Biological and Chemical Agents of Terrorism

“Desired” Characteristics of a Bioweapon

1. High morbidity and mortality
2. Person-to-person transmission
3. Ability to cause large outbreaks
4. Low infectious dose and high infectivity, especially by aerosol
5. Effective vaccine unavailable or in limited supply
6. Potential to cause high anxiety
7. Availability of pathogen or toxin
8. Large scale production
9. Environmental stability

Biological Agents of Highest Concern
Category A

1. Variola major (Smallpox)
2. *Bacillus anthracis* (Anthrax)
3. *Yersinia pestis* (Plague)
4. *Francisella tularensis* (Tularemia)
5. Botulinum toxin (Botulism)
6. Filoviruses and Arenaviruses (Viral hemorrhagic fevers)
Other Biological Agents of Concern
Category B

- Coxiella burnetti (Q fever)
- Brucella species (brucellosis)
- Burkholderia mallei (glanders)
- Burkholderia pseudomallei (melioidosis)
- Rickettsia prowazekii (Typhus fever)
- Alphaviruses (viral encephalitis)
- Ricin toxin (castor beans)
- Epsilon toxin of Clostridium perfringens
- Staphylococcus enterotoxin B
- Chlamydia psittaci (psittacosis)
- Water safety agents
- Food safety agents

Other Biological Agents of Concern
Category C

- Nipah virus
- Hantaviruses
- Tickborne hemorrhagic fever viruses
- Tickborne encephalitis viruses
- Yellow fever
- Multidrug-resistant tuberculosis

Biological Agents of Terrorism
Smallpox (Variola)

Smallpox as a Bioweapon

• British use of blankets from smallpox patients
• Aralsk accident – Soviet Union
  Vozrozhdeniye Island in summer 1971
  • Monterey Institute Report, 2002
  • Soviets stockpiled > 20 tons in the 1970s (Alibek p.112)

Preparation as a Bioweapon

• Soviets cultured highly virulent strains
• Mixed with stabilizing agents, keeping it viable for more than a year
• Aralsk accident suggests that variola virus strains have been developed which are stable in the air and can survive considerable UV exposure
Microbiology

- Orthopox virus
- Related viruses include monkeypox, camelpox, and ectromelia (mousepox)
- Not related to chickenpox (varicella-zoster)

Strains

- India-1967
  - Soviet strain isolated from Indian outbreak in 1967
  - Monkeys exposed to India-1 developed symptoms within 1 – 5 days

  Source: Alibek, K. Biohazard p111 et seq.

Genetic modifications

- Mousepox - addition of a gene that produces IL-4 [interleukin 4], could enable the virus to overcome both natural and vaccine-induced immunity. IL-4 shut down cell-mediated immunity. 60% of the previously immune mice died.
- Veepox and Ebolapox – genetic chimaeras
Specimens

- Digital photographs of rash to state health department
- Scrapings of skin lesions, papular, vesicular or pustule fluid, crusts, blood samples and tonsillar swabs – ONLY after consultation with the state health department
- Specimens should be collected ONLY by a person who has been successfully vaccinated within the past 3 years
- See Guide D in Smallpox Response Plan

CDC Smallpox | Laboratory Testing

Smallpox

- Transmission
  - Person-to-person – easily transmissible
  - Fomites
  - Aerosol device
- Period of Communicability
  - from the earliest lesions to last scab dropping off
- Infective aerosol dose
  - ID₅₀< 5 variola virions of India-1967
  - 10-100 organisms
- Incubation period
  - 7-19 days typically 10-14 days

Smallpox

- Duration of illness
  - 4 weeks
- Mortality
  - 20 – 50% in unvaccinated people
- Persistence of organism
  - Variola can remain viable and infectious for a long time in cool temperatures
  - Important for infection control and disposal of bodies.
- Vaccine efficacy
  - >95% produce Abs
Vaccine

• Vaccinia – different from variola virus but related
• Induces protection against variola and monkeypox viruses
• Smallpox Vaccination slides
• Effective in preventing or decrease the severity of disease if administered within 3-4 days of exposure

Treatment

• No specific treatment known
• Cidofovir IV possibly
• Other antivirals ??

