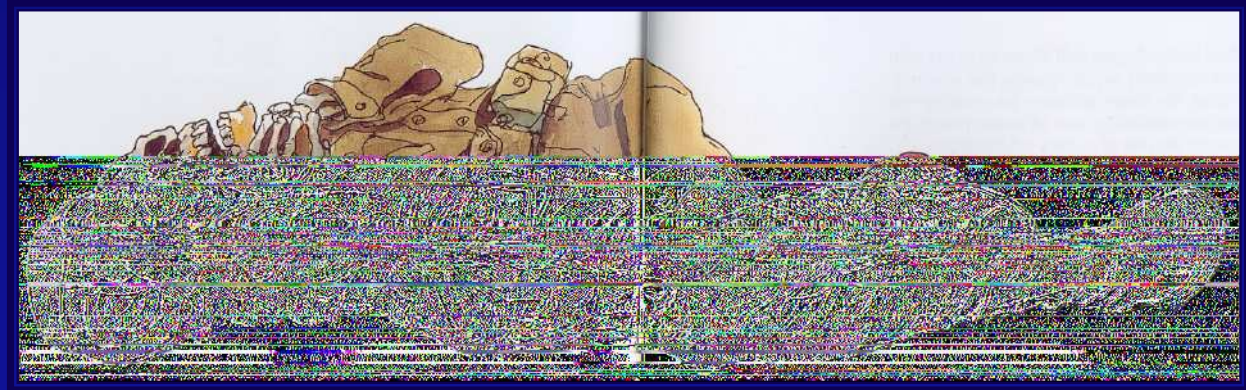


BIOLOGICAL WARFARE AND TERRORISM

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The dark side of microbiology

- Definition
- Historical perspective
- How real is the threat
- Biological agents
- Anthrax overview
- Smallpox, hemorrhagic viruses
- Clues to a potential biocrime
- Are we prepared
- Conclusion



Bioterrorism

- Intentional or threatened use of
 - Viruses, bacteria, fungi, or toxins from living organisms
- To produce death or disease in
 - Humans, animals, or plants

Once upon a time ...

Biological warfare is not new *even without a precise understanding*

- In Greek and Roman's times
- 14th Century: Plague at Kaffa, Ukraine



Biological warfare is not new

- 1754-1767, Smallpox blankets



Modern Biological Warfare

- During World Wars : Use of biological weapons
 - WWI, Germans infected French cavalry (B.mallei, B.anthraxis)
 - WWII, Japanese Program over Manchuria (B.anthraxis, V.cholerae, Shigella, Salmonella, Y.pestis)
- After WWII
 - US, Soviet Union and others initiated research in biological weapons

Biological Warfare Agreements

- 1925 Geneva Protocol
 - Use of biological weapons prohibited
 - Basic research, production and possession not proscribed
 - = ineffectiveness
- 1972 Biological Weapons Convention
 - Development, production, stockpiling « prohibited » (Qty)
 - Destruction of stocks required
- 1975 Geneva Conventions Ratified
 - > 100 signatory nations (Iraq and Soviet Union included)
 - Relative ineffectiveness

*By the end of the XXth century,
running to the next millennium ...*

Bioterrorism : How real is the Treat ?

Hoax versus Actual BT Event

A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars

Torok T et al, JAMA 1997; 278:389-395

- 1984, Oregon
- 751 cases of Salmonellosis
- Eating at salad bars in 10 restaurants
- Criminal investigation identified perpetrators as followers of Bhagwan Shree Rajneesh
- Significant role of
 - local public health dept.
 - early contact between local-state-national levels
 - Importance of notifying public health dept. of any suspicious clusters of illness

Shigellosis caused by intentional contamination The unhappy laboratorian in Dallas (1996)

- 12 (27%) of 45 laboratory workers in a large medical center had severe diarrheal illness
- 8 (67%) had positive stool cultures for *S.dysenteriae* type 2
- Eating muffins or donuts in staff break room implicated
- PFGA patterns indistinguishable for stool, muffin, and laboratory stock isolates
- Criminal investigation identified the provider of pastry coated with *Shigella* to her coworkers

