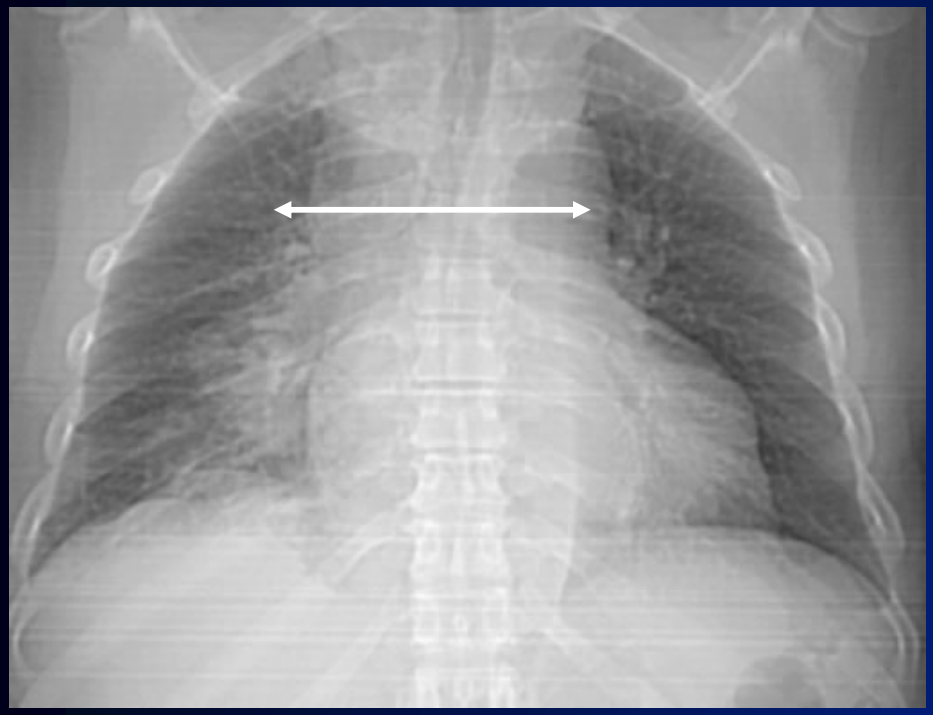
Index of Suspicion

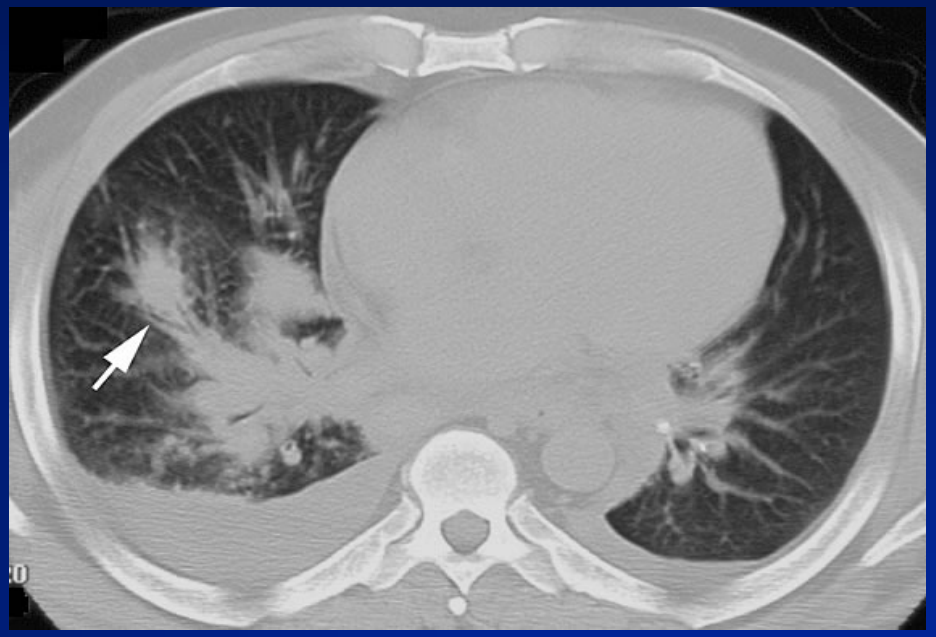
- Early recognition is the key to prevention
- A BW attack doesn't have to be large scale to have a significant impact
- A small outbreak may warn of a follow-on
- Everyone should know some basic epidemiology

