Plague

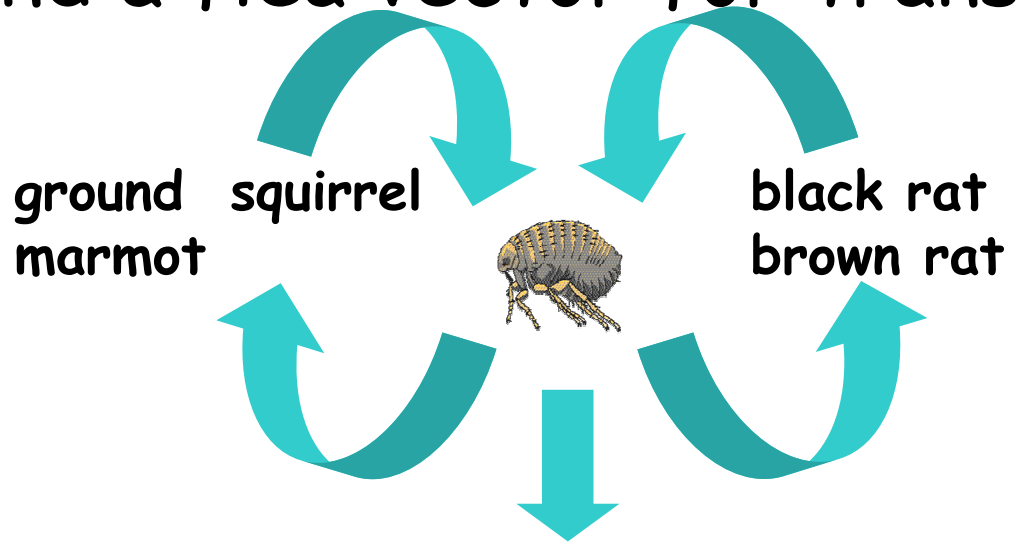
Professor Michael Prentice
University College Cork
Ireland

Lecture Overview

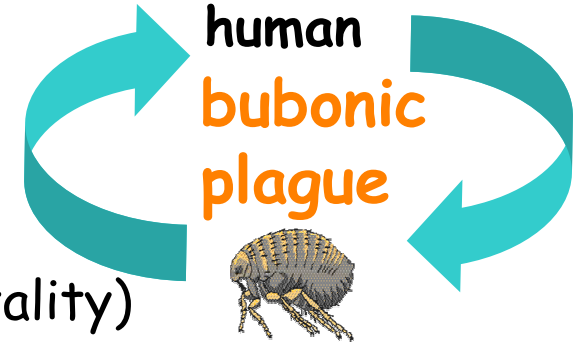
- What is plague ?
- What is *Yersinia pestis*?
- How did *Y. pestis* emerge?
- Brief clinical diagnostic overview
  - bioterrorism

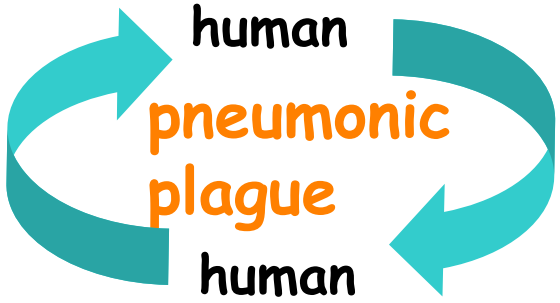
  - Based on Prentice MB, Rahalison L. Lancet 2007; 369: 1196-207

Plague - zoonosis requiring bacteraemia and a flea vector for transmission



80-95% of cases
10-20% mortality
(Septicaemic without Bubo: increased mortality)





