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BIOLOGICAL THREAT REDUCTION

Biological Agents: An Overview

Laboratory Biosafety and Biosecurity Workshop

Cairo, Egypt
3-5 April 2007

www.biosecurity.sandia.gov

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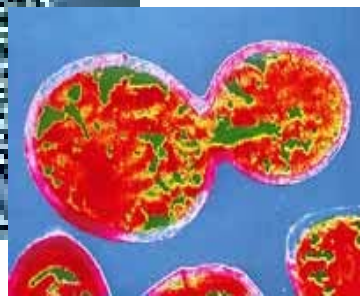
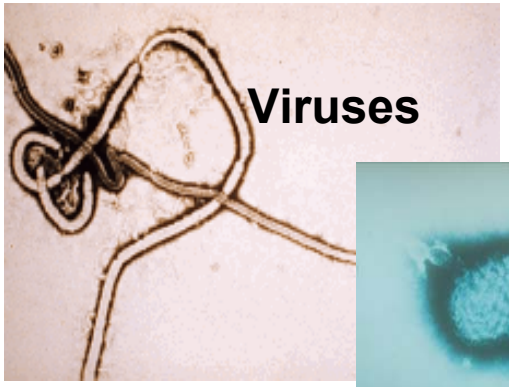
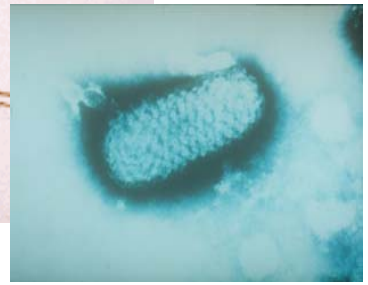
Types of Biological Agents

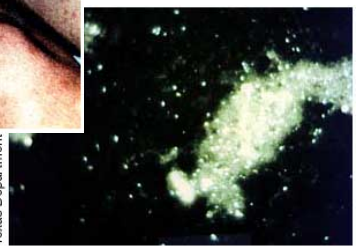



Fungi

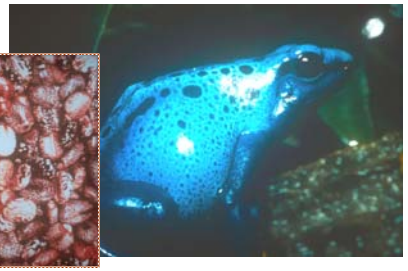



Viruses

Bacteria

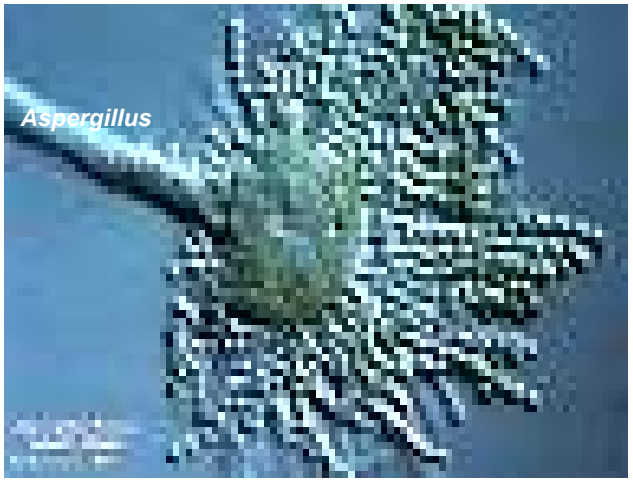


Obligate intracellular parasites:
Rickettsiae and Chlamydia

Proteins:
Toxins and Prions

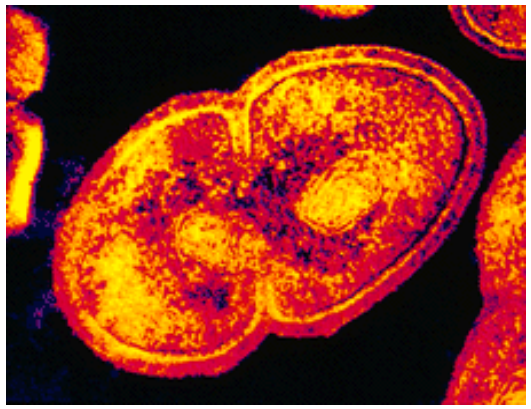
Fungi

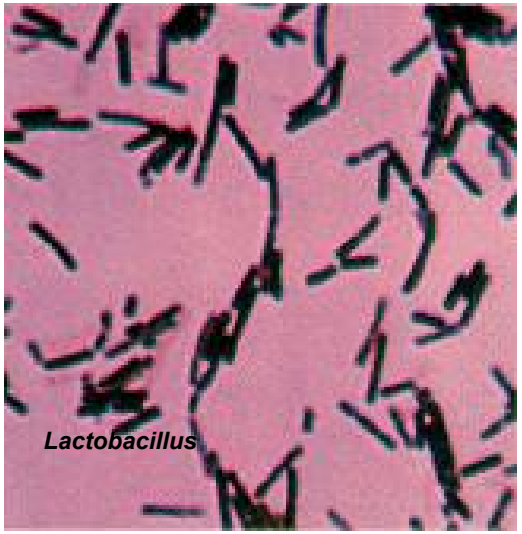


- **Size:** highly variable
- **Complexity:** moderate
- **Chemical:** DNA/RNA, complex metabolic, structural & enzymatic chemistry
- **Toxins:** often important - wide range of mycotoxins
- **Risk:** mainly RG2; C*occidioides, Histoplasma* are RG3
- **Pathology:**
  - Inflammation
  - Granuloma formation
  - Necrosis

