

## Biological and Chemical Agents of Terrorism

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### “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

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### Biological Agents of Highest Concern Category A

- Variola major (Smallpox)
- *Bacillus anthracis* (Anthrax)
- *Yersinia pestis* (Plague)
- *Francisella tularensis* (Tularemia)
- Botulinum toxin (Botulism)
- Filoviruses and Arenaviruses (Viral hemorrhagic fevers)

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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

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Other Biological Agents of Concern  
Category C

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

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Biological Agents  
of Terrorism

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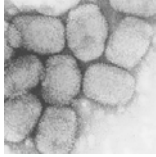
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## Smallpox (Variola)



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## 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)

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## Preparation as a Bioweapon



- Soviets cultured highly virulent strains
- Mixed with stabilizing agents, keeping it viable for more than a year<sup>1</sup>.
- Aralsk accident suggests that variola virus strains have been developed which are stable in the air and can survive considerable UV exposure

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## Microbiology



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

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## 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.

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## 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<sup>1</sup>.

- Veepox and Ebolapox – genetic chimaeras

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## 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](#)

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## 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
  - ID<sub>50</sub> < 5 variola virions of India-1967
  - 10-100 organisms
- Incubation period
  - 7-19 days typically 10-14 days

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## 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

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## 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<sup>1</sup>

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## Treatment



- No specific treatment known
- cidofovir IV possibly
- other antivirals ??

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## Infection Control



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

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## 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

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## 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

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## Anthrax



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## 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

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## 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](#)

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## 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

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## Microbiology

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



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## 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



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## Toxins

- Edema factor is an adenylate cyclase, similar to the pertussis toxin
- Edema toxin increases membrane permeability by  $\uparrow$  cAMP and also  $\downarrow$  ATP in M $\Phi$  and neutrophils
- Exact target for lethal toxin currently unknown



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## 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
  - Oblenski strain – genetically engineered to be resistant to vaccine induced immunity!

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## 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

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## Anthrax



- Transmission
  - Cocontaminated animal skins, tissue or soil
  - Aerosol device
- Period of Communicability:
  - Not communicable between humans
- Infective dose
  - ID<sub>50</sub> 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]

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## 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

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## Specimen collection



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

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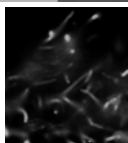
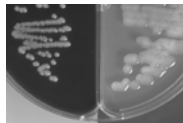
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## Laboratory Tests



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



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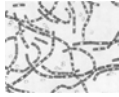
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## Vaccine



- 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

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## Treatment



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

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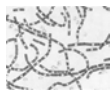
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## Post-Exposure Prophylaxis



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

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## Decontamination



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

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## Quarantine/Isolation



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

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## 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](http://www.cdc.gov/ncidod/eid/vol7no6/jernigan.htm)

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# *Yersinia pestis*



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## *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

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## Preparation as a Bioweapon



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

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## 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



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## 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*

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## 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

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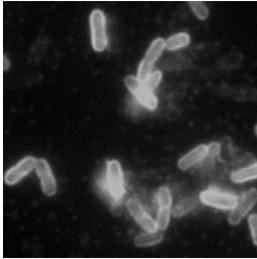
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Yersinia pestis – Direct Fluorescent Antibody (DFA)



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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)

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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

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## 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

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## Treatment - Adults



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

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## 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

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## Environmental Control



- Terminal cleaning of hospital rooms
- No need for environmental decontamination

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## 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](#)
- *Yersinia pestis* - Etiological Agent of Plague. A review of the microbiology of *Y. pestis*. Clin. Micro. Review 10( 1) 35 – 66 Jan 1997

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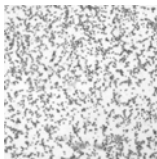
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## *Francisella*



*tularensis*

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## *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

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## 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

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## 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/Palaeartica* (type B) is avirulent

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# 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)

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# Tularemia



- 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

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## 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

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## 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

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## References



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

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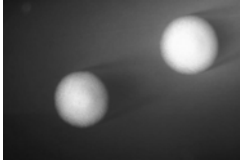
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## *Clostridium botulinum*



*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 Paris<sup>1</sup>
- Iraq was reported as having botulinum toxin<sup>2</sup>

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### Preparation as a Bioweapon



- Inhalational botulism demonstrated in primates<sup>1</sup>
- Attempted uses as a terrorism weapon<sup>1</sup>
- Toxin absorbed through mucosal membranes into bloodstream

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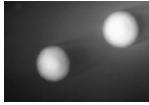
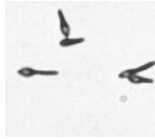
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## Microbiology

- *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



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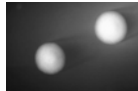
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## Botulinum toxin

- 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 inhaled or 70 µg orally
- Incubation period
  - 12-36 hours for ingested toxin
  - 12-72 hours for inhaled toxin?



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## 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



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## 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

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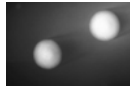
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## Laboratory Tests



- 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

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## Treatment



- Passive immunization with specific antitoxin
- Supportive medical care

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## 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

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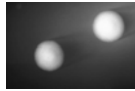
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## Decontamination



- 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

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## References



- CDC Botulinum Toxin Information
- Arnon et al. Botulinum Toxin as a Biological Weapon - JAMA 285; 1059-1070. 2001
- Botulinum Toxins-Ch. 33- DOD

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## Hemorrhagic Fever Viruses

**Arenaviruses**  
**Bunyaviruses**



**Filoviruses**  
**Flaviviruses**

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## Hemorrhagic Fever Viruses



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

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## 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

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## 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.

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## Hemorrhagic Fever Viruses

Virus	Person-to-person	Incubation period	Mortality %
Ebola	Yes	2 - 21	50 - 90%
Marburg	Yes	2 - 14	23 - 70%
Lassa fever	Yes	5 - 16	15 - 20%
New World Arenavirus	Yes	7 - 14	15 - 30%
Rift Valley fever	No	2 - 6	< 1%
Omsk HF	No	2 - 9	0.5 - 10%
Kyasanur Forest	No	2 - 9	3 - 10%

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## 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

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## 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)

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## 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

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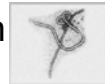
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## Treatment and Prevention



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

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## 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

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## 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](#)

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## 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](#)

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## Further References



- Center for the Study of Bioterrorism and Emerging Infections - Saint Louis University, School of Public Health
- Control of Communicable Diseases Manual 17<sup>th</sup> Edition. J. Chin Ed. APHA 2000
- CDC Video: "The History of Bioterrorism"

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## Photographic credits

- CDC Public Health Image Library

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