Secondary to bacteraemia
Primary by droplet infection

Adapted from Prof RW Titball

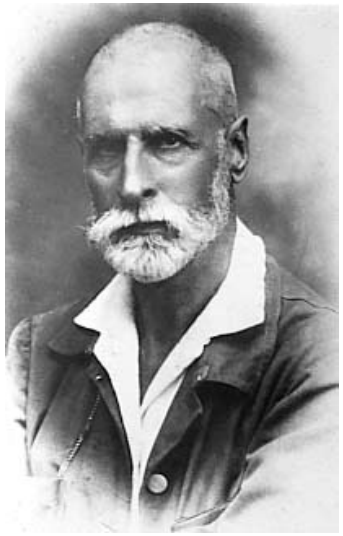
Yersinia pestis

causative agent of plague

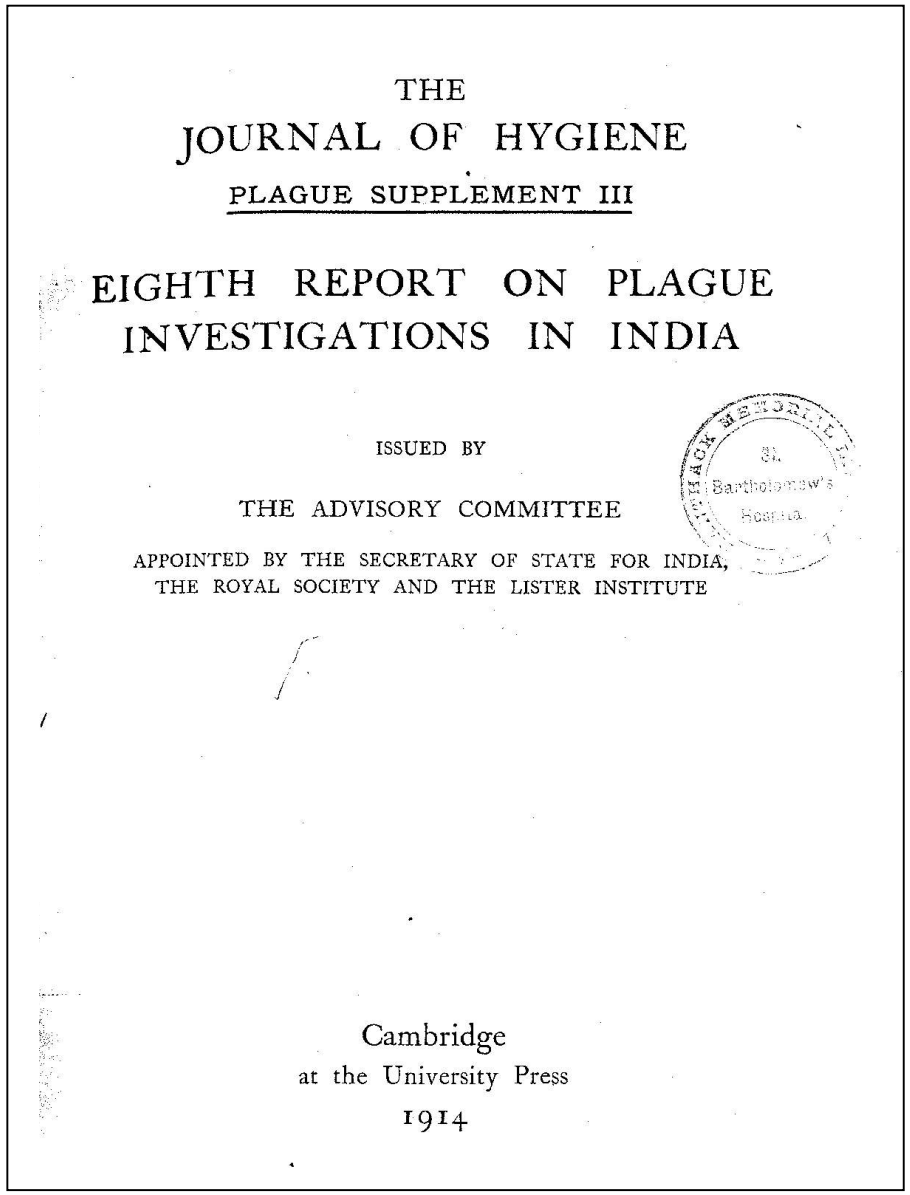
- Alexandre Yersin 1894 Hong Kong
  - (Kitasato)

