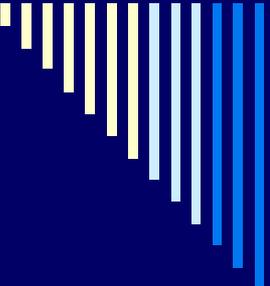


Attenuated strains of *Yersinia pestis* Biosafety Considerations

Joseph A. Kanabrocki, Ph.D.

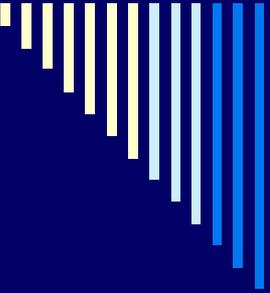
June 16, 2010





Outline

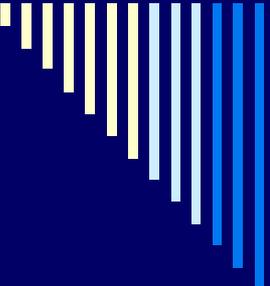
- Review of experiments under III-A-1:
What constitutes a microorganism that can cause “disease”
- Overview of *Yersinia pestis*
- Presentations
- Questions for Discussion



NIH Guidelines for Research Involving Recombinant DNA Molecules (*NIH Guidelines*)

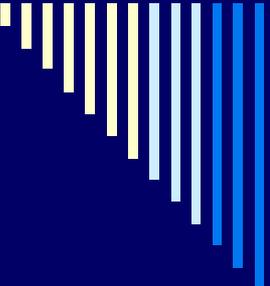
- **Section III-A-1-a:**

The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.



Applicability of Section III-A-1-a to Attenuated Strains

- **Does the deliberate transfer of antibiotic resistance with the potential to compromise use of the drug extend to agents capable of causing any disease or should it be limited to those agents that raise public health concerns?**
- **Does the proposed manipulation of the organism, for example introduction of multiple antibiotic resistance markers, change the assessment of whether it raises a public health concern?**



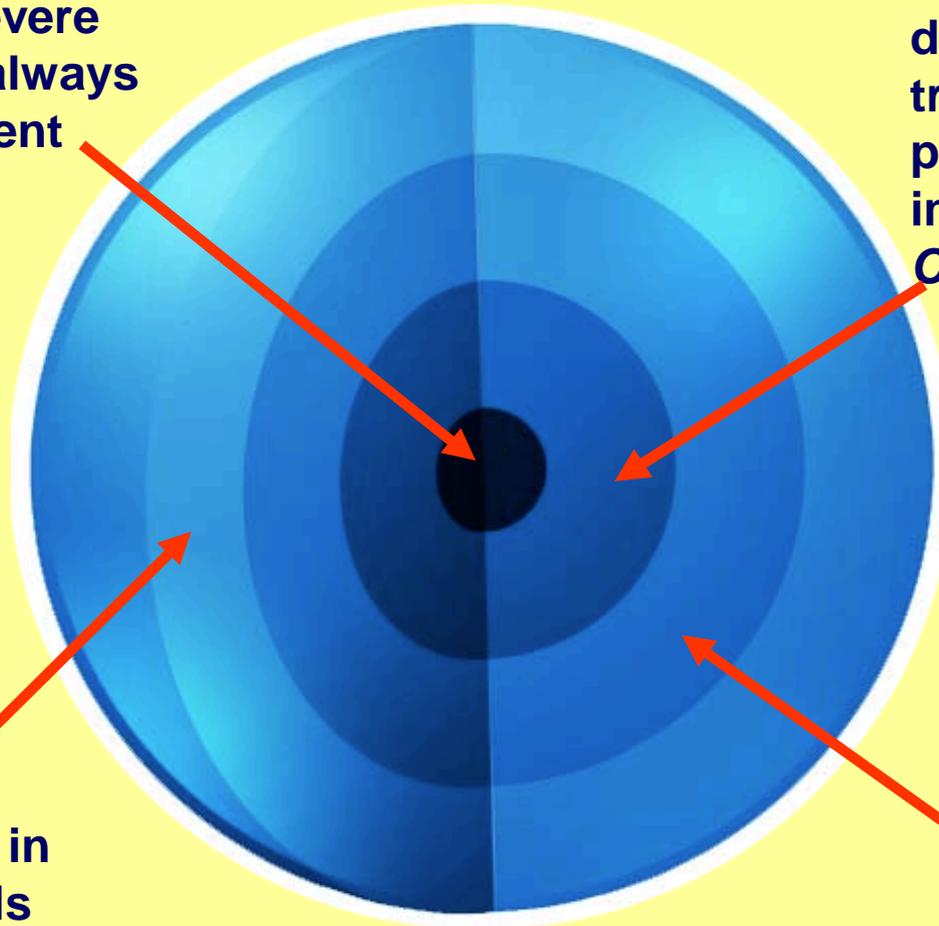
Applicability of Section III-A-1-a to Attenuated Strains

- How does one define a public health pathogen
 - Is it defined by pathogenicity?
 - Is it defined by the susceptible population?
i.e. the ability to cause disease in healthy adults, children, pregnant women and/or immunocompromised individuals?
 - Should route of transmissibility be a factor?