Biological Terrorism?
Epidemiologic Clues

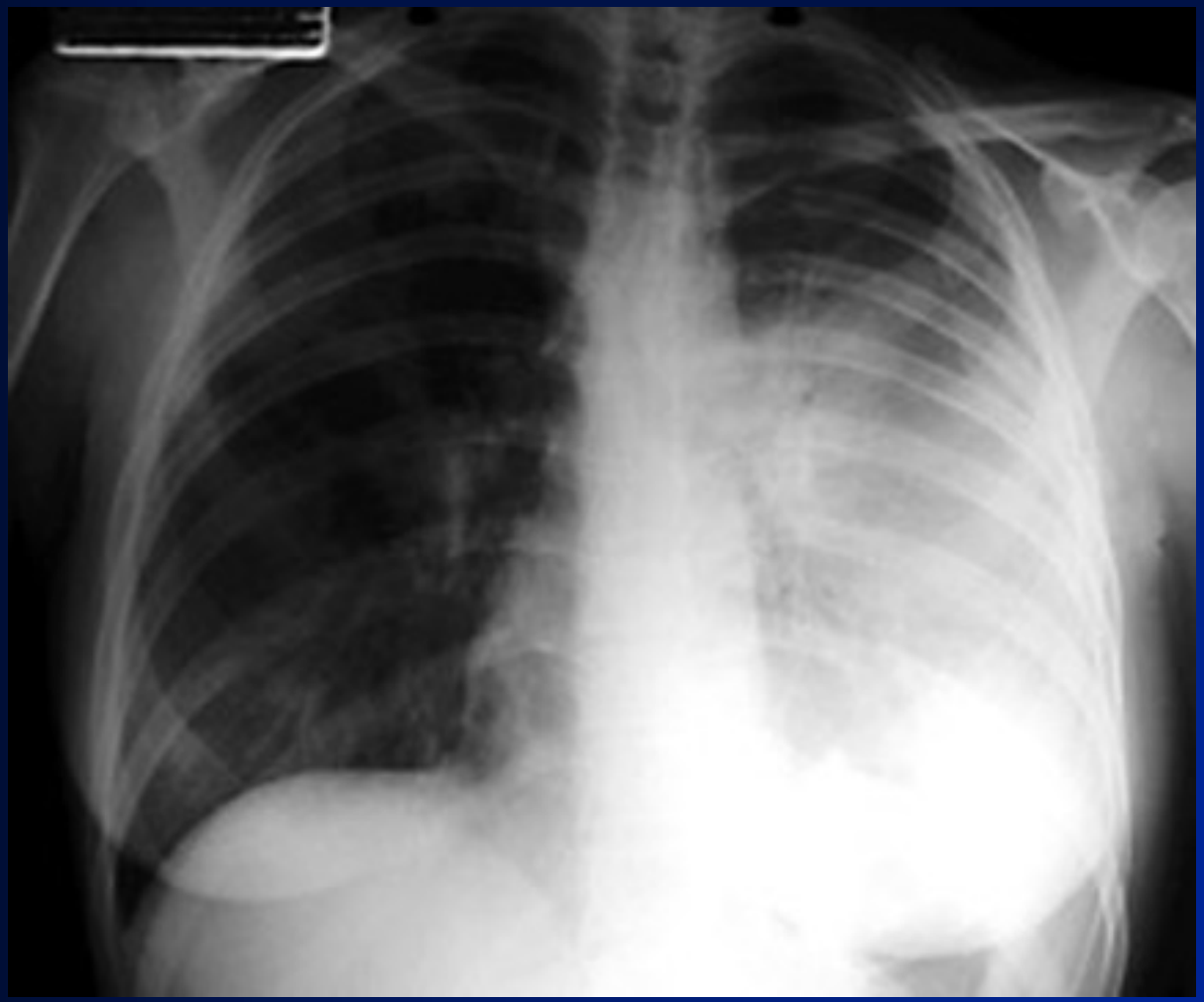
- Unusual disease
- Apparent aerosol route of transmission
- Geographic distribution: unusual location for disease; localized area
- High morbidity and/or mortality relative to number at risk
- Direct evidence
- Massive point source or multiple point sources
- Serial epidemics
- Unusual clinical presentation
- Animals: multiple dead species, reverse spread
- Lower attack rates among protected