Kolavic et al. JAMA 1997;278:396

Miller. Clin Microb Newsletter 2001;23:179

Chemical & Biological Terrorism

- 1991 : Minnesota, ricin toxin (hoax)
- 1994-95 : Tokyo, Sarin and biological attacks
- 1995 : Arkansas, Ricin toxin (hoax)
- 1995 : Ohio, *Yersinia pestis* (sent in mail)
- 1997 : Washington DC, « Anthrax » (hoax)
- 1998 : Nevada, non-lethal strain of B.anthraxis
- 1998 : Multiple « Anthrax » hoaxes

Agent selection considerations

- « Catastrophic » public health consequences
 - High morbidity and mortality
 - Mass casualties which overwhelm medical systems
 - Contagious

« Terror » perspective

- Distress of population
- Panic among those who decide
- Irrational measures
- Paralysis of public utilities
- Financial loss

Biological Agents of Highest Concern (Category A)

- *Variola major* (Smallpox)
- *Bacillus anthracis* (Anthrax)
- *Yersinia pestis* (Plague)
- *Francisella tularensis* (Tularemia)
- Botulinum toxin (Botulism)
- Filo- and Arenaviruses (Viral hemorrhagic fevers)
 - Easily disseminated or transmitted,
 - High mortality, social disruption,
 - Special preparation needed

Biological Agents of Highest Concern (Category A)

- **ALL suspected or confirmed cases should be reported to health authorities IMMEDIATELY**

➤ Info: <http://www.cdc.gov>

Why these biologics as weapons

- Infectious via aerosol
- Organisms fairly stable in the environment
- Susceptibility of civilian unprotected population
- Delayed onset
- High morbidity and mortality
- Person-to-person transmission (smallpox, plague, VHF)
- Difficult to diagnose and / or treat
- Previous development for BW

Other advantages of biologics as weapons

- Its easy to do but hard to trace
 - Small amounts - devastating effects
 - Easy to obtain
 - Inexpensive to produce
 - Potential for dissemination over large geographic area
 - Invisible, odorless, tasteless
 - Difficult to detect
 - Creates panic
 - Can overwhelm medical services
 - Perpetrators escape easily

Overt Attack

(Announced release of biological agent)

■ Problems

- Verifying if an attack has taken place
- Influx of « worried well » - need triage
- Need for infection control
- Prophylaxis drugs & vaccines limited

Covert Attack

(Biological agent released secretly)

- Problems with recognition :
 - Symptoms overlap common illnesses
 - Delayed onset from time of exposure
 - Victims present to different centers
 - Potential secondary spread

Likely Scenarios

- Aerosol release
- Major city, large event, or key function
- Recognition of attack through epidemiologic patterns or lab specimen