Infection Control

• Patient isolation
• Respiratory/Airborne and contact precautions
• Contacts - Quarantine and observe
Infection Control

- Aerosol infection control procedures
- Disinfection of surfaces: 10% bleach
- Disposal of bodies by cremation as soon as possible
  - Variola survives in cool, humid conditions such as ice arenas – do not store there

References

- CDC Smallpox Home – the most up-to-date information
- CDC Rash Testing Protocol Revised Jan 2004
- Medical Aspects of Chemical and Biological Warfare-Smallpox
  Department of Defense

Anthrax
**Anthrax as a Bioweapon**

- Japanese – Unit 731 worked on developing anthrax as a weapon and tested on Allied soldiers 1934 -1945
- Svedlovsk – accidental release of anthrax from a bioweapons facility -April 1979 to May 1979
- Aum Shinrikyo – 1990 to 1995 staged a dozen unsuccessful attacks, many using *B. anthracis*
- US – October 2001 Anthrax spores sent via mail

**Preparation as a Bioweapon**

- Preparation
  - Culture and concentrate large quantities in liquid culture
  - Induce spore formation by drying/reduce essential nutrients
  - Grind to a fine powder (1 – 5 microns) without killing it
- Particle size of < 3 microns to enter the alveoli

Reference: Anthrax as a Biological Weapon, 1999

**Anthrax as Bioweapon**

“A strategic attack against a densely populated city using 50 kilograms of anthrax spores, which have a mortality rate of about 90 percent, could result in about 100,000 fatalities.”

Dr. Ken Alibek November 6, 1998
Microbiology

• Gram positive bacillus
• Non-hemolytic
• Aerobic
• Form endospores when environment is stressful
• Spores germinate in a suitable environment

Virulence

• Capsule
  • Antiphagocytic
  • Without this the bacteria are attenuated
• Toxins – plasmid coded virulence factors
  • Edema Toxin = Protective Antigen + Edema Factor
  • Lethal Toxin = Protective Antigen + Lethal Factor
• Others: hemolysins, phospholipases

Toxins

• Edema factor is an adenylate cyclase, similar to the pertussis toxin
• Edema toxin increases membrane permeability by ↑ cAMP and also ↓ ATP in MΦ and neutrophils
• Exact target for lethal toxin currently unknown
Microbiology - Strains

• Various strains of *B. anthracis* have been developed for different purposes
  • Ames – widely used in the US; also the strain used in the 2001 anthrax letters
  • Sterne – attenuated strain used for development of animal vaccines
  • Anthrax 836 – developed by the Soviets
  • Ob lensk strain – genetically engineered to be resistant to vaccine induced immunity

Genetic modification of *B. anthracis*

• Genes have been added to alter the bacteria making them immune from vaccine protection
  • Ref: (Vaccine 15(17-18):1846-1850, Dec 1997, Pomerantsev AP, Staritsin NA, Mockov YV, Marinin LI., Expression of cereolysine ab genes in *Bacillus anthracis* vaccine strain ensures protection against experimental hemolytic anthrax infection

Anthrax

• Transmission
  • Cocontaminated animal skins, tissue or soil
  • Aerosol device
• Period of Communicability:
  • Not communicable between humans
• Infective dose
  • ID_{50} is about 8,000 – 40,000 spores, but may be lower
• Incubation period
  • 1 – 7 days, although maybe as long as 60 days [4 days with range 4 – 6 days in US 2001 attacks]
Anthrax

- Duration of illness
  - variable
- Mortality
  - 20 – 50% in unvaccinated people
  - 60% in the 2001 US attacks with appropriate therapy
  - Near 100% if untreated
- Persistence of organism
  - Years in the soil
- Vaccine
  - Effective against cutaneous and inhalational anthrax

Specimen collection

- Notify lab that anthrax is suspected
- Blood or CSF
- Nasal swabs are not useful, other than for epi studies

Laboratory Tests

- Blood culture
- Blood smear – Gram stain
- Direct Fluorescent Antibody (DFA) assay
- Polymerase Chain Reaction (PCR) to detect antigen
Vaccine

- Made from an avirulent, non-capsulated strain of *B. anthracis* which expresses PA
- 3 doses 2 weeks apart, then three doses at 6, 12 and 18 months. Then annual booster
- Only for persons 18 – 65 years

Treatment

- Assuming the anthrax is sensitive to doxycycline and ciprofloxacin
- Doxycycline is preferred (to reduce risk of ciprofloxacin-resistance)

Post-Exposure Prophylaxis

- Doxycycline (preferred) or ciprofloxacin
- 60 days if antibiotic used alone
- Vaccine and antibiotics
  - 3 doses of vaccine
  - Antibiotic for 7 – 14 days after 3rd dose of vaccine
Decontamination

• Decontamination of environmental surfaces
  • A number of different sporocidal agents are available depending upon the environmental surface/item
  • Hypochlorite (bleach) is effective for surfaces
  • Ref: Inactivation of anthrax spores