Widened Mediastinum



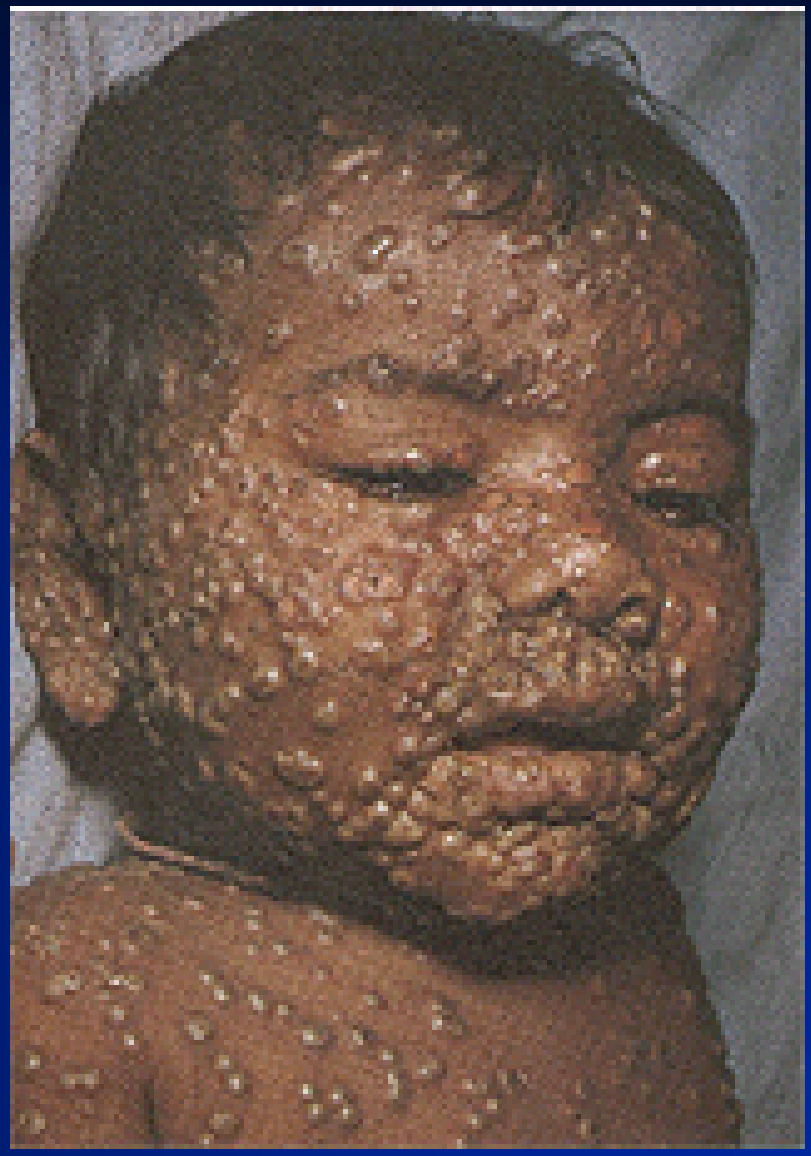


Pneumonia with Hemoptysis

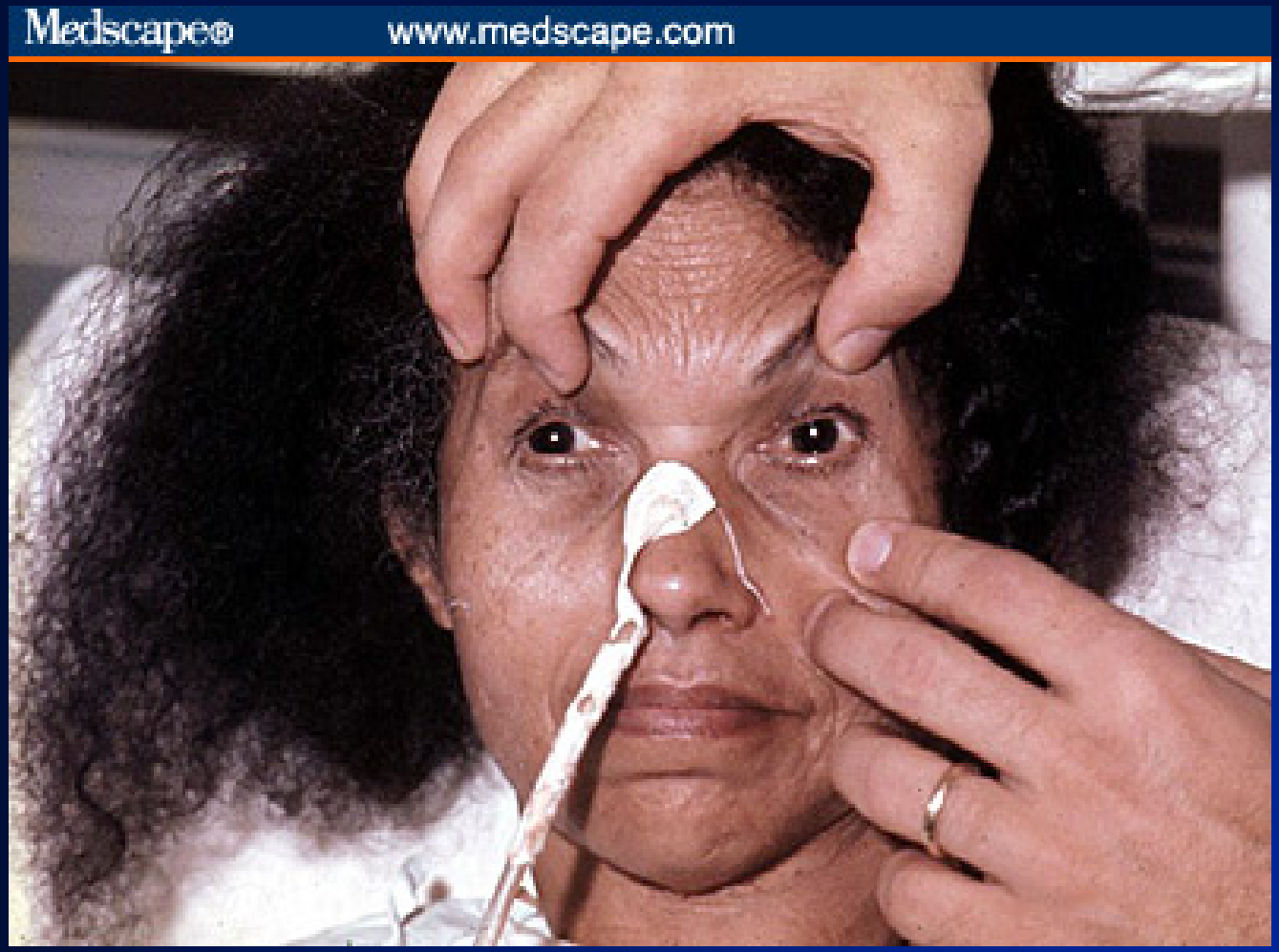


Febrile Rash

- Centrifugal
- Synchronous
- Umbilicated



Descending Flaccid Paralysis



Febrile Bleeding Diathesis

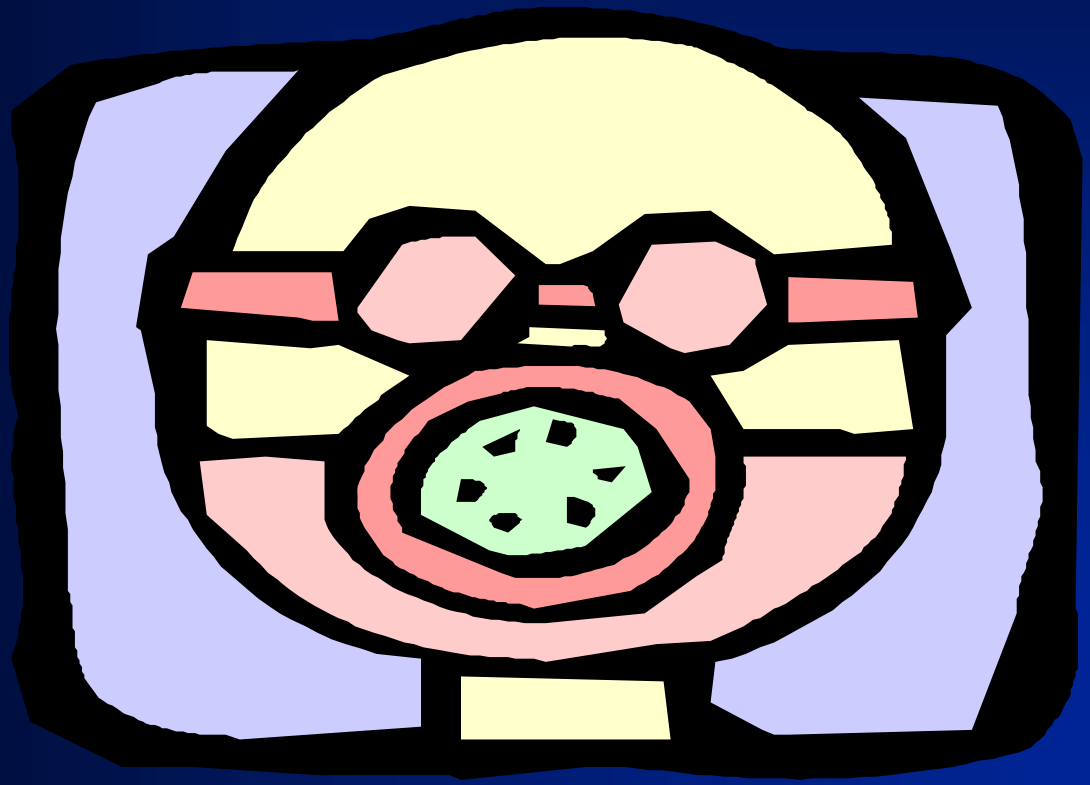
Biological Terrorism Diseases

Diagnostic Associations

Agent	Association
Anthrax	Wide mediastinum
Plague	Hemoptysis*
Smallpox	Exanthem
Botulism	Flaccid paralysis*
VHFs	Bleeding diatheses*

