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.anthracis)
  - WWII, Japanese Program over Manchuria (B.anthracis, 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
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
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: Navada, non-lethal strain of B.anthracis
- 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
(Anounced 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:**
  - Capsule and toxin
- On sheep's blood agar
  - Non-hemolytic
  - Large colonies
  - Firmly adherent
  - Non-motile
  - Non encapsulated
  - Gram+ spore forming rods

On nutrient agar/CO₂
- 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 \( \oplus \) edema toxin
    - \textit{Adenylate cyclase activity \( \oplus \) fluid accumulation}
    - Lethal factor + protective antigen \( \oplus \) lethal toxin
      - \textit{Zinc metalloprotease activity \( \oplus \) stimulates macrophages to release TNF\( \alpha \) and interleukin-1\( \beta \) + 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 (woolsorter’s disease)
Anthrax in Europe

Figure 1 Distribution of animal and human cases in Europe. The number of known or suspected animal cases of anthrax can be used to categorize the anthrax situation in countries. Countries in which more than 25 human cases were reported between 1991 and 1995 are shown (four-pointed star), as are locations where cases reported in the medical literature and acquired abroad or from imported products were diagnosed, 1991–2000 (five-pointed star). Adapted from Hugh-Jones [17], Paulet et al. [21], Ringertz et al. [22], and Mallon and McKee [23].
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 vesicules (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*

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

<table>
<thead>
<tr>
<th>Cases</th>
<th>Florida</th>
<th>NY city</th>
<th>New Jersey</th>
<th>Washington</th>
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<td><strong>Confirmed</strong></td>
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<td>3</td>
<td>0</td>
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<td>1</td>
<td>2</td>
<td>5</td>
<td></td>
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<tr>
<td><strong>Suspected</strong></td>
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<td>0</td>
<td>3</td>
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<tr>
<td>Inhalational</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>22</td>
</tr>
</tbody>
</table>

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. anthracis*
    - 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. anthracis*) 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  Clinical labs
A  BSL-3

Levels: D, C, B, A

Clinical labs

BSL-3

BSL-4

BSL-3
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 !!