Quarantine/Isolation

• Standard barrier precautions
• No need to isolate
• No need to vaccinate contacts

References

• Anthrax Lab Protocol
• JAMA -- Anthrax as a Biological Weapon, 1999
• JAMA -- Anthrax as a Biological Weapon, Update, 2002
• Fact Sheet for Parents-Anthrax
• Fact Sheet for Clinicians-Anthrax
• Acceptable Biological Specimens Needed for Testing for Anthrax
• CDC Anthrax References
• BT related anthrax attack
  www.cdc.gov/ncidod/eid/vol7no6/jernigan.htm
**Yersinia pestis**

**Yersinia pestis as a Bioweapon**
- Invading Tartar armies catapulted the bodies of plague victims into the city of Kaffa
- Japanese Unit 731 experimented with plague on POWs; also dropped canisters of plague infected fleas in Manchuria
- US tried to manufacture plague as a weapon but could not retain virulence
- Soviets developed a strain which retained virulence in aerosol

**Preparation as a Bioweapon**
- Soviets powdered *Y. pestis* and placed in small spray cans.
- Soviet arsenal was 20 + tons of powdered *Y. pestis*
Microbiology

• Gram negative rods or coccobacilli
• Do not form spores
• Characteristic “safety pin” appearance on Gram, Wright or Wayson stains
• Carbohydrate-protein envelope called capsular antigen F1 - develops above 33°C
• Sensitive to sunlight and heating

Virulence

• Virulence factors encoded on the chromosome and 3 plasmids
  • Damage host cells
  • Inhibit phagocytosis and other host defence mechanisms
• Soviets were reported to have developed multi-drug resistant strains of *Y. pestis*

Diagnosis

• Based on symptoms and exposure history
• Treatment should not wait for lab results
• Specimens
  • Peripheral blood smear, sputum & bubo aspirate
  • Gram, Wright or Wayson stain
  • DFA for capsular antigen F1
  • Culture of blood, bubo aspirate, sputum and CSF (must wait 48 hours)
• Confirm by specific ‘phage lysis
Yersinia pestis – Direct Fluorescent Antibody (DFA)

Specimens

- Peripheral blood smear, sputum and bubo aspirate
  - Gram, Wright or Wayson stain
  - DFA for capsular antigen F1 Culture of blood, bubo aspirate, sputum and CSF (must wait 48 hours)
- Culture of blood, bubo aspirate, sputum or CSF (must wait at least 48 hours)

Pneumonic Plague

- Transmission
  - Respiratory Transmission via
    - Large droplets or fomites
    - Human-to-human: close contact 2 – 5 feet
- Period of Communicability
  - Until 48 hours after initiation of antibiotics and favorable progress
- Infective dose
  - 100-500 organisms
- Incubation period
  - 1-3 days, maybe as long as 6 days
Pneumonic Plague
- **Duration of illness**
  - 1-6 days
- **Mortality**
  - 100% fatal unless treatment is initiated early
- **Persistence of organism**
  - *Y. pestis* does not pose an environmental hazard
    - Very sensitive to sunlight and heating
    - Does not survive outside the host for long
- **Vaccine**
  - Ineffective against pneumonic plague
  - No longer available in the US

Treatment - Adults
- Contained casualty setting
  - Streptomycin
  - Gentamicin
  - Alternatives
    - Chloramphenicol
    - Doxycycline
    - Ciprofloxacin
- For Mass Casualty settings
  - Doxycycline; Ciprofloxacin

Plague – Infection Control
- Respiratory isolation for patients for first 48 hours of antibiotic treatment and clinical improvement
- Contacts
  - Close contacts – doxycycline or other approved antibiotic
  - Monitor for signs of disease
  - Not isolation
Environmental Control

• Terminal cleaning of hospital rooms

• No need for environmental decontamination

References

• CDC Plague Information
• CDC Laboratory Protocol for Y. pestis
• Plague – a military review of the medical and epidemiological aspects of plague
• Plague as a Biological Weapon. JAMA 283 (17):2281-2290, May 3, 2000

Francisella

*tularensis*
**F. tularensis as a Bioweapon**

- Studied by Japanese, US, Canada & UK during WW2
- Aerosolized tularemia stockpiled by the US military in the late 1960's (destroyed by 1973)
- The Soviet Union produced antibiotic and vaccine resistant strains into the early 1990s.
- 50 kg dispersed over a city of 5 million would result in 19,000 deaths and 250,000 incapacitating illnesses