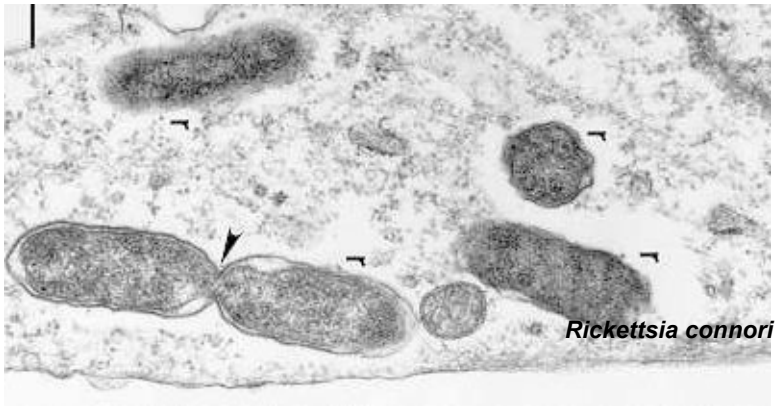
Bacteria

- **Size:** 0.2 μm - 500 μm
- **Complexity:** moderate
- **Chemical:** DNA/RNA, simple structural & metabolic components, limited enzyme systems
- **Toxins:**
  - exo**: diphtheria, botulinum,**
  - endo**: lipopolysaccharide (LPS) of many pathogenic Gm- bacteria**
- **Risk:** RG2, few RG3 (e.g., *B. anthracis*, *Brucella* sp., *F. tularensis*)
- **Pathology:**
  - Septicemia
  - Invasive
  - Cell death





Rickettsiae and Chlamydia

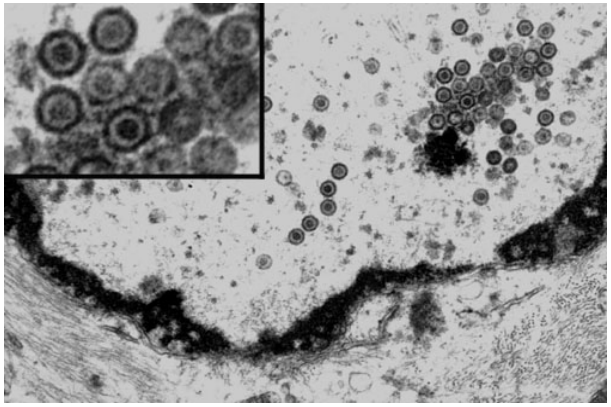

Rickettsia connori

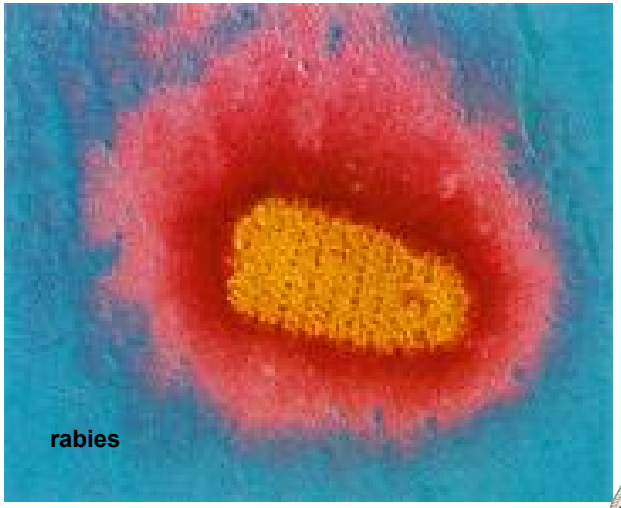
- **Size:**
  - 0.3 μm – 0.6 μm (Rickettsia)
  - 0.3 μm – 1.0 μm (Chlamydia)
- **Complexity:** similar to Gram-negative bacteria
- **Chemical:** DNA/RNA, similar to Gram negative bacteria but can't metabolize energy
- **Toxins:** one (a Chlaymdia) but not important in pathogenesis
- **Risk:** RG3 (Rickettsia) and RG2 (Chlamydia)
- **Pathology:**
  - Vasculitis
  - Cell death
  - Inflammation

Viruses

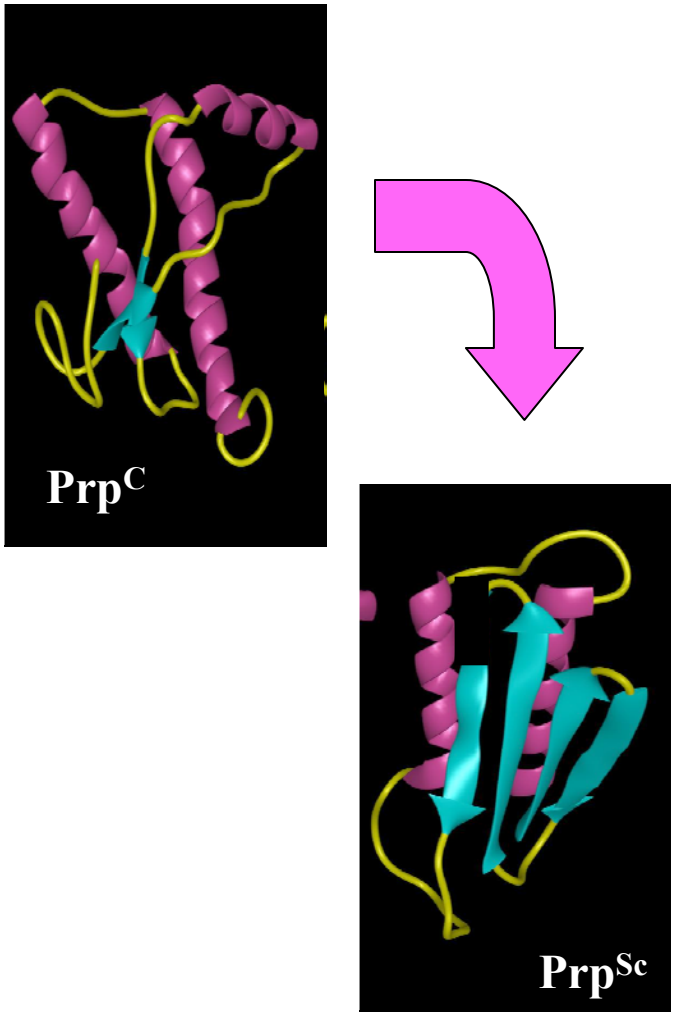
- **Size:** 20 nm - 400 nm
- **Complexity:** simple
- **Chemical:** DNA or RNA; protein capsid; lipids, viral carbohydrates in membrane envelope; few other chemicals
- **Toxins:** no, but genes may code for toxic products
- **Risk**: most RG2, several RG3, a few RG4
- **Pathology:**
  - Inflammation