* when seen in multiple patients from the same location

II.
Protect Yourself and Your Patients



Protection Against Biological Agents

- **Physical**
  - Protective suits
  - Hepa-filter masks
- **Chemical**
  - Pre- & post-exposure antibiotics
- **Immunological**
  - Passive (e.g. botulinum antitoxin)
  - Active (e.g. anthrax & vaccinia vaccines)

Personal Protective Equipment

Medical Biological Defense
BW Vaccine Status

Licensed

- Anthrax (Bioport)
- Smallpox (Acambis)

IND

- Tularemia LVS
- Q-Fever CMR *(Coxiella burnetii)*
- Venezuelan Equine Encephalitis (VEE)
- Eastern Equine Encephalitis (EEE)
- Western Equine Encephalitis (WEE)
- Botulinum Toxoids

Emerging

- Botulinum (recombinant C fragment)
- Anthrax (Recombinant PA)
- VEE, EEE, WEE (recombinant clones)
- Staphylococcal Enterotoxins (recombinants)
- Plague (F1-V antigen)
- Ricin (A Subunit)
- SEB mutagen
- Naked DNA Multi-Valent Vaccines

III.
Assess the Patient

Relevant History

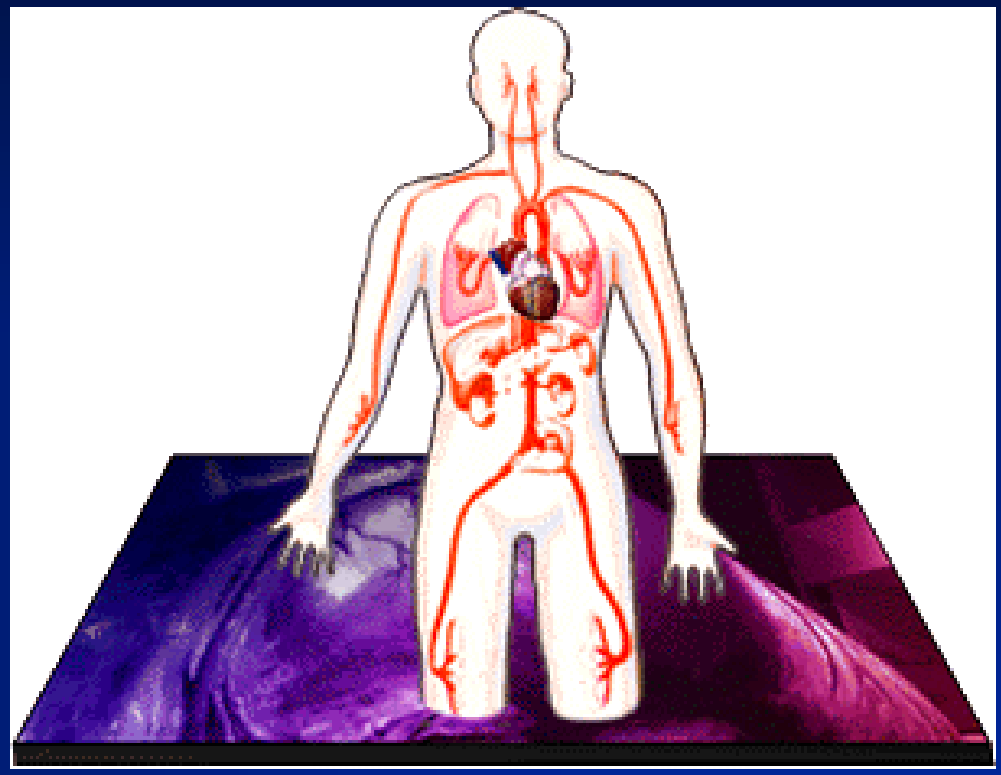
- Other unit members ill
- Unusual dissemination devices
- Uncontrolled food sources
- Vector exposure
- Immunization history
- Travel history
- Occupational exposure
- Protective equipment status

“AMPLE” History

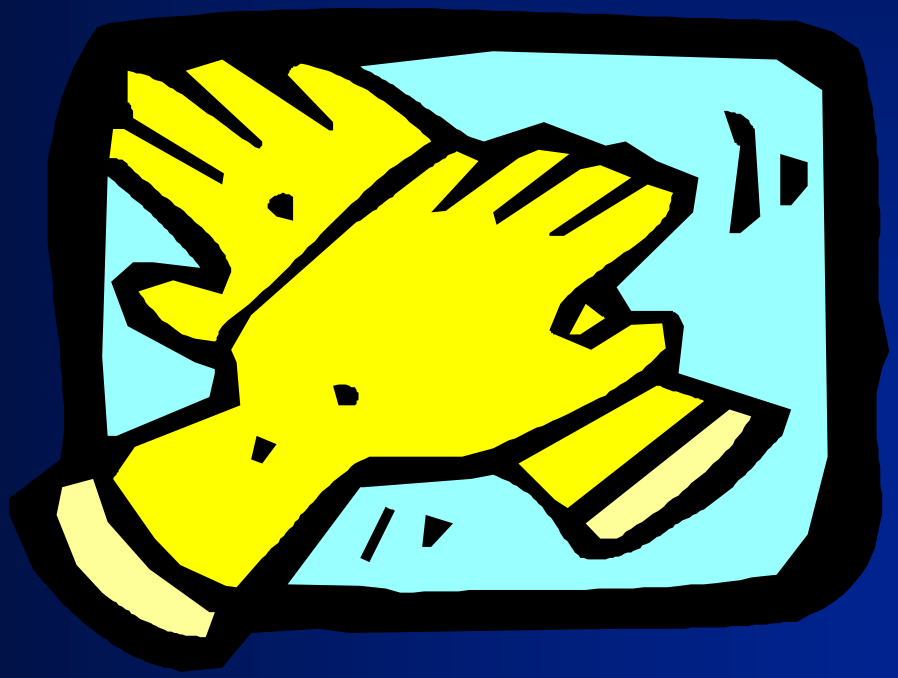
- **Allergies, Arthropods**
- **Medications, MOS (occupation), MOPP status**
- **Past med history / Immunizations**
- **Last Meal, Food Procurement**
- **Events**
  - Environment on battlefield
  - Travel history
  - Other unit members
  - Munitions

Physical Exam

- **Respiratory**
- **Neuromuscular**
  - Central & peripheral
- **Vascular/Hematologic**
- **Dermatologic**