From catastrophic terrorism with mass casualties

To microevents producing civil unrest, disruption, disease, death



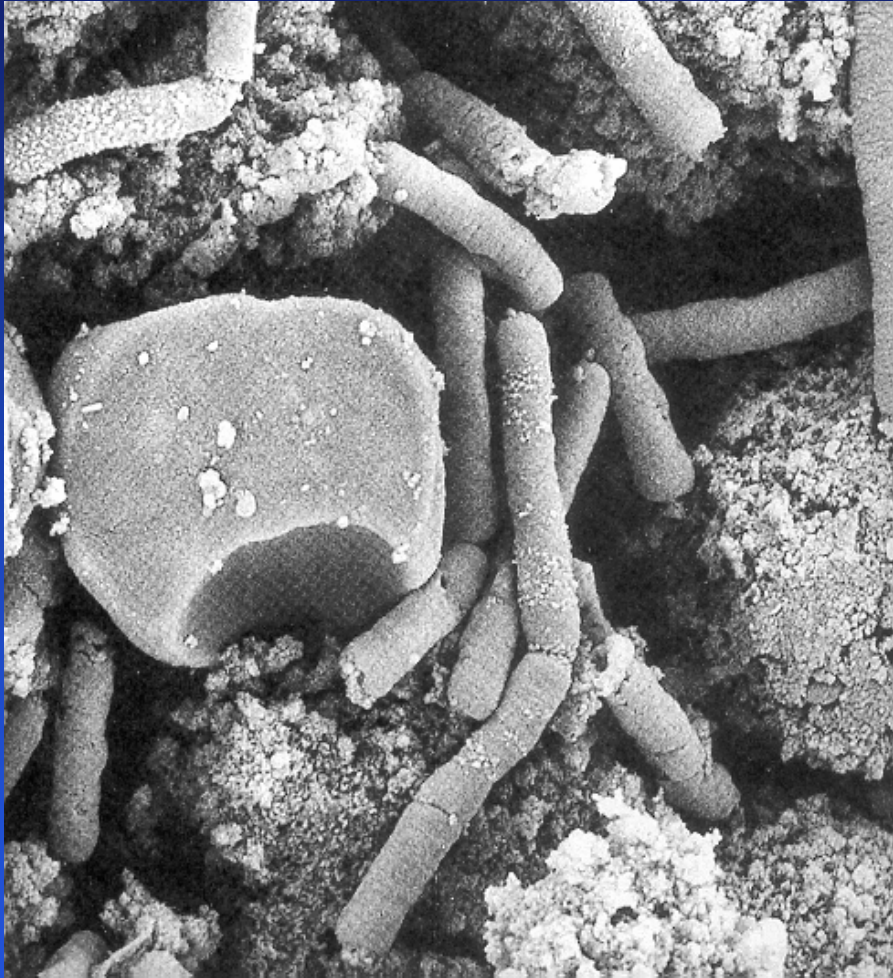
**From an ancient disease
to bioterrorism**

**From an endemic disease in
poor countries to terror in
rich countries**

Anthrax : overview

- Naturally occurring anthrax
 - Bacillus anthracis
 - A zoonose
 - Man, accidental victim
- Anthrax as a bio-weapon
 - Why
 - How
 - Facts

The anthrax bacteria : *Bacillus anthracis*



- Illustrious bacterium :
Koch, 1877
Pasteur, 1881
- Survival :
Spores
- Weapons :
Caspule and toxin



- On sheep's blood agar
 - Non-hemolytic
 - Large colonies
 - Firmly adherent
 - Non-motile
 - Non encapsulated
 - Gram+ spore forming rods




- On nutrient agar/ CO_2
- Encapsulated bacilli
 - India-ink staining

Pathogenesis, Major factors of virulence

- The capsule
 - 3 genes *capA*, *capB* and *capC*, carried on a plasmid
- Edema and lethal toxins
 - Both must be present for disease to occur
 - Encoded on another plasmid
 - Edema factor + protective antigen → edema toxin
 - *Adenylate cyclase activity* → *fluid accumulation*
 - Lethal factor + protective antigen → lethal toxin
 - *Zinc metalloprotease activity* → *stimulates macrophages to release TNF α and interleukin-1 β + proinflammatory cytokines*

Anthrax : a zoonose

- Worldwide
 - Particularly in poor countries
- Primarily disease of herbivores
- Systemic infection ↑ 
- Contamination :
 - While grazing on contaminated land (spores)
 - Eating contaminated feed or drinking from contaminated water holes
- Soil reservoir
- Control : vaccination to livestock