www.moh.gov.vn

http://100years.vnu.edu.vn:8080/BTDHQGHN



www.pasteur-international.org



THE
JOURNAL OF HYGIENE
PLAGUE SUPPLEMENT III

EIGHTH REPORT ON PLAGUE INVESTIGATIONS IN INDIA

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA, THE ROYAL SOCIETY AND THE LISTER INSTITUTE

Cambridge
at the University Press
1914

1896/7 Ogata, Simond
fleas in disease transmission

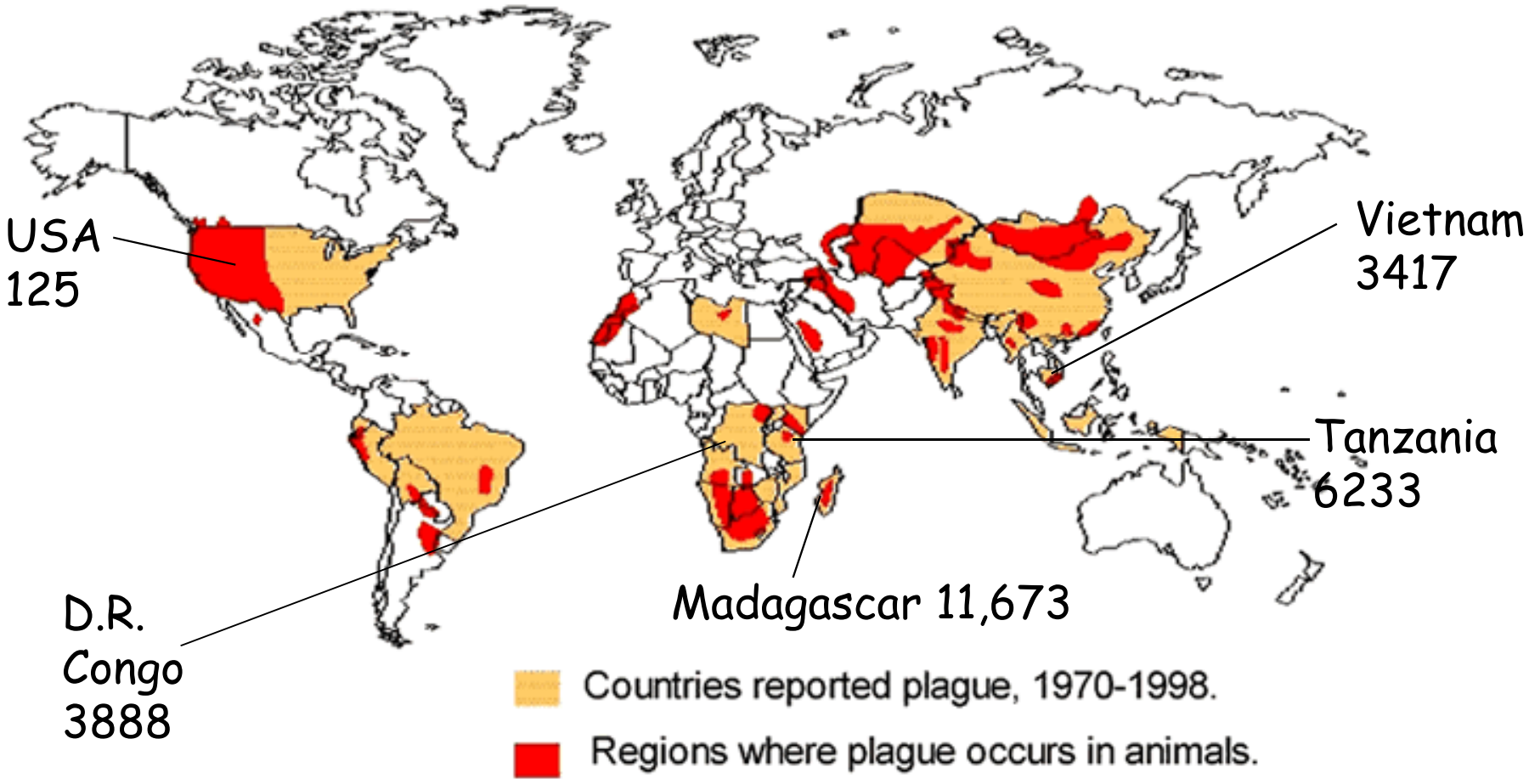
1906 First report on plague
investigations in India

1914 Eighth Report
Blockage of flea
proventriculus by bacteria
(Bacot & Martin)

Specific control measures
against human disease
(rat exclusion, quarantine)

Passive antibody therapy,
crude vaccine (Haffkine)

Total reports to WHO 1987-2001: 36,876



www.cdc.gov/ncidod/dvbid/plague/world98.htm

Ariz. Biologist Likely Died of Plague

By JACQUES BILLEAUD
The Associated Press
Saturday, November 10, 2007; 5:08 PM

PHOENIX -- A wildlife biologist at Grand Canyon National Park most likely died from the plague contracted while performing a necropsy on a mountain lion that later tested positive for the disease, officials said Friday.

Eric York, 37, who worked in the park's cougar collaring program, became ill on Oct. 30 and called out sick from for a couple of days before being found dead in his home Nov. 2. Tests were positive for the pneumonic plague.

Officials said 49 people who came in contact with York were given antibiotics as a precaution. None have shown symptoms of the disease.

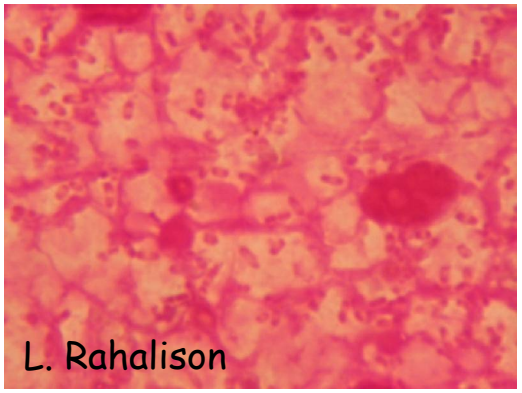
York, whose family lives in Massachusetts, had skinned the cougar and was exposed to its internal organs during the necropsy he performed three days before developing symptoms, said David Wong, an epidemiologist for the U.S. Public Health Service.

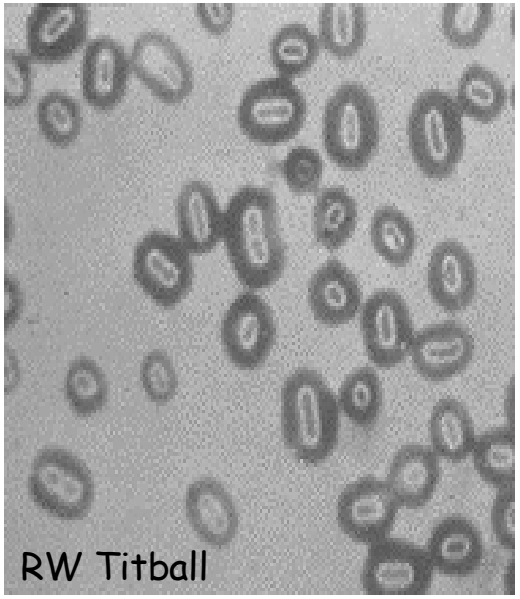
The cougar, which had died from the plague, was believed to have remained in back-country areas where park visitors wouldn't normally go, officials said.