IV.
Decontaminate as Appropriate



Microbial Elimination
Terminology

- **Sterilization**
  - Elimination of all microbial life
- **Disinfection**
  - High-level disinfectants
    - Kill all except high levels of endospores
  - Intermediate-level disinfectants
    - Kill tubercle bacilli, vegetative bacteria, viruses
  - Low-level disinfectants

Decontamination After a Biological Attack

- **Personnel**
  - Decon rarely needed
  - Less relevant than for Chem attack
  - Soap & water
- **Materiel**
  - Often unnecessary
  - Less relevant than for Chem attack
  - 5.0% bleach more than adequate
  - 0.1% bleach kills anthrax spores

V.
Establish a Diagnosis

Diagnostic Matrix

Immediate, Respiratory

- Nerve agents
- Cyanide
- Mustard
- Lewisite
- Phosgene
- SEB inhalation

Delayed, Respiratory

- Inhalational anthrax
- Pneumonic plague
- Pneumonic tularemia
- Q Fever
- SEB inhalation
- Ricin inhalation
- Mustard
- Lewisite
- Phosgene

Immediate, Neurological

- Nerve agents
- Cyanide

Delayed, Neurological

- Botulism – peripheral symptoms
- VEE – CNS symptoms

Syndromic Diagnosis

Syndrome	Agents
• Neurological	• Botulinum toxin, VEE
• Bleeding	• VHF, ricin, plague
• Dermatologic	• Smallpox, plague, VHF, T-2 mycotoxin, anthrax
• Pneumonia	• Tularemia, brucellosis, Q fever, plague

Establishing a Diagnosis

- **Clinical**
- **Epidemiological**
- **Laboratory**
- **Radiology**
- **Consultants**
  - Infectious disease
  - Neurology
  - Hematology
  - Preventive medicine

Obtaining Clinical Specimens

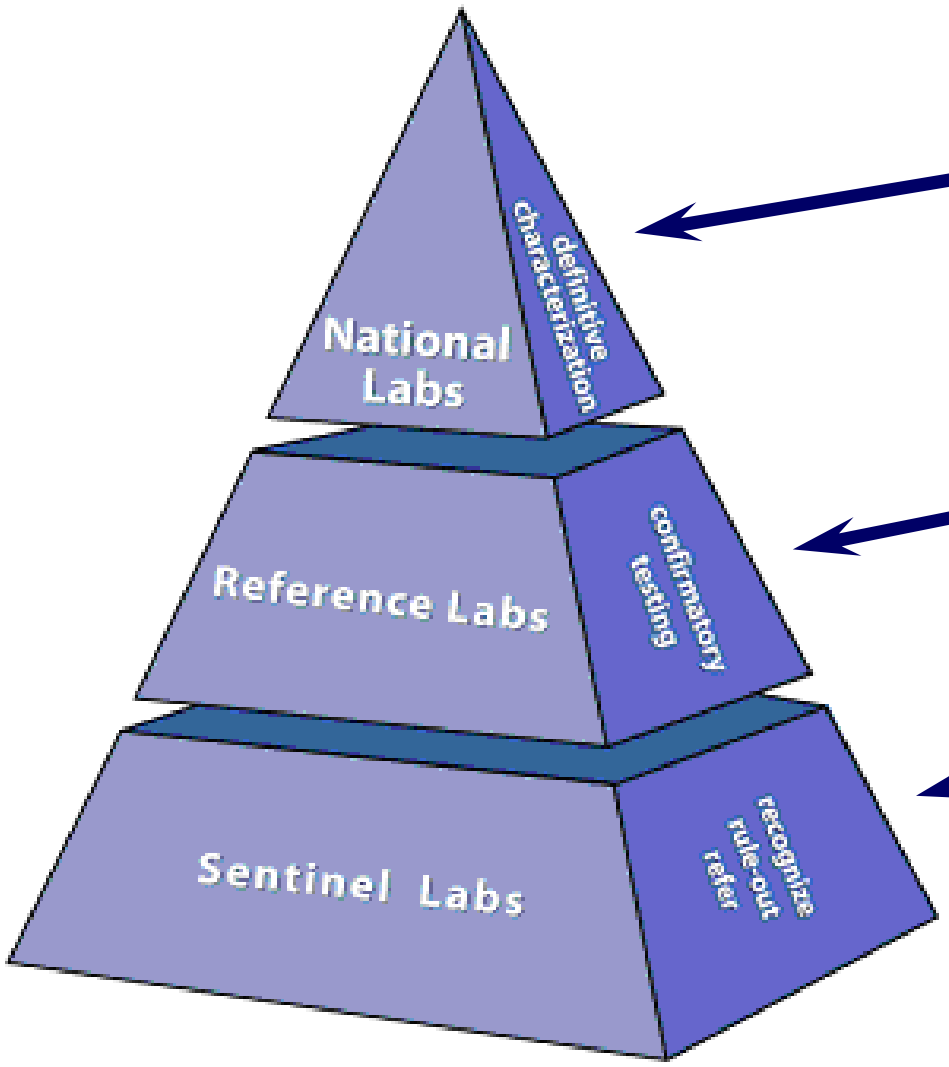
- **Immediate post-exposure period (0-24 hours)**
  - Swab: nares, hairy portions of face (PCR, culture)
  - Serum: archives (PCR, bacterial culture)
  - Sputum: bacterial culture
- **Acutely ill patient (>24 hours)**
  - Swab: nares and throat (PCR, cultures, ELISA)
  - Blood, urine, sputum (PCR, cultures, toxin assays)
- **Critically ill patient**
  - Swab: throat
  - Blood, urine, sputum, feces
- **Deceased**
  - Autopsy: spleen, lymph nodes, kidney, liver, brain, lung

Diagnostics

Rapid and Confirmatory

- Development and evaluation of diagnostic assays
- Technologies field-tested with Army Area Medical Laboratories (AML)
- DoD Reference laboratory for biological agent confirmation

Laboratory Response Network



Definitive characterization

Confirmatory testing

Recognize, rule-out, refer

CDC

Action Items

- What is the bio-safety level of my lab?
- Is my lab active in the Lab Response Network?
- Where is the nearest higher level lab?
- What guidelines should be followed to package and ship biological agents?
- Whom should I call?
- Review your current protocols and safety practices
- Incorporate biologic event response plan into SOPs
- Keep updated and train staff