Herpes simplex





Prions



- "Infectious proteinaceous particles"
- Abnormal isoform of a normal cellular protein
- PRP^{C} ---(increased β sheet)---> PRP^{Sc}
- No prion-specific nucleic acids or virus-like particle
- Risk: RG2 (animal)
  RG3 (human)
- Transmissible spongiform encephalopathy (TSE)

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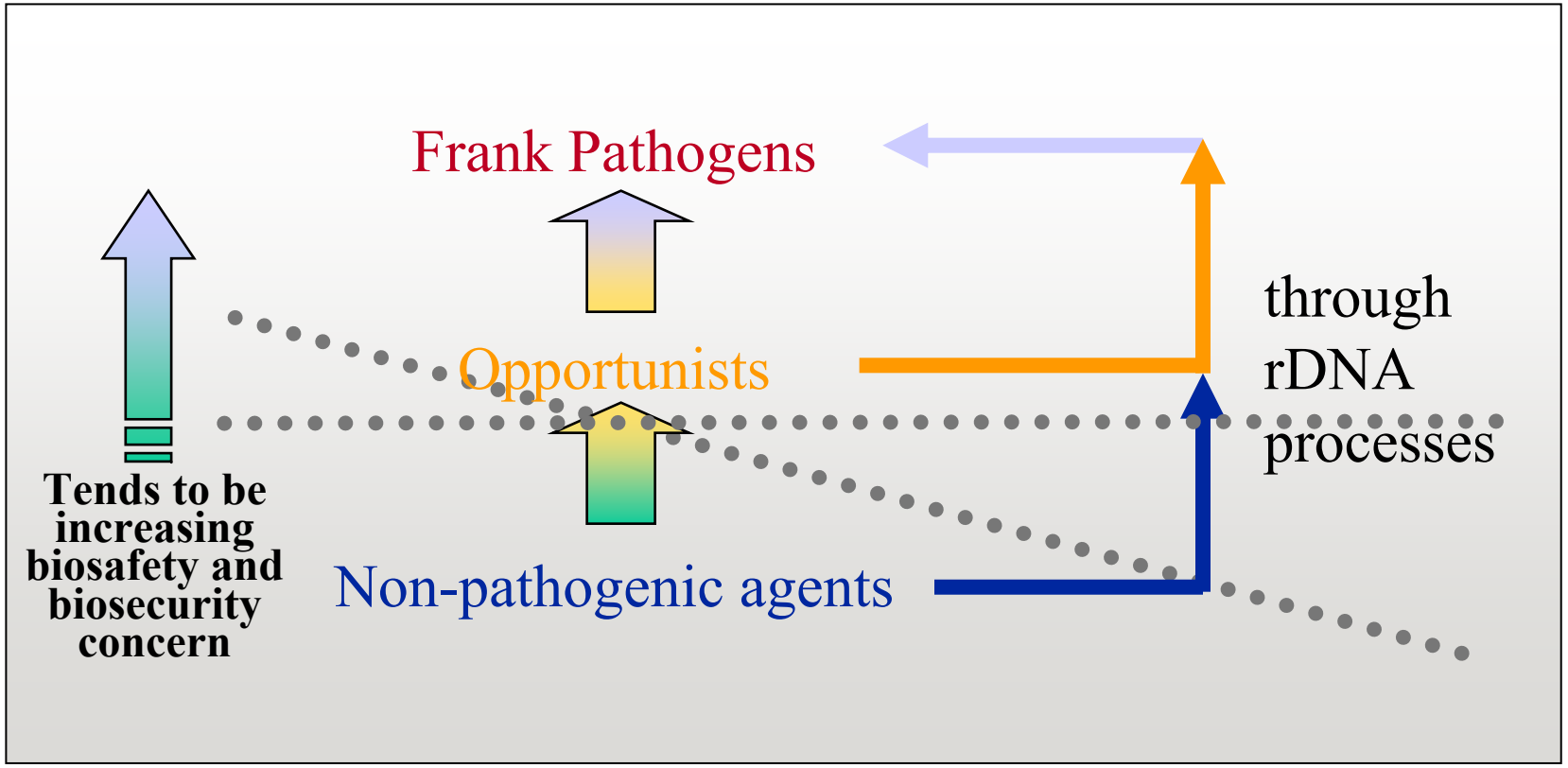
Koch's Postulates

- Robert Koch applied these four precepts in 1884 to establish the etiology of tuberculosis.

- The only way to conclusively demonstrate microbial etiology of a disease:
  - The organism must be found in all animals suffering from the disease, but not in healthy animals;
  - The organism must be isolated from a diseased animal and grown in pure culture;
  - The cultured organism should cause disease when introduced into a healthy animal;
  - The organism must be re-isolated from the experimentally infected animal.