Eric York, 37, is seen in this undated photo provided by the Grand Canyon National Park. A wildlife biologist at Grand Canyon National Park,

Yersinia pestis

bacteriology and genetics





- Gram-negative rod
- Only member of the Enterobacteriaceae to infect an insect
- capsule visible by light microscopy
- Non-motile, rough LPS

Pathogenic *Yersinia* sp

Psychrotrophs, 46-50% G+C%

- *Yersinia pestis*
  - flea-borne systemic pathogen
- *Yersinia pseudotuberculosis*
  - enteric pathogen
- *Yersinia enterocolitica*
  - enteric pathogen

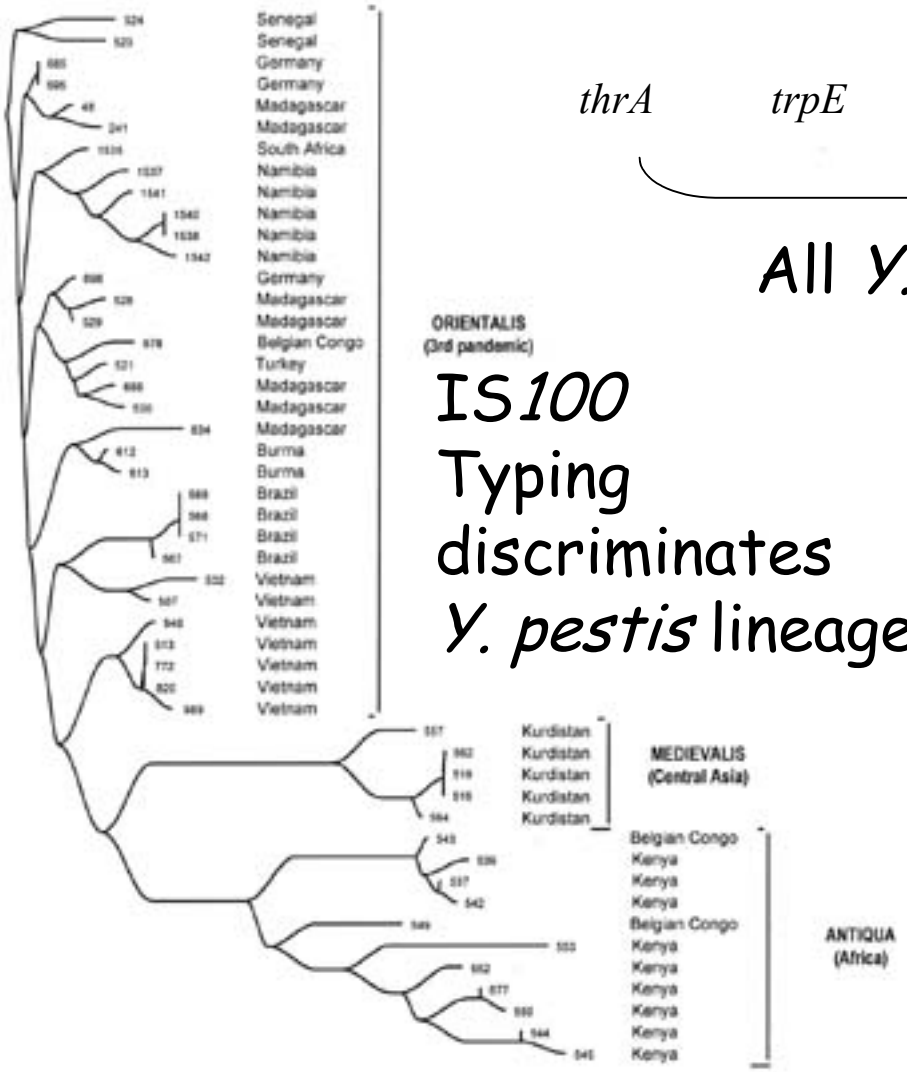
} DNA-DNA + Biochemistry

Multi Locus Sequence Typing (MLST):