VI.
Render Prompt Treatment

Field Expedient Therapy

Immediate, Respiratory

Cyanide

Delayed, Respiratory

Inhalational anthrax

Pneumonic plague

Pneumonic tularemia

Q Fever

Immediate, Neurological

Nerve agents

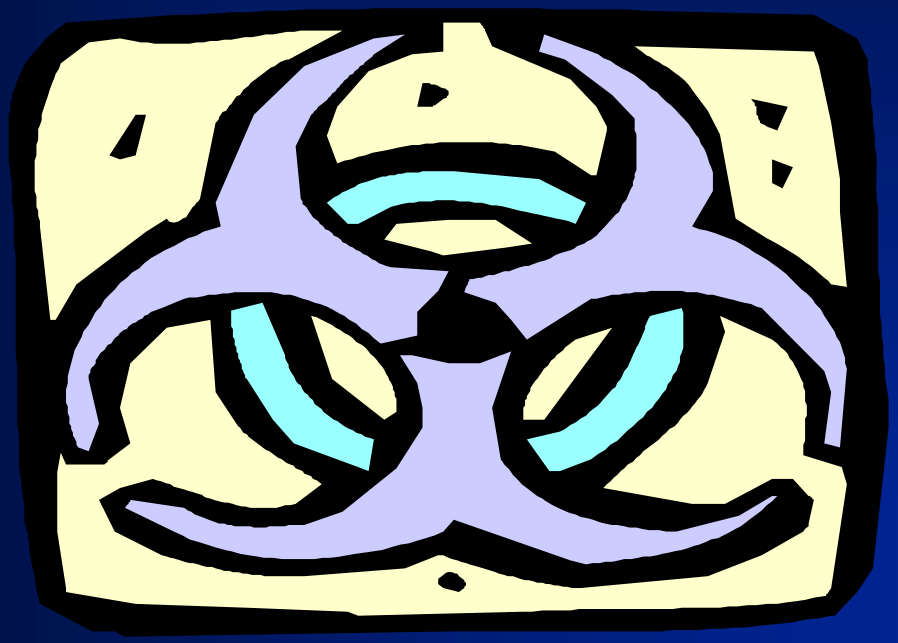
Delayed, Neurological

Botulism – peripheral symptoms

Treatment for Non-specific Febrile Illness Caused by Biological Agents

Agent	Treatment
Brucellosis	Doxy
Q-Fever	Doxy
Tularemia	Doxy or Gent
Prodromal Plague	Doxy or Gent
Prodromal Anthrax	Doxy or Cipro
VEE	None

VII.
Practice Good Infection Control



Laboratory Biosafety

BSL-2	BSL-3	BSL-4
Anthrax*	Brucella	Ebola
Cholera	Plague	Marburg
Tularemia B	Tularemia A	Arena Viruses
Toxins	Q-Fever	TBE viruses
	VEE	Flaviviruses

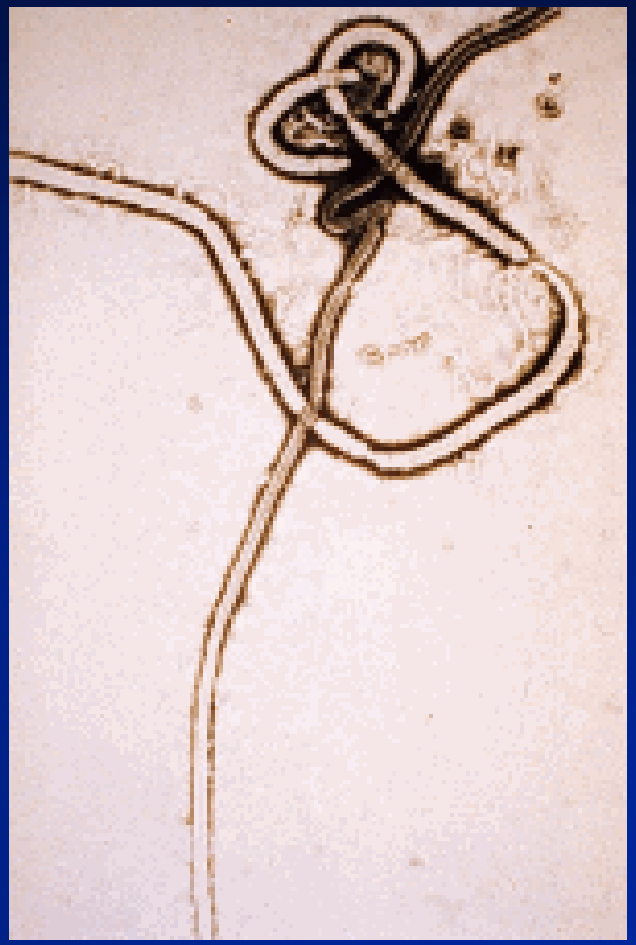
*BSL 2/3

Hospital Isolation Precautions

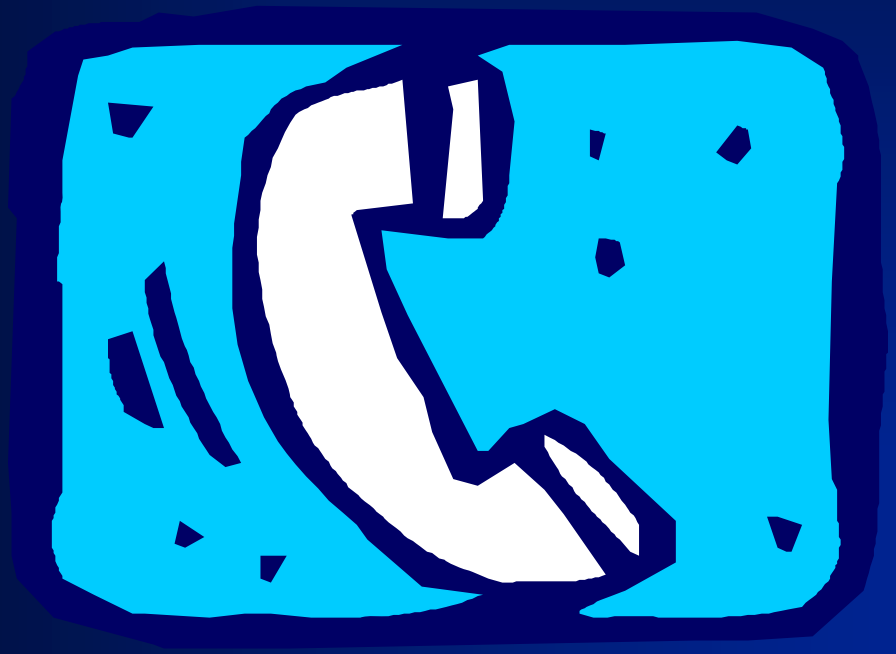
- **Standard Precautions**
- **Transmission-Based Precautions**
  - Airborne Precautions
  - Droplet Precautions
  - Contact Precautions