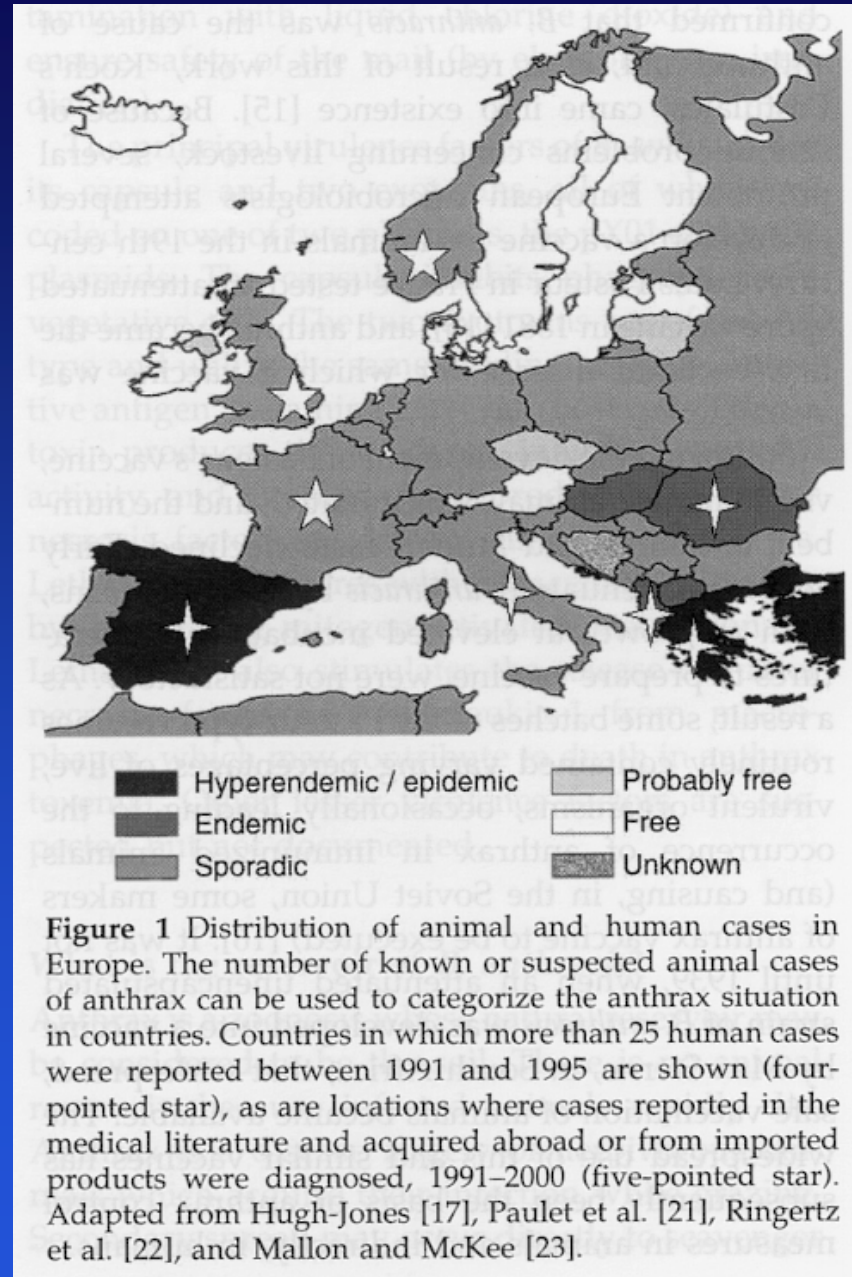
Man : accidental victim of anthrax

- 20,000 - 100,000 cases/year
 - USA : <1 case/year for the past 20 years
- By handling products or consuming undercooked meat from infected animals
- From inhalation of spores
 - From contaminated products : such as wool
 - During a BT attack, intentional release of spores

Man : accidental victim of anthrax

- 3 Forms occur in humans
 - Cutaneous : the most common form occurring naturally (95 %)
 - Gastrointestinal (1%)
 - Inhalational (wool sorter's disease)