What are the Boundaries of Attenuation and Avirulence

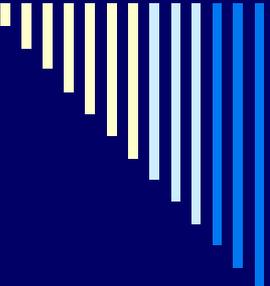
Able to cause severe disease almost always requiring treatment

Able to cause mild disease that may require treatment and is of public health importance (e.g. *Chlamydia trachomatis*)



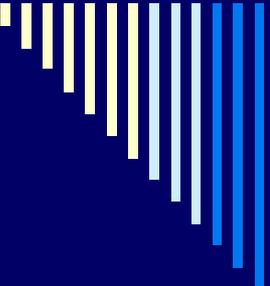
Rarely, if ever causes disease in humans, animals or plants

Attenuated, unlikely to cause clinical disease requiring treatment in the general population – not generally transmissible



Yersinia pestis

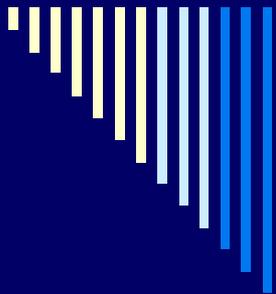
- **Causative agent of bubonic/pneumonic plagues throughout human history**
- **Remains endemic in many parts of the world but mainly Africa and China**
- **In 2006, 13 cases and 2 deaths reported from New Mexico, Colorado, California and Texas**
- **Zoonotic disease with rodents being the primary reservoir; agent is transmitted by fleas**
- **More recently, focus is on its potential as a bioweapon**



Yersinia pestis

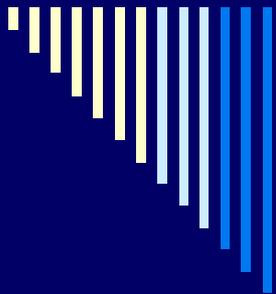
Antibiotic Resistance

- **To date only documented resistant strains in Madagascar**
 - **One strain isolated from 16 year old boy with bubonic plague was resistance to streptomycin, chloramphenicol, tetracycline, sulfonamide, ampicillin, kanamycin, and spectinomycin**
 - **Second case was 14 year old boy who was infected with strain resistant to streptomycin**



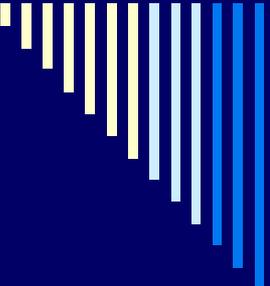
Yersinia pestis attenuated strains

- The wild-type strain is regulated as a Select Agent (SA) by the CDC
 - Strains excluded from the SA regulations are:
 - *pgm⁽⁻⁾* strains (*pigmentation deficient*)
 - 102 Kb chromosomal deletion of a high pathogenicity island that encodes in part a siderophore (an iron chelator and transporter)
 - *lcr⁽⁻⁾* strains (*low calcium response*)
 - lacking a virulence plasmid (pCD1) that encodes *Yersinia* outer membrane proteins (Yop) which inhibit phagocytosis and inflammation



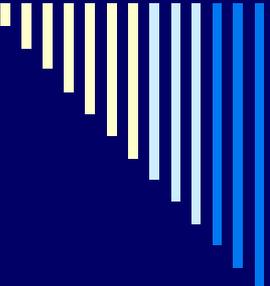
Yersinia pestis attenuated strains

- **Both strains are considered to be attenuated yet a recent fatal LAI (2009) was reported involving experiments with a *Y. pestis pgm*⁽⁻⁾ strain.**
 - **Animal models demonstrate that if there is supplementation of serum iron at the time of inoculation, virulence of the *pgm*⁽⁻⁾ strain can be rescued**
(Lee-Lewis H., Anderson D.M., 2010, Infect Immunol 78(1):220-30)



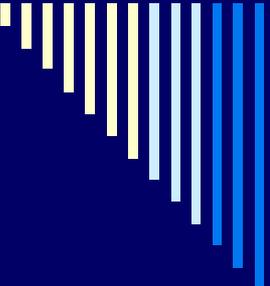
Attenuated Strains of *Y. pestis* - Questions

- Are attenuated strains of *Y. pestis* considered to be disease agents in humans?
 - Evidence for *lcr*⁽⁻⁾ strains
 - Evidence for *pgm*⁽⁻⁾ strains
- Is the evidence sufficient or what additional evidence is needed to establish that the *lcr*⁽⁻⁾ strain is avirulent in humans such that it cannot cause human disease?



Attenuated Strains of *Y. pestis* - Containment

- Appendix B of the *NIH Guidelines* lists *Y. pestis* (all strains) as a Risk Group (RG) 3 agent, therefore most rDNA experiments with this agent should be performed at BL3 containment.
 - Should attenuated recombinant strains of *Y. pestis* be generally contained at BL3 or at BL2 ?
 - Should different containment and/or biosafety practices be specified for *pgm*⁽⁻⁾ strains versus *lcr*⁽⁻⁾ strains?



Presentations

- **Olaf Schneewind, M.D., Ph.D., Louis Block Professor and Chair Dept. of Microbiology, University of Chicago**
- **Martin Schriefer, Ph.D., Laboratory Chief, CDC Fort Collins (*via teleconference*)**
- **Denise Gangadharan, Ph.D., Deputy Associate Director for Science, Division of Select Agents and Toxins, Center for Disease Control and Prevention, Atlanta, GA (*via teleconference*)**