The Dividing Line

- **Based on pathogenicity for humans**



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Sources of Pathogens and Toxins

- Natural environment
  - Endemic
  - Outbreaks
- Legitimate facilities
  - Culture collections
    - More than 400
    - 62 countries
  - Laboratories
  - Pharmaceutical companies
  - Biotechnology companies
  - Clinical and diagnostic laboratories
- Synthetic biology
  - De-novo synthesis
    - Polio virus
    - Ebola virus
  - Modifying less virulent pathogens

ATCC

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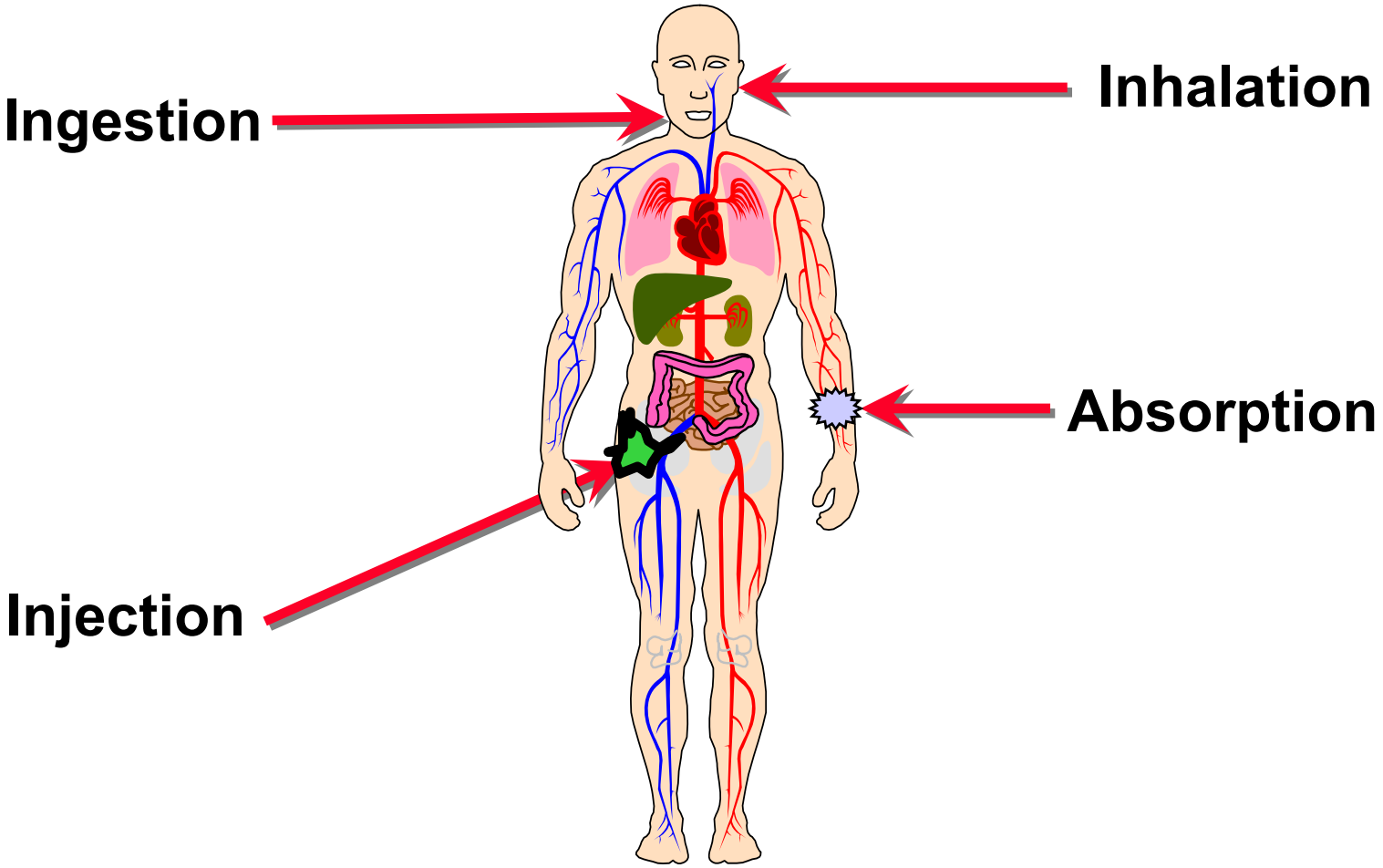
Routes of Entry



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Overview of Selected Agents

- **Fungi**
  - ***Coccidioides immitis***
- **Bacteria**
  - ***Bacillus anthracis***
  - ***Yersinia pestis***
  - ***Francisella tularensis***
  - ***Brucella spp.***
- **Rickettsiae and Chlamydia**
  - ***Rickettsia rickettsii***
- **Viruses**
  - **Hemorrhagic Fever viruses**
  - **Variola major virus**
  - **Avian influenza virus, H5N1**
  - **Foot-and-mouth Disease virus**
- **Proteins**
  - **Botulinum toxin**
  - **Prions**

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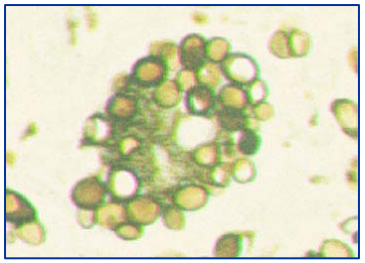
Fungi: *Coccidioides immitis*

- Coccidioidomycosis ("Valley fever" or "desert fever")
- Zoonotic
- Endemic to arid and semi-arid regions of the Western Hemisphere
- Highly stable – easy to produce spores
- Usually asymptomatic, 30-40% of infected become ill
- Not contagious
- 5-10 out of every 1000 infected develop life-threatening infection
- 10th most common laboratory-acquired infection
- In US: BSL3 recommended