**Preparation as a Bioweapon**

- Vaccine-resistant and antibiotic-resistant strains have been developed
- Dried and aerosolized
- Considered by the Soviets as an ideal weapon as it can rapidly incapacitate the health system

**Microbiology**

- Gram negative coccobacillus
- Survival
  - Does not form spores, but is hardy, especially at low temperatures.
  - Can survive for weeks in the environment (water, hay, carcasses)
- Two subspecies (biovars) of *F. tularensis*.
  - *F. tularensis* subsp. Tularensis (type A) is virulent
  - *F. tularensis* subsp. Holarctica/Palaearctica (type B) is avirulent
Tularemia

- **Transmission**
  - From infected animals or contaminated soils
  - Bite of arthropods including ticks
  - Aerosols?

- **Period of Communicability:**
  - No human-to-human transmission

- **Infective dose**
  - 10-50 organisms for *F. tularensis* Tularensis

- **Incubation period**
  - 1-21 days (average: 3-5 days)

- **Duration of illness**
  - ~2 weeks, more if untreated even months

- **Mortality**
  - Untreated 30 – 60 % mortality

- **Persistence of organism**
  - Months in cold, moist soil
  - Probably a short duration in an indoor environment or if aerosolized outside

- **Vaccine**
  - Not generally available in the US

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**Diagnosis**

- Respiratory secretions and blood
- Smears – Gram or Wright stain, direct fluorescence, or immunohistochemical staining
- Cultures for confirmation
  - Pharyngeal washings, sputum – not blood
- ELISA, agglutination, PCR, PFGE and other specialized techniques useful for species confirmation and epi studies
Treatment and Prophylaxis

• Treatment for 10 days
  • Aminoglycosides: Streptomycin is the drug of choice, but gentamicin may be more readily available
  • Fluoroquinolones such as ciprofloxacin have demonstrated activity against F. tularensis

• Prophylaxis for 14 days
  • Doxycycline or ciprofloxacin

Infection Control

• Drainage and secretion precautions
• Isolation of patient or contacts not needed
• BSL-2 laboratory conditions
• Standard disinfection of clothes or linens used by patients

References

• CDC Tularemia Information
• Tularemia as a Biological Weapon, JAMA 285 (21): 2763, 2001
• Tularemia-Ch 24-DOD
• Alibek, K. Biohazard. 1999 Delta Books
**Clostridium botulinum**

*toxin*

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**C. botulinum toxin as a Bioweapon**

- Aerosol
- Food or water contamination
- Botulinum toxin found in a terrorist safe house of the Red Army Faction in Paris¹
- Iraq was reported as having botulinum toxin²

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**Preparation as a Bioweapon**

- Inhalational botulism demonstrated in primates¹
- Attempted uses as a terrorism weapon¹
- Toxin absorbed through mucosal membranes into bloodstream
**Microbiology**

- *Cl. botulinum* is a Gram positive, spore-producing, anaerobic bacillus
- Produces 7 antigenically distinct toxins, A – G
- Most outbreaks in humans due to toxins A, B and E
- Not transmitted human-to-human

**Botulinum toxin**

- **Transmission**
  - Not contagious and not transmitted between person
  - Waterborne transmission considered unlikely
- **Infective dose**
  - 1 gm of crystalline toxin can kill > 1 million people
  - Lethal amount for a 70kg (154 lb) human estimated to be 0.70 – 0.90 µg inhalationally or 70 µg orally
- **Incubation period**
  - 12-36 hours for ingested toxin
  - 12-72 hours for inhaled toxin?

**Botulinum toxin**

- **Duration of illness**
  - Death in 24-72 hours if not appropriately treated
- **Mortality**
  - High without respiratory support
- **Persistence of organism**
  - Toxin is easily destroyed by heat
  - Aerosolized toxin estimated to decay at <1% to 4% per min.
- **Vaccine**
  - Anti-toxin against specific toxin – no cross-protection
  - Vaccine – toxoid available through an IND only
Specimen collection

• Contact the MDCH before collecting specimens
• Foodborne
  • Serum, Stool, Gastric aspirate, Vomitus, Suspected food/drink
• Inhaled
  • Nasal swabs taken within 24 hours of exposure for immunologic testing

Laboratory Tests

• Mouse bioassay
  • Takes time (up to 4 days)
  • Can be confounded by various factors e.g. use of saline enema, medications taken by patient
• Anaerobic culture of Cl. botulinum
  • Takes longer (7 – 10 days)
• ELISA
  • From nasal swabs taken within 24 hours