Isolation Precautions

- **Airborne precautions**
  - Smallpox
- **Droplet precautions**
  - Pneumonic plague
  - Smallpox
- **Contact precautions**
  - Viral hemorrhagic fevers
  - Smallpox



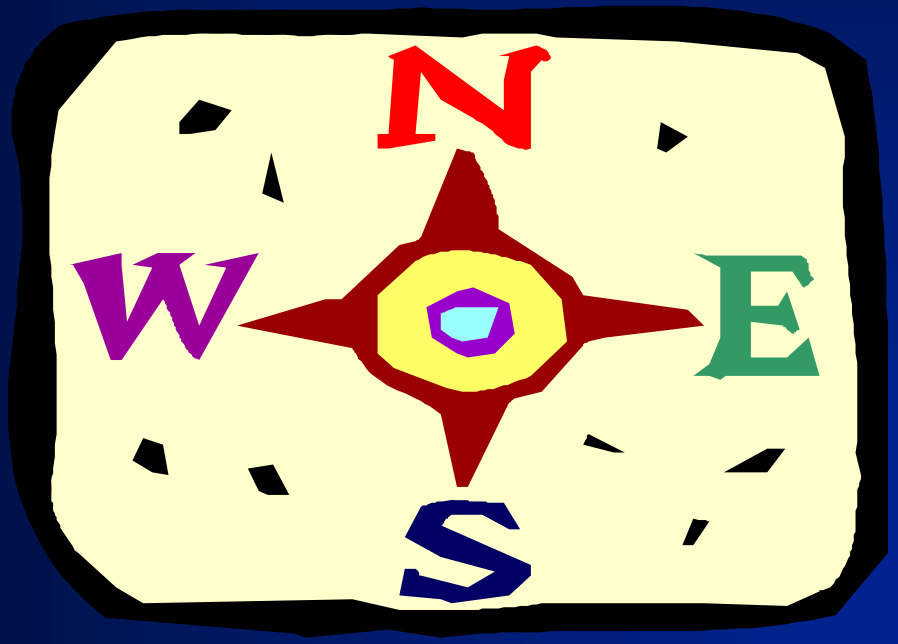
VIII.
Alert the Proper Authorities



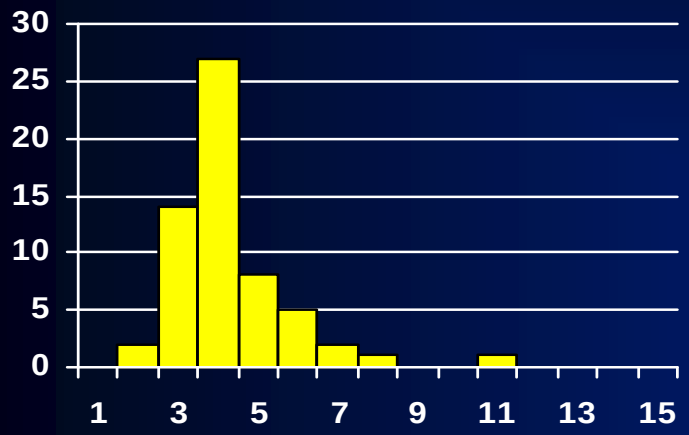
Who to Alert?

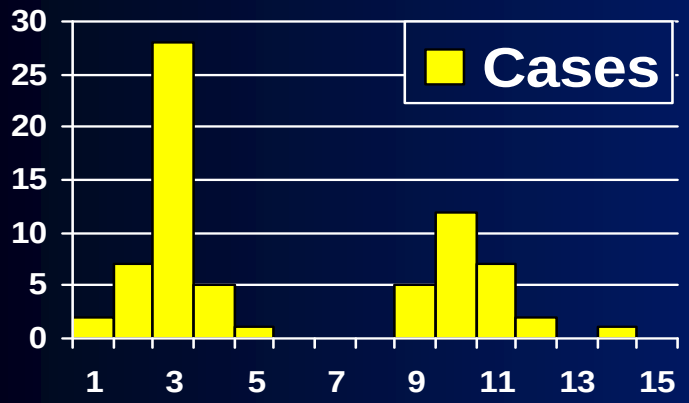
- Your Command
- Medical personnel
- Preventive medicine / public health personnel
- Laboratory
- Law enforcement
- Follow local Emergency Response Plan