Coccidioides immitis

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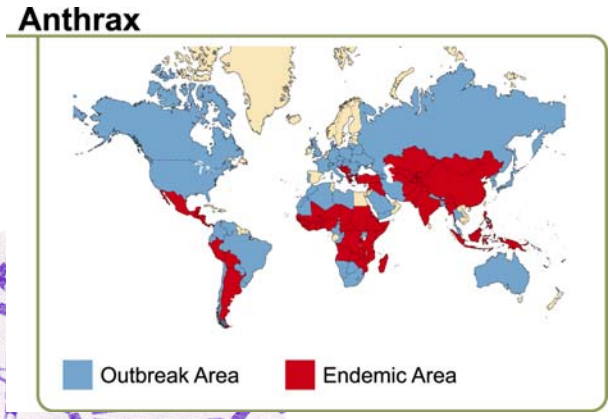
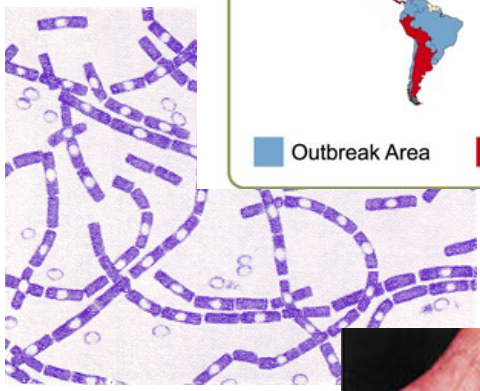
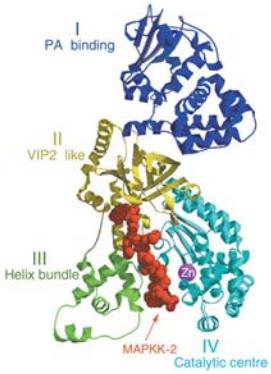
Bacteria: *Bacillus anthracis*

- Anthrax
  - Cutaneous
  - Gastrointestinal
  - Pulmonary
- Zoonotic
- Global distribution
  - Especially Middle East, Africa, Asia, US
  - Many less virulent strains
- Very stable – spore former
- ID_{50}: 8000-50,000 spores
- Mortality rate: 90 – 100% (pulmonary)
- Vaccine available
- Antibiotics effective if administered shortly after exposure
- In US: often used in BSL2 laboratories







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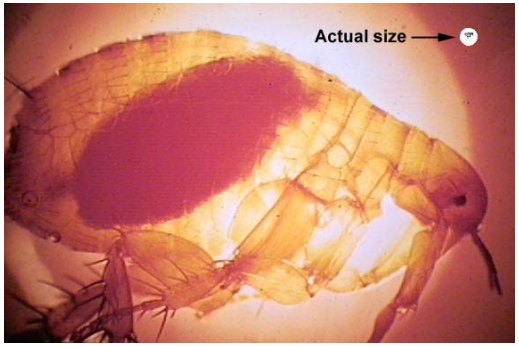
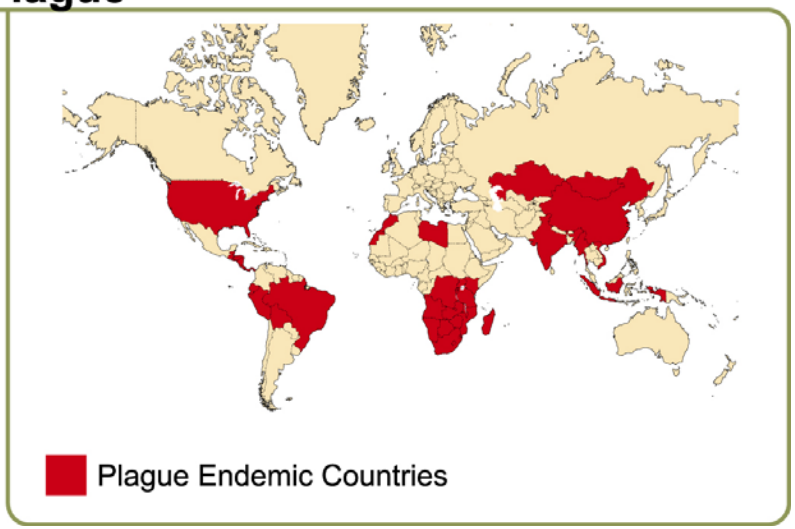
Bacteria: *Yersinia pestis*

- Plague
  - Bubonic
  - Septicemic
  - Pneumonic
- Zoonotic
- Global distribution
  - Except Australia and Antarctica
  - Virulence highly variable
- Less stable – easily degraded by drying and sunlight
- ID_{50}: 100 – 20,000 organisms
- Mortality rate: ~100% for pneumonic if treatment not started within 24 hours of exposure
- No licensed vaccine in US; vaccine available in FSU and Mongolia
- In US: BSL2 for samples; BSL3 for large amounts



Plague



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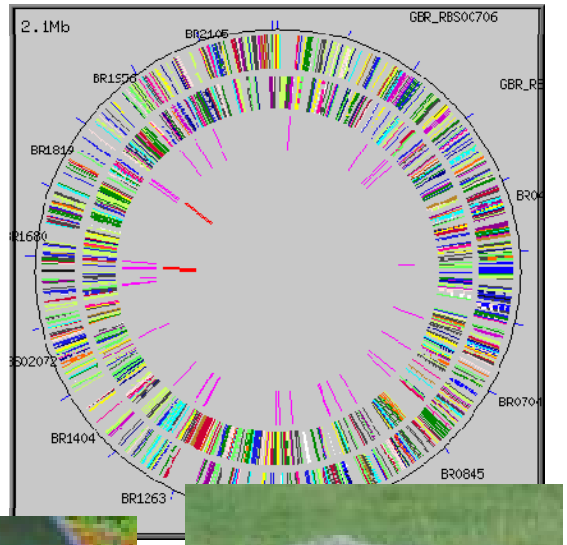
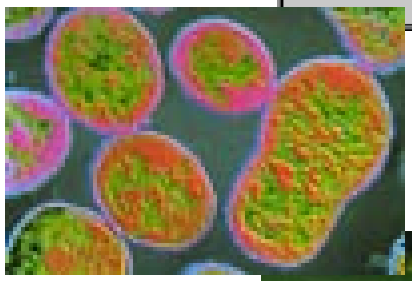
Bacteria: *Francisella tularensis*