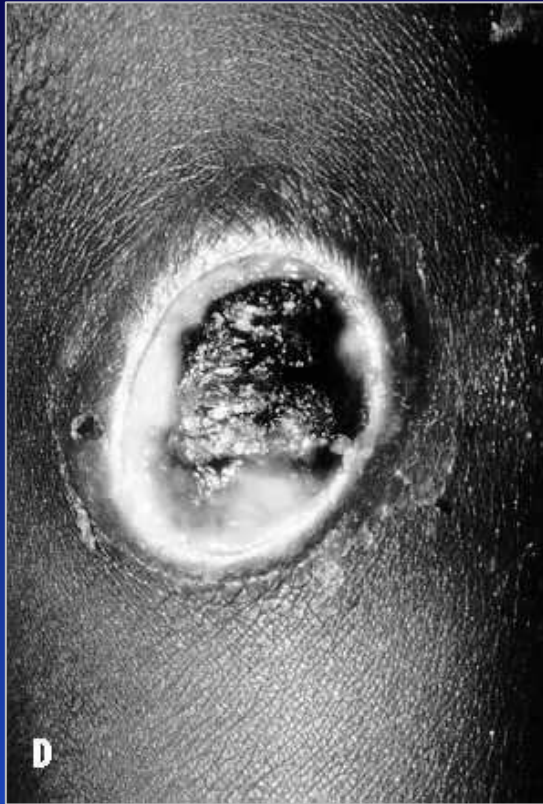
Anthrax in Europe



Man, accidental victim : *cutaneous anthrax*



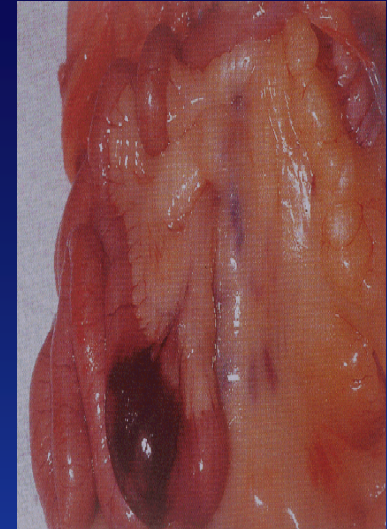
In an aerosol bioterrorist attack, few cases would probably emerge



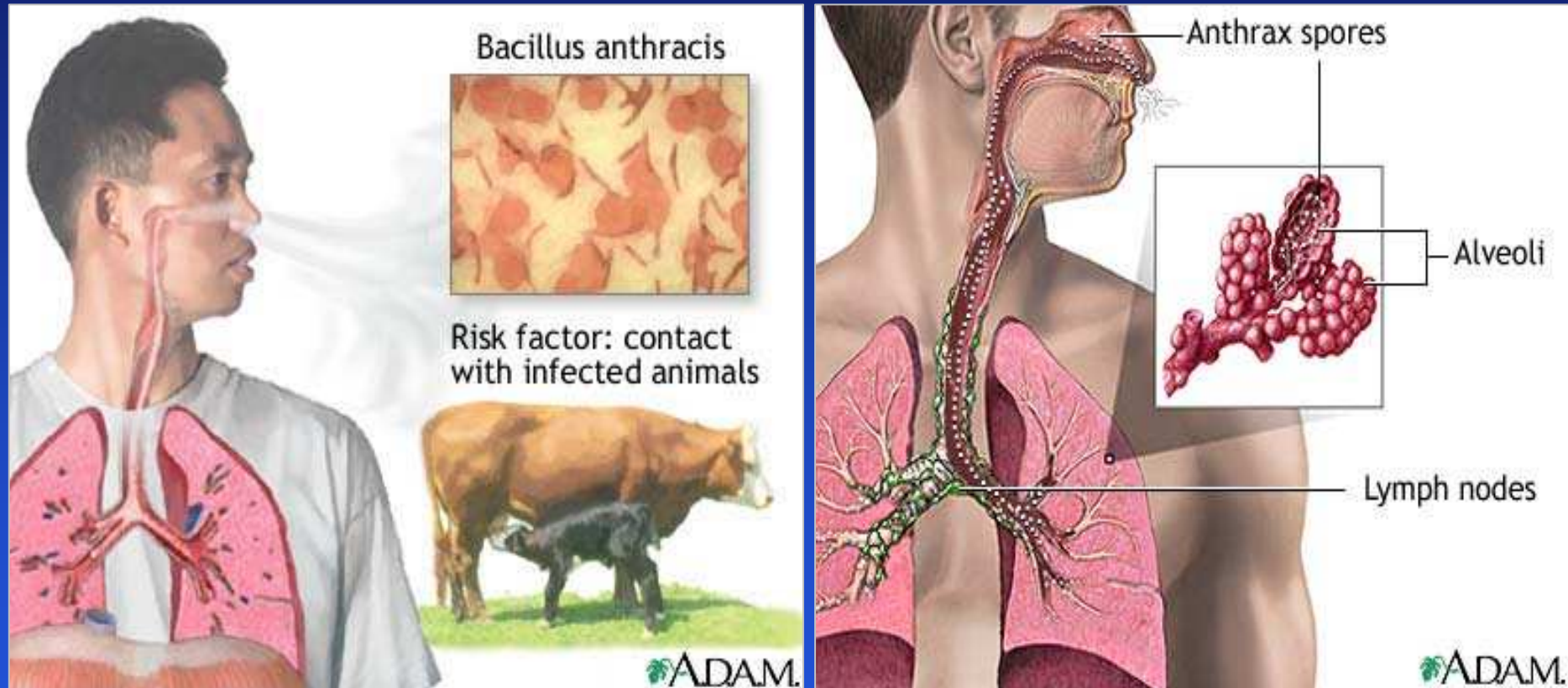
- Incubation : hours to 7 days
- Small papule -> ulcer surrounded by vesicles (24-48h)
- Painless eschar with edema
- Death 20% untreated, rare if treated