Y. pestis derived from *Y. pseudotuberculosis*

thrA *trpE* *glnA* *tmk* *dmsA* *manB*

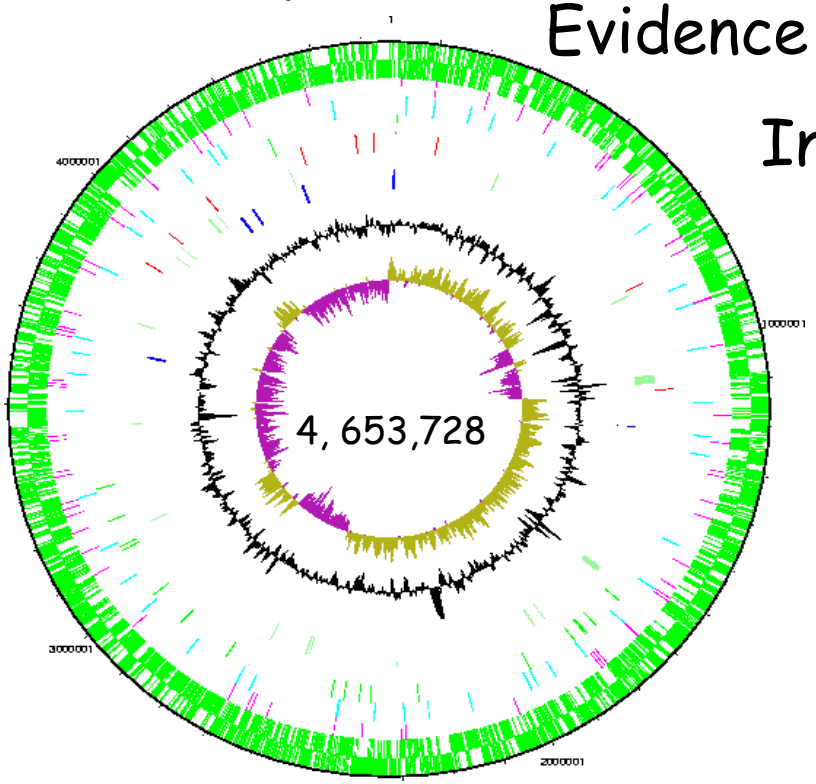
All *Y. pestis* alleles identical (clonal)