Treatment

• Passive immunization with specific antitoxin
• Supportive medical care
Infection Control

• Standard precautions
• Isolation not needed, but persons with suspected meningitis require droplet precautions
• Contacts: no actions
• Others exposed: purge with cathartics, gastric lavage and/or high enema & close observation

Decontamination

• Clothing and skin – wash with soap and water after exposure
• Contaminated objects can be
  • Left isolated and avoided until toxin has decayed
  • Cleaned with 0.1% hypochlorite solution (bleach)
• Heating to 85°C for 5 minutes will detoxify
• Contaminated food or drink

References

• CDC Botulinum Toxin Information
• Botulinum Toxins-Ch. 33- DOD
Hemorrhagic Fever Viruses

- Arenaviruses
  - Lassa
  - Machupo
  - Junin
- Bunyaviruses
  - Rift Valley fever
- Filoviruses
  - Ebola
  - Marburg
- Flaviviruses
  - Kyasanur Forest

HF viruses as bioweapons

- Marburg, Ebola, Lassa and others weaponized by Soviet Union
- Yellow fever & Rift Valley fever viruses weaponized by the US (also N. Korea?)
- Successful infection of primates via aerosols
Microbiology

- RNA viruses with a lipid envelope.
- Survival is dependent on host - natural reservoir.
- Human cases/outbreaks of VHF occur sporadically and irregularly.
- Humans are not the natural reservoir.
- Humans-to-human transmission can occur with some of the viruses.

Hemorrhagic Fever Viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Person-to-person</th>
<th>Incubation period</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebola</td>
<td>Yes</td>
<td>2 – 21</td>
<td>50 - 90%</td>
</tr>
<tr>
<td>Marburg</td>
<td>Yes</td>
<td>2 - 14</td>
<td>23 – 70%</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>Yes</td>
<td>5 - 16</td>
<td>15 – 20%</td>
</tr>
<tr>
<td>New World Arena</td>
<td>Yes</td>
<td>7 - 14</td>
<td>15 – 30%</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>No</td>
<td>2 - 6</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Omsk HF</td>
<td>No</td>
<td>2 - 9</td>
<td>0.5 – 10%</td>
</tr>
<tr>
<td>Kyasanur Forest</td>
<td>No</td>
<td>2 - 9</td>
<td>3 – 10%</td>
</tr>
</tbody>
</table>

Hemorrhagic Fever Viruses

• Period of Communicability
  • Duration of illness from blood, tissues and fluids from an infected person
• Infectious dose
  • 1 – 10 virus particles
• Duration of illness
  • 7-16 days
• Persistence of organism
  • Unstable in the environment
• Vaccine
  • No vaccines
Diagnosis

• Diagnosis based on clinical criteria and judgment
• Tests on serum and plasma available only at BSL-4 laboratories e.g. CDC or USAMRIID
  • ELISA (Ag-capture and IgM detection by Ab-capture)
  • RT-PCR (inactivate samples with chloroform and methanol)
  • Viral culture (takes 3 – 10 days)

Specimen shipping

• Sample for serology - 10-12 ml
  • ship on dry ice
• Tissue for immunohistochemistry
  • formalin-fixed or paraffin block
  • ship at room temperature
• Tissue for PCR/virus isolation
  • ante-mortem, post-mortem; ship on dry ice
  • ship serum cold or on dry ice in a plastic tube

Treatment and Prevention

• No approved antivirals
  • Ribavirin has some activity against Arenaviruses and Bunyaviruses, but not against Filo- or Flavi-viruses (IND protocols)
• Supportive care
• No vaccines
Infection Control and Decontamination

VHF viruses are highly infectious!!

• Infection Control
  • Stringent barrier nursing
  • Hazard labeling of specimens sent to BL-4 lab
• Decontamination
  • Autoclaving
  • Liberal disinfection - hypochlorite (1:100 dilution household bleach) or phenolic disinfectants

References

• CDC Viral Hemorrhagic Fevers Information
• VHF-Medical Management DOD
• Viral Hemorrhagic Fevers as a Bioterrorism Weapon-JAMA 287(18);2391-2405 May 8, 2002

Further References

• CDC Emergency Preparedness & Response Site
• Public Health Foundation: Home
• US Dept of Defense “Blue Book”
• Medical Aspects of Chemical and Biological Warfare
• MDCH Clinical Aspects of Critical Biological Agents
Further References

- Center for the Study of Bioterrorism and Emerging Infections - Saint Louis University, School of Public Health
- CDC Video: "The History of Bioterrorism"

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- CDC Public Health Image Library