Gastrointestinal anthrax

- Ingestion of contaminated meat
- Incubation : hours or up to 7 days
- Fever, acute gastroenteritis, vomiting, bloody diarrhea
- Intestinal eschar similar to anthrax lesion (hemorrhagic)
- Progression to generalized toxemia
- Mortality rate 50-100% despite treatment



Man, accidental victim : *inhalational anthrax*





- Inhalation of spores
- Incubation: 1 to 43 days
- Initial symptoms (2-5 d)
 - Fever, cough, myalgia, malaise
- Terminal symptoms (1-2d)
 - High fever, severe respiratory distress, septic shock
 - Hemorrhagic mediastinitis

**Mortality ~ 100%
in 24-36 hours
despite aggressive
treatment**



Anthrax

- Diagnosis, Culture : blood or sputum
 - Gram + spore forming rod
 - Gram stain direct smear
 - ELISA to detect antigen in blood
 - PCR for virulence genes (under investigation)
- Isolation : standard precautions, no quarantine needed
 - No human to human transmission

Presumptive Identification of *Bacillus anthracis*

Type of Sample	Presumptive Id Characteristic	Method
<i>Clinical sample</i>	1) G+ rods AND	Gram stain
	2) Capsule	India ink stain
<i>Isolate</i>	1) Spore-former AND	Gram stain
	2) Colony morphology AND	Observation on SBA
	3) Non-hemolytic AND	Observation on SBA
	4) non-motile	Motility medium/wet-mount

Anthrax : Treatment

Unlikely to be effective after symptoms

- Antibiotics
 - Penicillin or doxycycline
 - Ciprofloxacin or other quinolones
- Supportive care
- Post-exposure
 - Vaccine x3 (0,2,4 wks) + Cipro or Doxy x30 d
 - OR antibiotics alone x60 d

Long term antibiotics necessary because of spore persistence

Anthrax, a bio-weapon - Facts

- 1979, Sverdlovsk (URSS): « accidental » release of spores from a military laboratory
 - Over 4 km around, at least 77 cases with 66 deaths
 - Over 50 km, attack on livestock (cattle, sheep,...)
- 1995, Tokyo : Aun Shinrikyo's sect
 - 8 failed attempts of attack with anthrax and botulism toxin
- 2001, USA

« Postal » Anthrax, USA (CDC, November 2001)

Cases	Florida	NY city	New Jersey	Washington	TOTAL
Confirmed					17
<i>Cutaneous</i>	0	4	3	0	
<i>Inhalational</i>	2	1	2	5	
Suspected					5
<i>Cutaneous</i>	0	3	2	0	
<i>Inhalational</i>	0	0	0	0	
TOTAL	2	8	7	5	22

5 deaths among these cases

Anthrax, a bio-weapon, how ?