IS*100* Typing discriminates *Y. pestis* lineage

Achtman et al PNAS 1999 23;96(24):14043-8

Y. pestis CO-92 Genome



Parkhill et al
Nature 2001

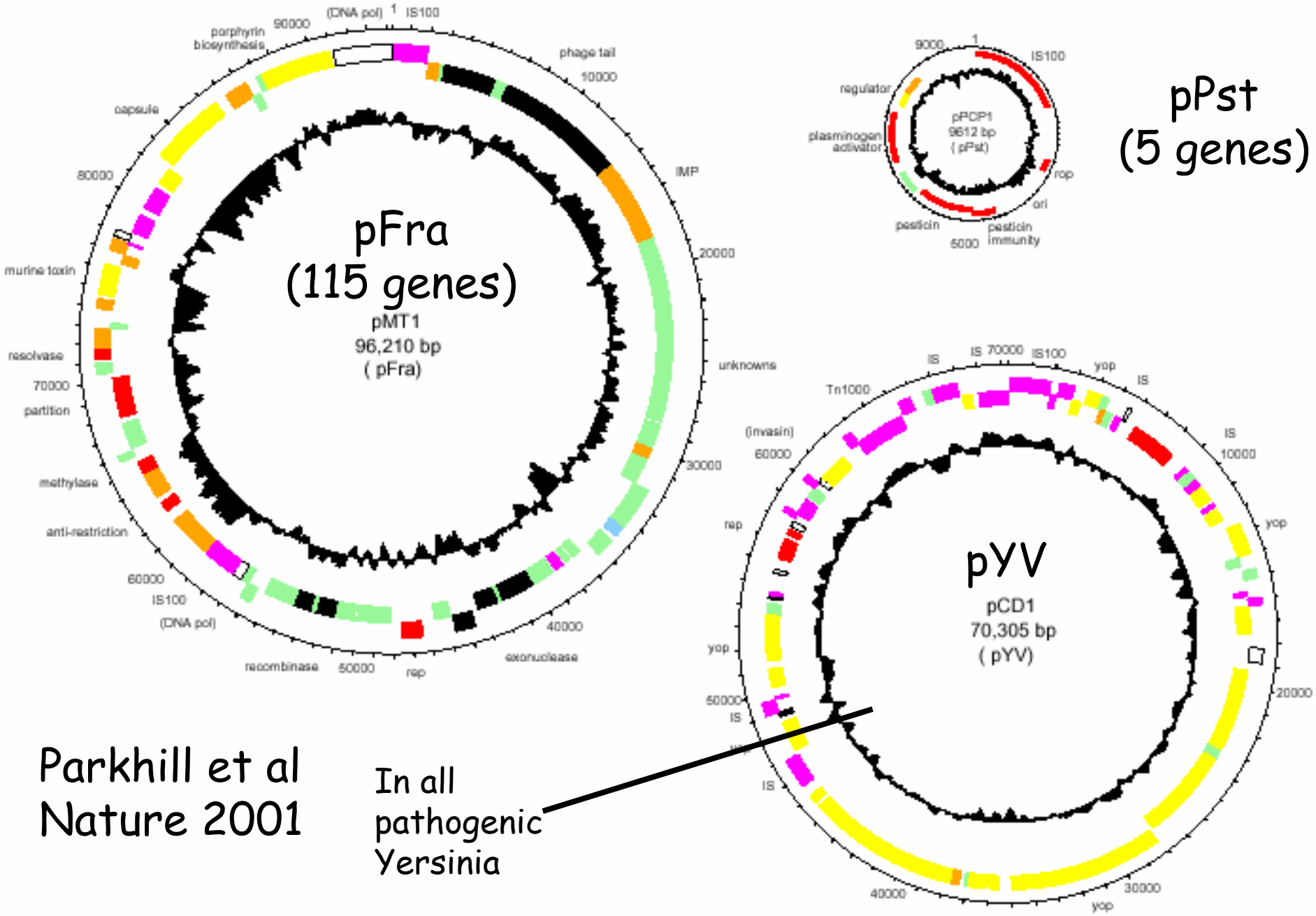
Evidence of gene loss: >140 pseudogenes

Interruption by IS elements

Frame shifting mutations

IS	Size	Copies
IS*1541*	0.7 kb	53 (72)
IS*100*	1.95 kb	43 (51)
IS*285*	1.3 kb	20 (22)
IS*1661*	2.0 kb	7 (8)
Others	0.3-13.8 kb	
rRNA	5.1 kb	5