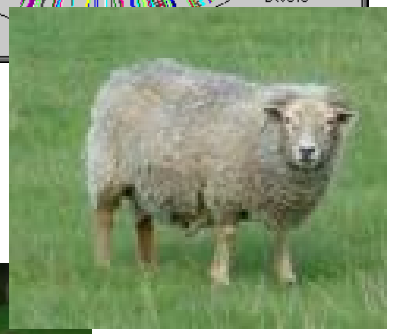
- Tularemia ("rabbit fever")
  - Ulceroglandular
  - Gastrointestinal
  - Pulmonary
- Zoonotic
- Distribution
  - North America, Europe, Middle East, Russia, and Japan
  - Rare in UK, Africa, Central and South America
- Persists in water but sensitive to UV radiation and oxidation
- ID_{50}: 10 – 25 organisms by respiratory routes
- Mortality rate: less than 20%
- Antibiotics
- In US: BSL3 most common

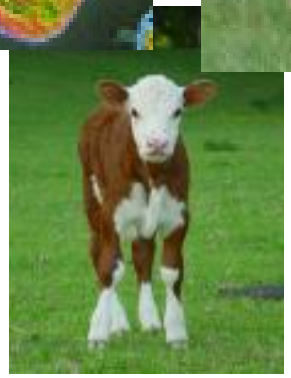
Bacteria: *Brucella spp.*

- Brucellosis
- Zoonotic
- Wide distribution
  - South America, Central America, Eastern Europe, Asia, Africa, Middle East
- Stable
- 6 species; 4 cause disease in humans
- ID_{50}: 10 – 100 organisms
- Low mortality
- Treatment with antibiotics
- In US: BSL 3 most common









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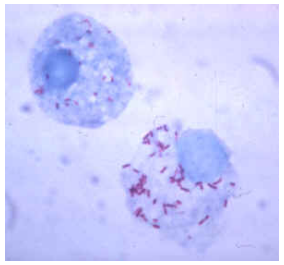
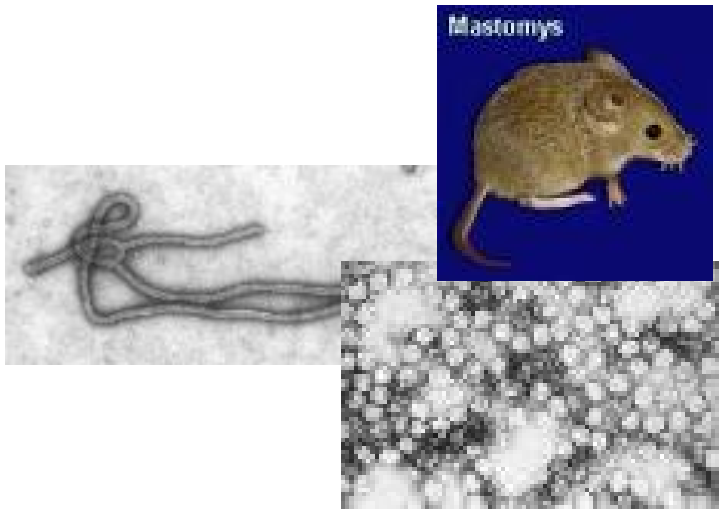
Rickettsiae: *Rickettsia rickettsii*

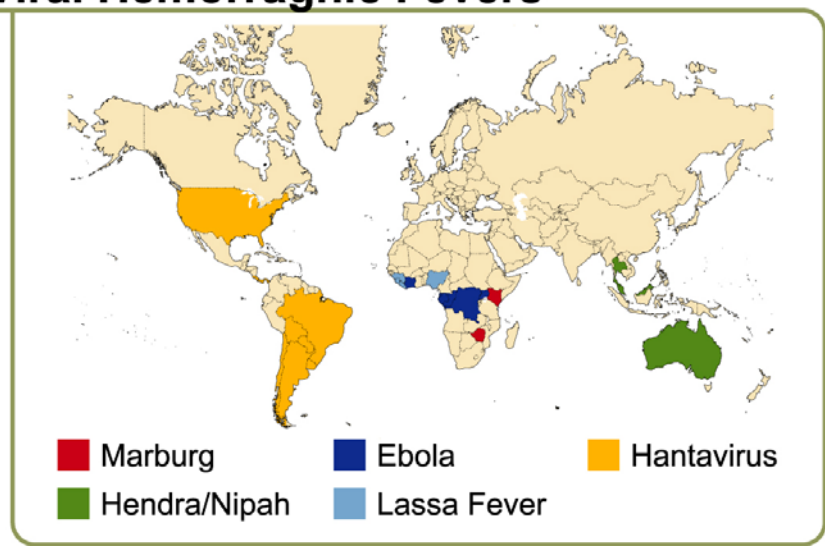
- Rocky Mountain Spotted Fever
- Zoonotic
- Distribution
  - US, Mexico, Central and South America
- Stable in tick blood at ambient T; 1 year survival
- ID_{50}: 1-10 organisms
- Mortality 15-20% if untreated
- Antibiotics: doxycycline; delayed diagnosis contributes to increase in fatalities
- In US: BSL3