- Infection via aerosol
- Duration of aerosol: few hours
- Low risk of secondary aerosol
 - *Could be improved as bio-weapon:*
 - *Multi-Resistant strains*
 - *Highly virulent strains*

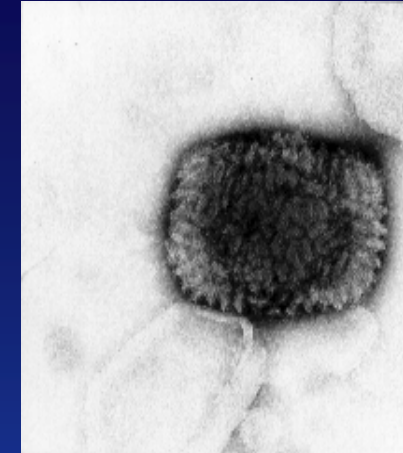
Quiz for a champion: What do ?

50 kg of Anthrax spores, released under the wind, by a plane over a urban population of 5,000,000 ?

- Sicken > 250,000 people
- Kill > 100,000 people

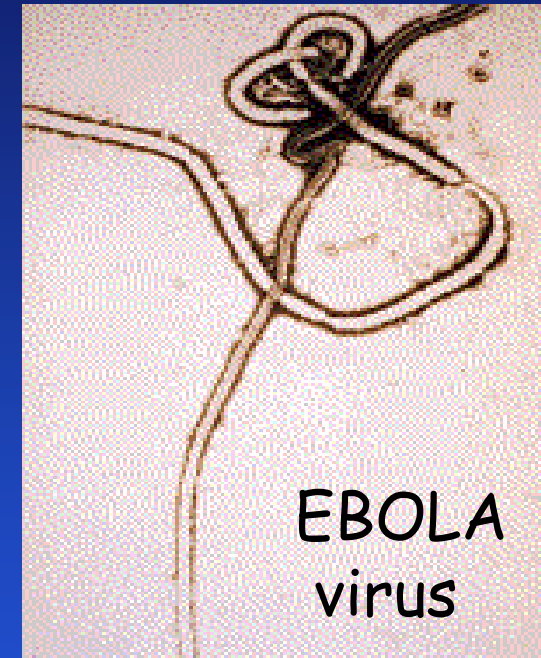
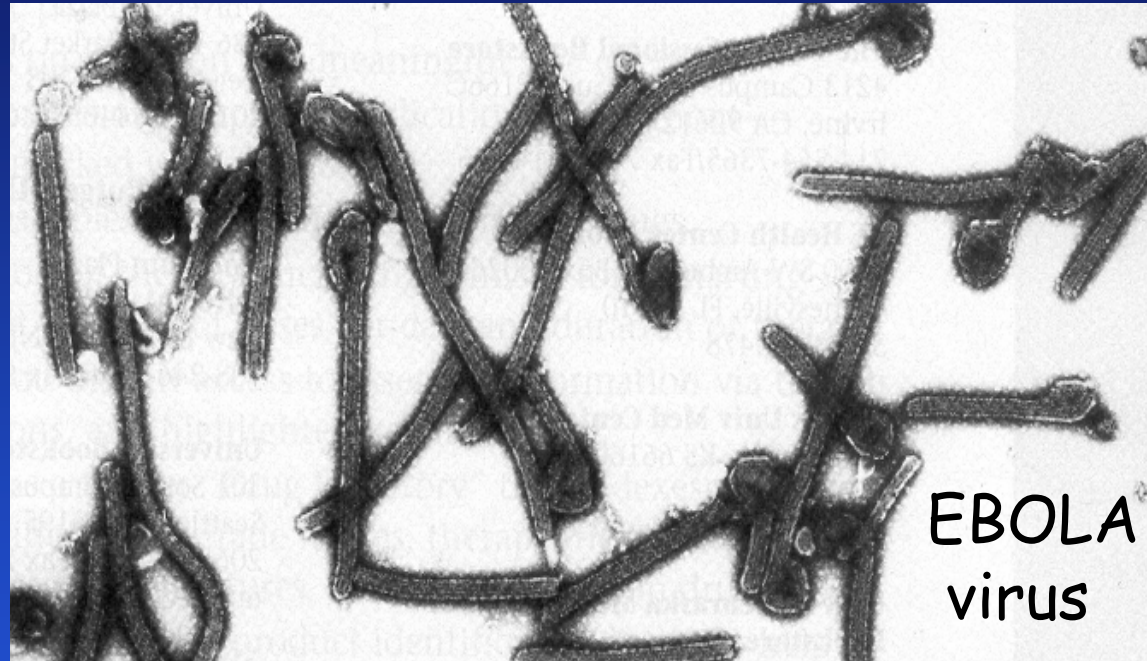
WHO, 1970