Yersinia pestis gene acquisition - plasmids



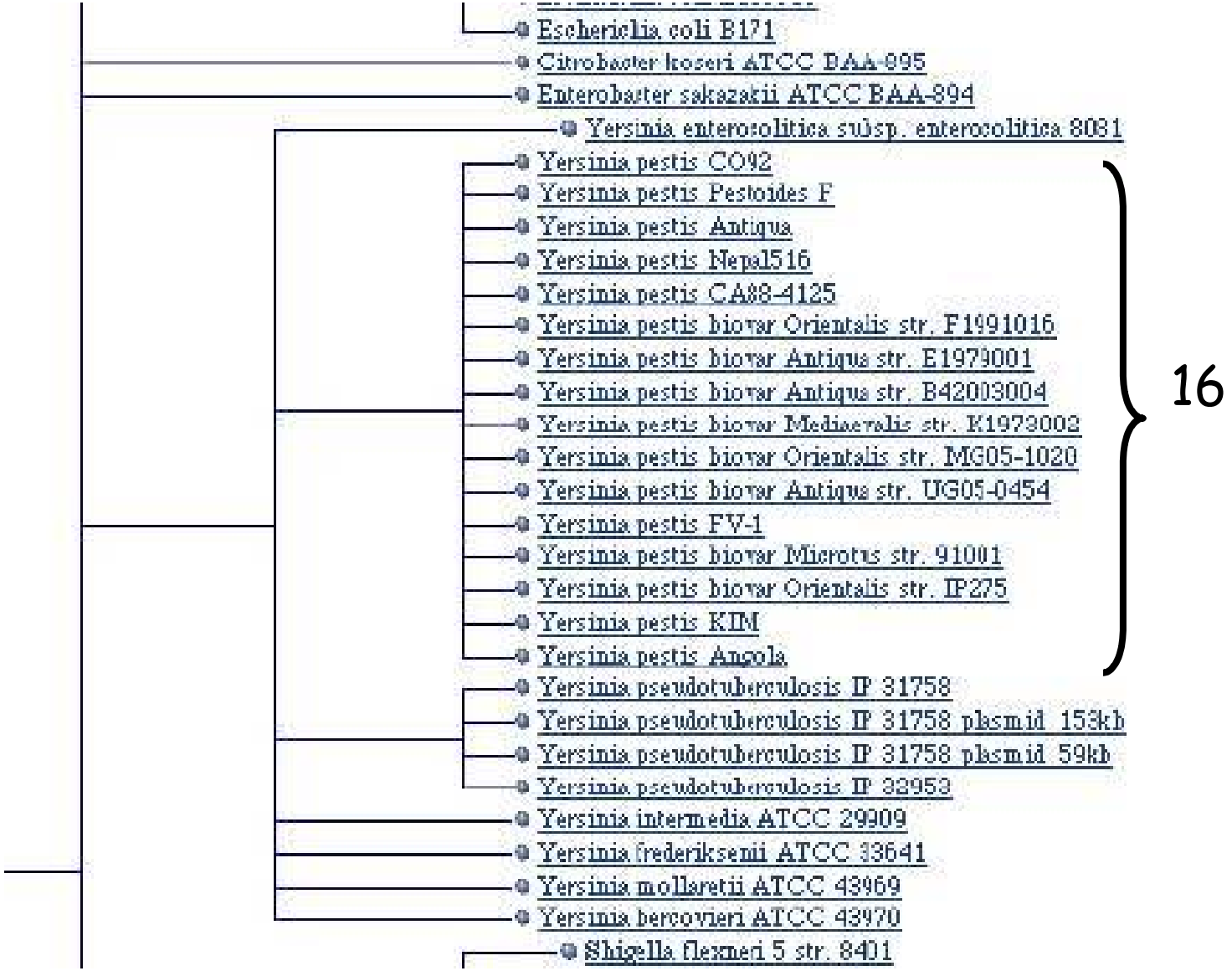
Parkhill et al
Nature 2001

In all pathogenic Yersinia

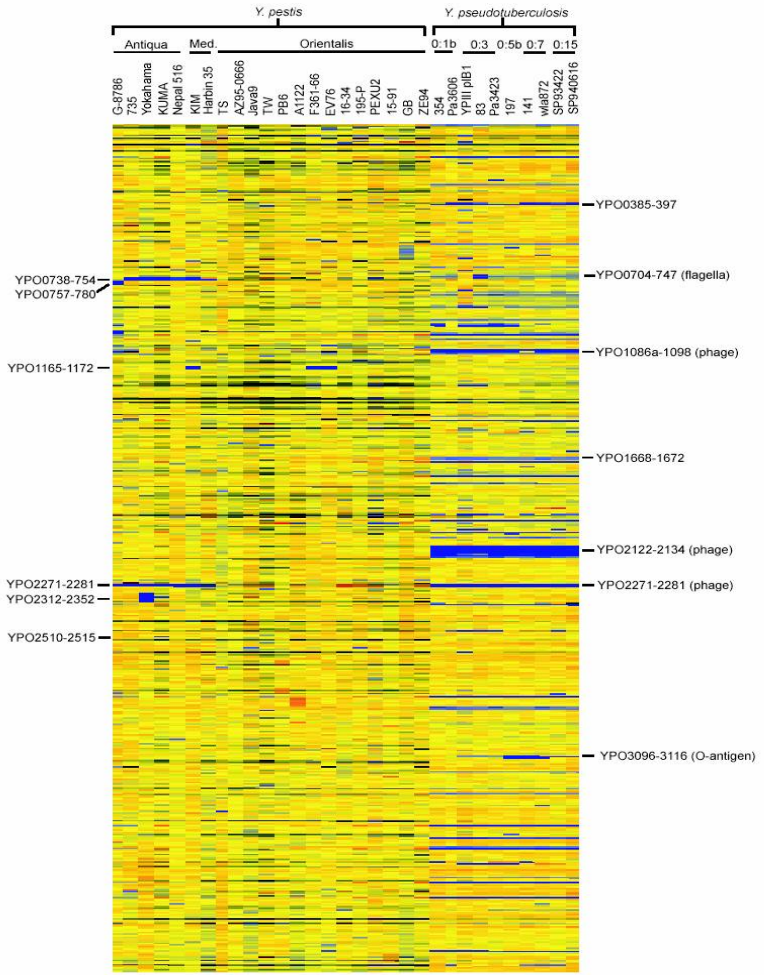
Multi-genomic comparison

- *Y. pestis* KIM
  - Deng et al JBact 2002; 184:4601-11
- *Y. pseudotuberculosis* IP 33953
  - LPS locus resembling *Y. pestis*
  - Chain, Carniel et al PNAS 2004;101:13826-31
- *Y. pestis* 91001 (avirulent to humans)
  - Song et al DNA Res 2004; 11:179-97

http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html



Microarray using *Y. pestis* genome sequence: *Y. pestis* and *Y. pseudotuberculosis* gene complement profiling



present

absent

duplicated

Gene complement similar
Acquisition of mobile
Elements by *Y. pestis* cf
Y. pseudotuberculosis

Hinchliffe et al
Genome Research 2003

DNA data on *Y. pestis* and phylogeny

- MLST and Single Nucleotide Polymorphism (SNP analysis)
- DNA sequence, gene content and gene order
- Different methods agree: **all** current *Yersinia pestis* strains are very closely related and derive from *Y. pseudotuberculosis*
  - it is a **clonal** pathogen
  - Worldwide spread in many hosts, must have spread recently to retain this similarity
  - **Recently emerged** pathogen (how?)

Phenotypic differences between the human pathogenic *Yersinia*

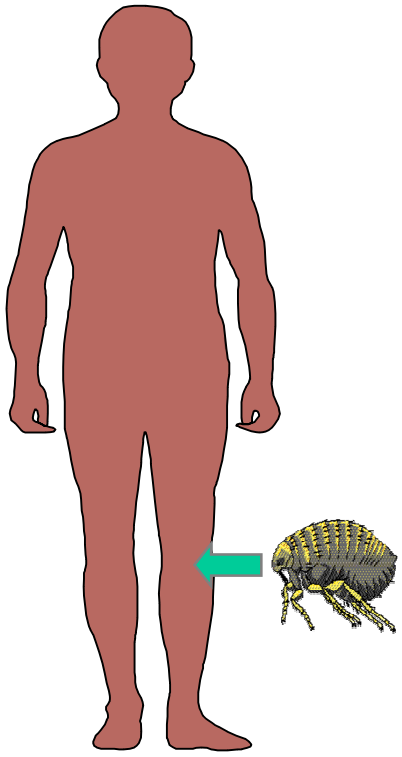
SubSpecies	*Y. pseudotuberculosis*	*Y. pestis*	
Invasin	Y	N	These genes are present in Y. p but inactivated = pseudogenes
Adherence Invasion locus	Y	N	
Yersinia adherence factor	Y	N	
urease	Y	N	
LPS O-side chain	Y	N	
Haemin storage	Y/ N	Y	
plasminogen activator	N	Y	These genes are only in *Y. pestis*
murine toxin	N	Y	
F1 capsular antigen	N	Y	