Viruses: Hemorrhagic Fever Viruses

- Viral Hemorrhagic Fevers
  - Filoviruses: Ebola and Marburg
  - Arenaviruses: Lassa, Junin, Machupo, Guanarito, Sabia
  - Hantaviruses
  - Dengue
  - Yellow fever
- Zoonotic
- Distribution varies by species
- Relatively unstable (enveloped viruses)
- ID_{50}: 1 – 10 virions
- Mortality varies
  - Ebola is highest: 50 – 90%
  - Lassa: 15 – 20%
- Generally only supportive treatment
  - Ribavirin somewhat effective for arenaviruses
  - Yellow fever vaccine
- In US: Most are BSL4





Viruses: Variola Major Virus

- Smallpox
- Reservoir: Human only
- Eradicated; officially available in only 2 laboratories
  - CDC (USA)
  - Vector (Russia)
- Very stable
- ID_{50}: 10 – 100 virions
- Mortality rate: ~30% (unvaccinated)
- Vaccine available
- Other treatments limited
  - Post-exposure vaccination (within 4 d
  - Supportive care
  - Cidofovir has shown promise
- BSL4

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Viruses: Avian Influenza H5N1 Virus

- Highly Pathogenic Avian Influenza (HPAI)
- Zoonotic
- Distribution
  - Spreading rapidly in environment
- Relatively unstable
  - But stable in water
- Highly infectious in poultry; apparently limited infectivity currently in humans
- Vaccine for animals available; human vaccine under development
- Treatment for humans:
  - Oseltamivir
  - Zanamavir
- In US: BSL3

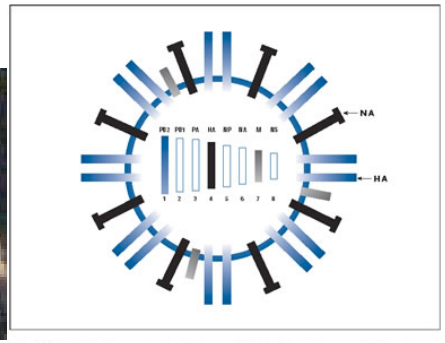
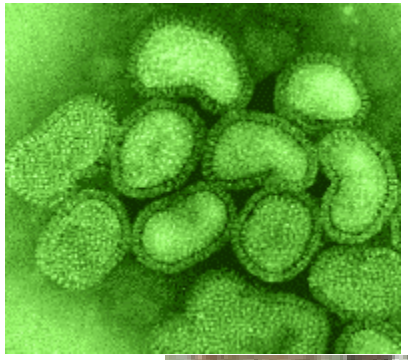


Figure 3. Model of the influenza virus showing the segmented nature of the viral genome and the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA).



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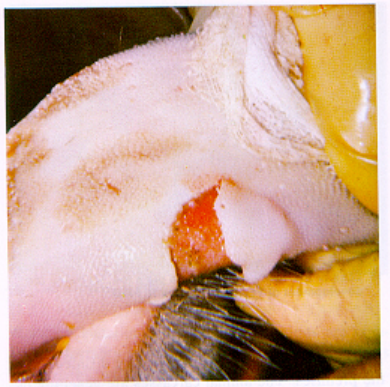
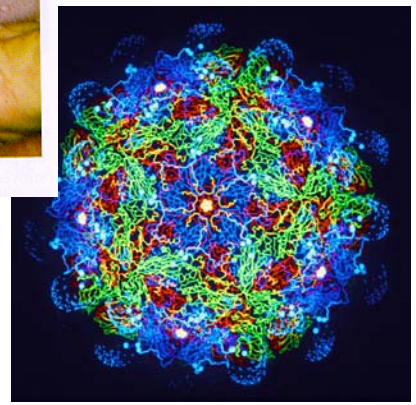
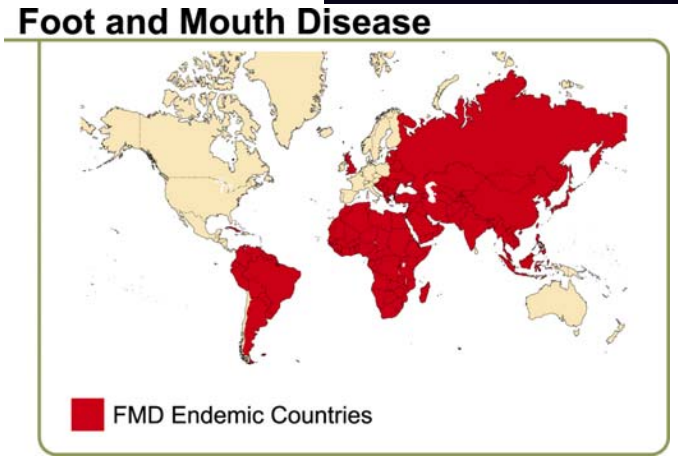
Viruses: Foot-and-Mouth Disease Virus

- Foot-and-mouth disease
- Animals only
- Wide distribution
  - South America, Europe, Asia, and Africa
- Very stable
- Highly infectious for cattle
  - ID_{50} < 10 virions
- High morbidity; low mortality for adult animals (< 5%)
- Vaccine
- In US: BSL3-Ag