IX.
Assist in the Epidemiologic Assessment



The Epidemiological Sequence



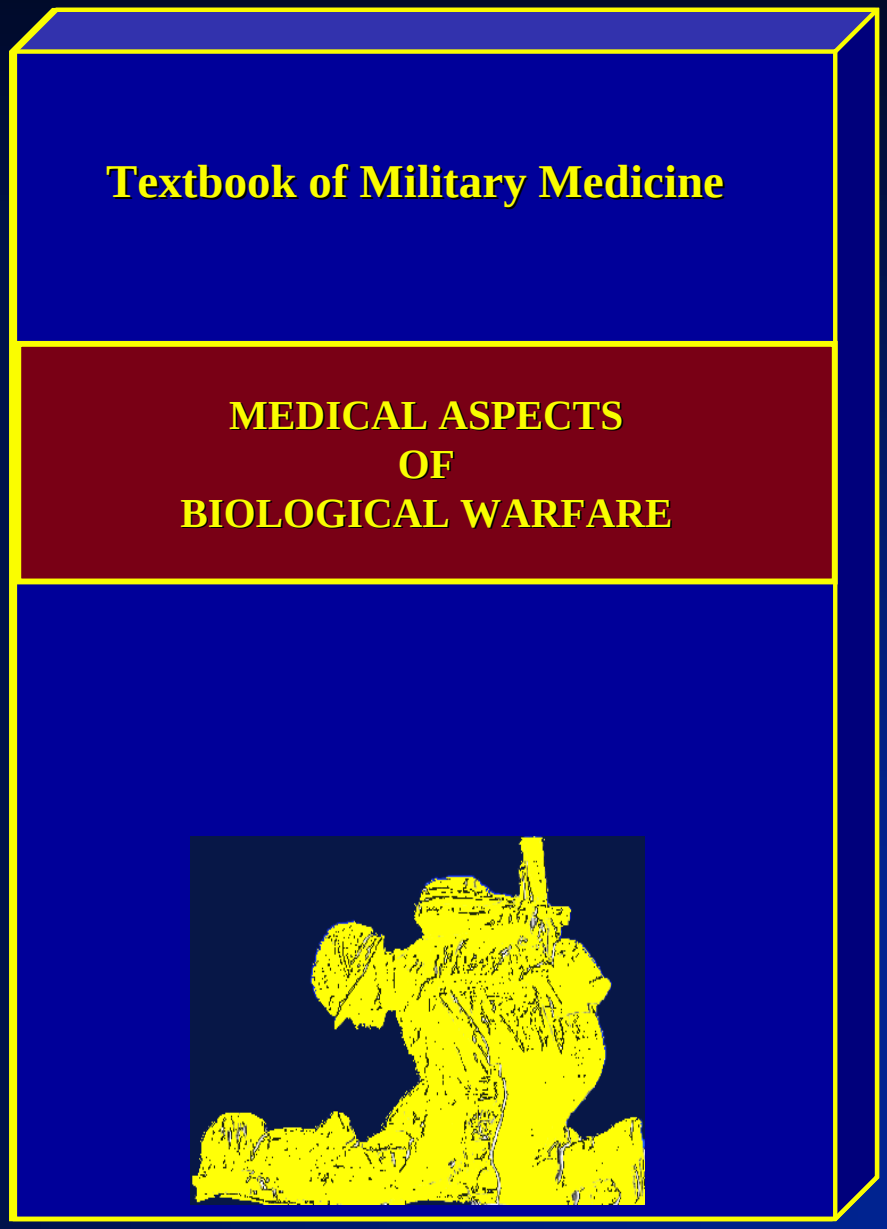


1. Prepare for field work
2. Establish the existence of an outbreak
3. Verify the diagnosis
4. Define and identify cases
5. Describe and orient the data in terms of time, place, and person
6. Develop hypotheses
7. Evaluate hypotheses
8. Refine hypotheses and carry out additional studies
9. Implement control and prevention measures
10. Communicate findings

CDC

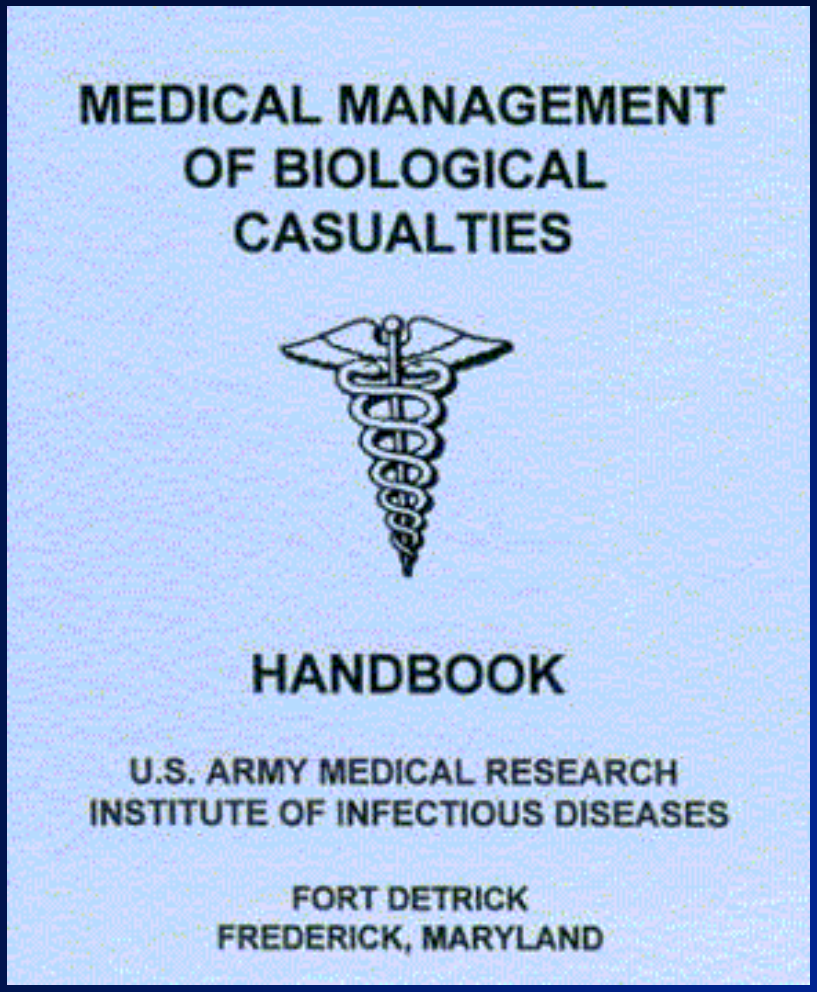
X.
Know and Spread the Information

Sources of Information



http://www.bordeninstitute.army.mil/published_volumes/biological_warfare/biological.html

The Blue Book



http://www.usamriid.army.mil/education/instruct.htm

Distance Learning

- **Advanced Topics on Medical Defense against Biological and Chemical Agents**
- **Biological and Chemical Warfare and Terrorism – Medical Issues and Response**
- **CME**
- **Formats:**
  - DVD
  - Video
  - Webcast
  - Satellite Broadcasts

Websites

- www.usamriid.army.mil
  USAMRIID website
- www.bt.cdc.gov
  CDC's bioterrorism preparedness and response website
- www.apic.org
  APIC's bioterrorism response plan
- www.nbc-med.org
  U.S. Army Surgeon General's site on NBC defense
- www.upmc-biosecurity.org
  Center for Biosecurity of the University of Pittsburgh Medical Center
- www.anthrax.osd.mil
  Anthrax Vaccine Implementation Program

Medical Management
Summary

1. Maintain an Index of Suspicion
2. Protect Yourself and Your Patients
3. Assess the Patient
4. Decontaminate as Appropriate
5. Establish a Diagnosis
6. Render Prompt Treatment
7. Practice Good Infection Control
8. Alert the Proper Authorities
9. Epidemiologic Assessment
10. Know and Spread the Information

Questions?

References

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