Smallpox, a weapon of horror



- 1980 - Global eradication
- Humans were only known reservoir
- Person-to-person transmission (aerosol/contact)
- Most contagious before onset of rash
- Up to 30% mortality in unvaccinated
- Immunity not life-long

Hemorrhagic Fever Viruses : Apocalyptic Agents ?



- **Filo- (Ebola, Marburg), Arena-, Bunya- and Flaviviruses**
- Spectacular in presentation, mysterious, exotic, frightening
- High fatality ratio: virus dependant (10-90%)
- Few or no therapeutic options

Bioterrorism, how serious is the threat ?

- > 11 September 2001, USA
- Bioterrorism:
 - A remote risk became a real possibility
- Letters with spores of *B.anthraxis*
 - Few cases, 5 deaths
 - Rather biocrime than bioterrorism
- Several lessons have been learned

Lessons learned

- Relatively easy to produce contagious material,
 - But, not easy to manufacture the adequate « spores »
 - Some organisms (*B.anthraxis*) easy to find worldwide
- Effect of a limited attack
 - Copycat phenomenon in other countries (letters claiming to contain contagious material)
 - Labs had to face unknown problems
 - How to use prophylactic antibiotics
- No country was really prepared

Our eyes were opened

Are we prepared ? Where do we go from here ?

Planning - Coordination - Preparedness

- Medical response
- Priorities for Public Health infrastructure
- Laboratory response

Are we prepared ?

- Emergency preparedness and response
- Enhance surveillance and epidemiologic capacity
- Enhance laboratory capacity, network for BT
- Consensus protocols, update
- Education, training, information
- Link between partners
- Communication is vital
- Create a national stockpile
- New tools for medical management + diagnostic

Laboratory Response Network for Bioterrorism



To provide an organized response system (detection-diagnosis)

Levels:

D

BSL-4

C

BSL-3

B

BSL-3

A

BSL-2
Clinical labs

For First Responders

The challenge =
To recognize the disease !

Why ?

Timely initiation of appropriate and
measured response is critical

Clues to a potential biocrime

Epidemiologic clues + characteristic syndromes
→ Natural or nefarious outbreak ?

- Geographical pattern
- Outbreak of rare or novel disease
- Outbreak outside an endemic area
- Seasonal disease during an off-season time
- Unusual resistance profile
- Unusual clinical presentation or age distribution, etc

What is next ?

Conclusion

Use of biological agents for terrorism

=

Real threat but low probability event

=

Very large, potentially devastating
consequences

BE PREPARED !!