18 Ruptured vesicle on bovine tongue.





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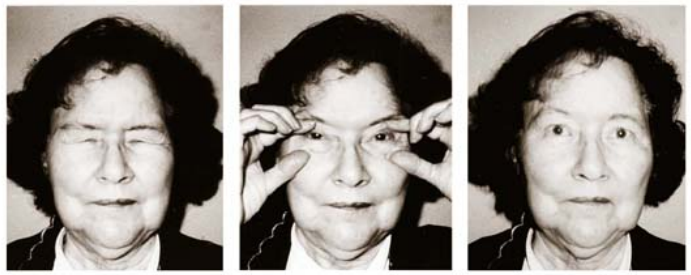
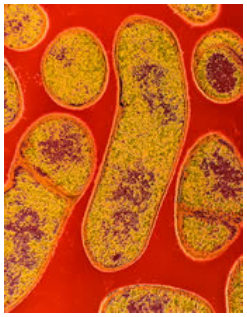
Proteins: Botulinum toxin

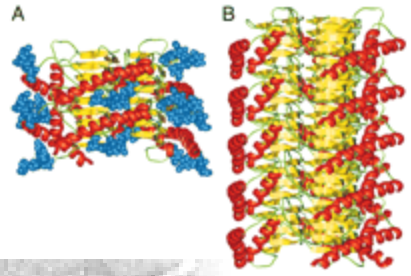
- Botulism
- Produced by *Clostridium botulinum*, *C. baratii*, and *C. butyricum*
  - Multiple types of toxin: A, B, C, D, E, F, G
  - Only A, B, E, and F produce human disease
- Zoonotic
- Degraded by heat and humidity – relatively stable for a protein
- LD_{50}: 0.001µg/kg
- Treatment
  - Antitoxin (limited supplies)
  - Supportive care

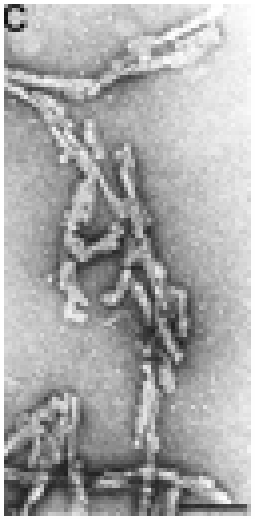


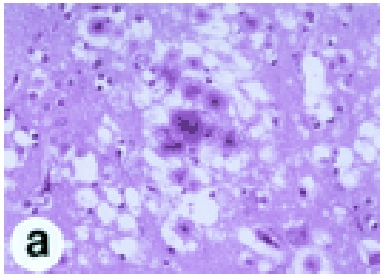


Proteins: Bovine Spongiform Encephalopathy Prion

- Mad cow disease
- Causative agent of BSE causes variant Creutzfeldt Jakob disease (vCJD)
- Diagnosed in cattle in:
  - Europe, Canada, US, Japan
- Long incubation period
- Extremely stable – not inactivated by conventional fumigants, very heat resistant
- Laboratory recommendations:
  - In US: BSL2 or BSL3
  - In UK: modified BSL3 (room need not be sealable, HEPA filters not required)
  - OIE: BSL3







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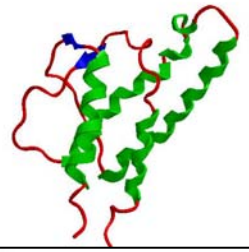
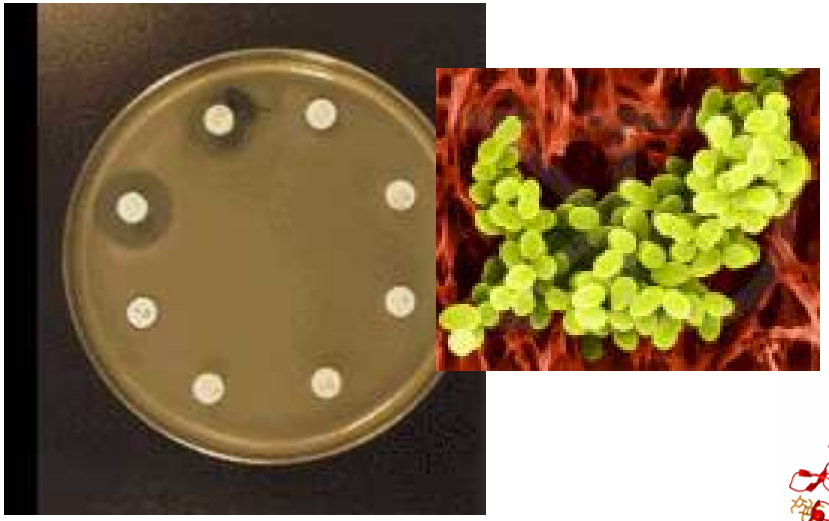
Biological Threats are Evolving

- Drug resistant agents
- Genetically-engineered agents





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Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus Suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox

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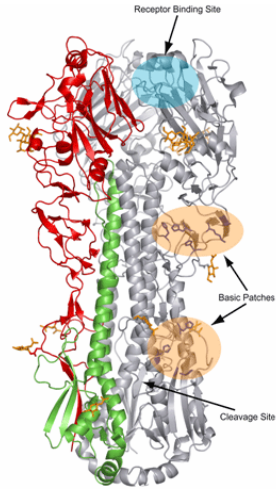

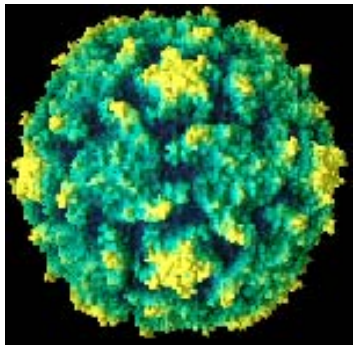

Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus

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International
BIOLOGICAL THREAT REDUCTION

Summary

- Types of agents
  - Fungi
  - Bacteria (including mycoplasma, rickettsae, chlamydia)
  - Viruses
  - Proteins
    - Prions
    - Toxins
- Almost all biological agents available in nature
- Many factors to consider when evaluating biological hazards
- Delayed onset of symptoms – many cause flu-like illness