Genes associated with enteric infection

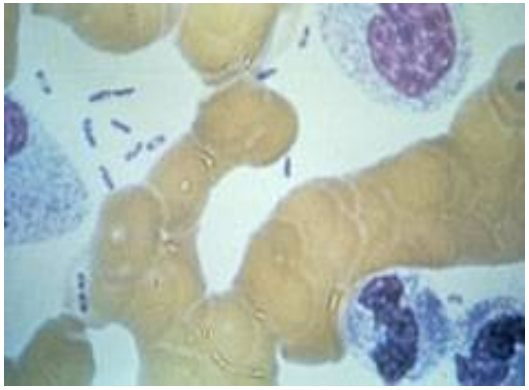
DNA data on *Y. pestis* and clues about pathogenicity

- Genes gained compared with *Y. pseudotuberculosis* are bacteriophage or plasmid
- Pseudogenes may represent unwanted baggage- genes necessary for enteric survival/infection being discarded
  - Gene loss can increase pathogenicity but no real evidence for increased pathogenicity from specific pseudogenes in *Y. pestis*
  - Focus on **phage** and **plasmid** acquired genes

Pathogenesis



- Flea bites infected rodent
- Infected flea leaves rodent and bites human, regurgitating the blood meal containing bacteria
- The bacteria are delivered subcutaneously.
- The bacteria have been growing in the flea at <<37°C

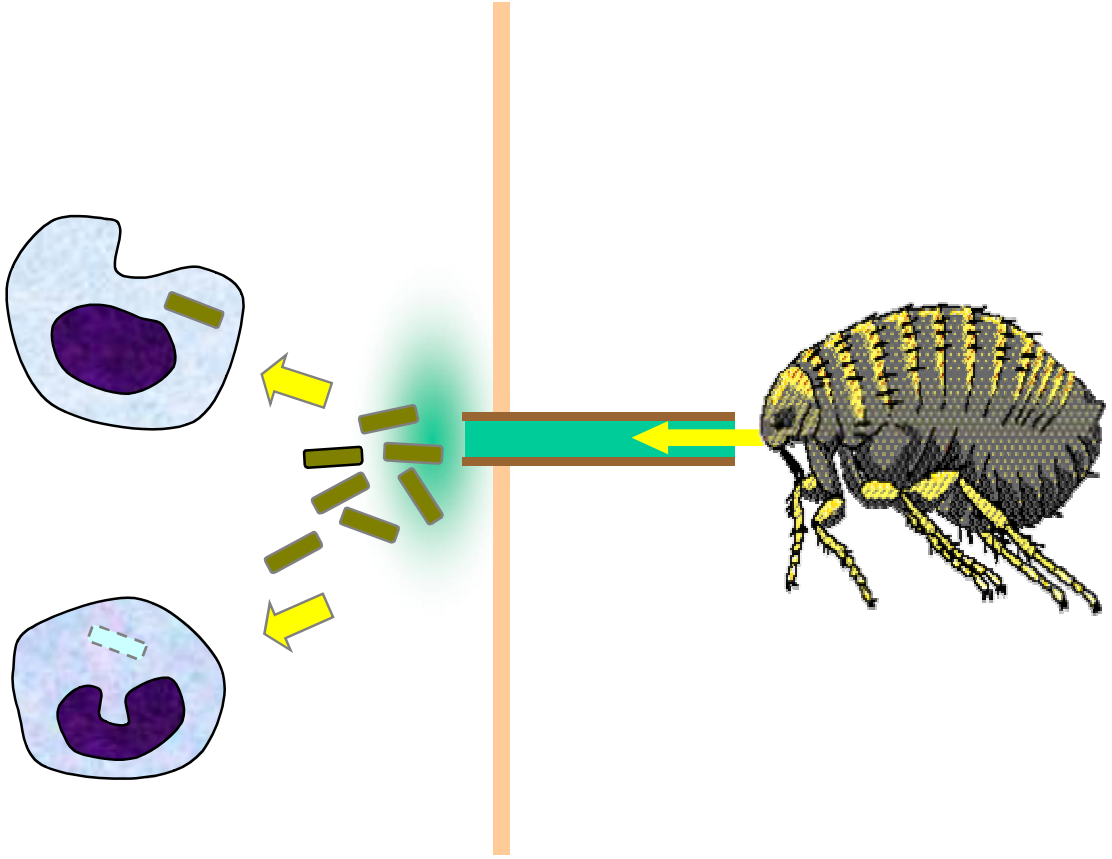


Adapted from Prof RW Titball

The fate of *Y. pestis*

Y.pestis is able to survive in macrophages

Neutrophils kill ingested *Y.pestis*



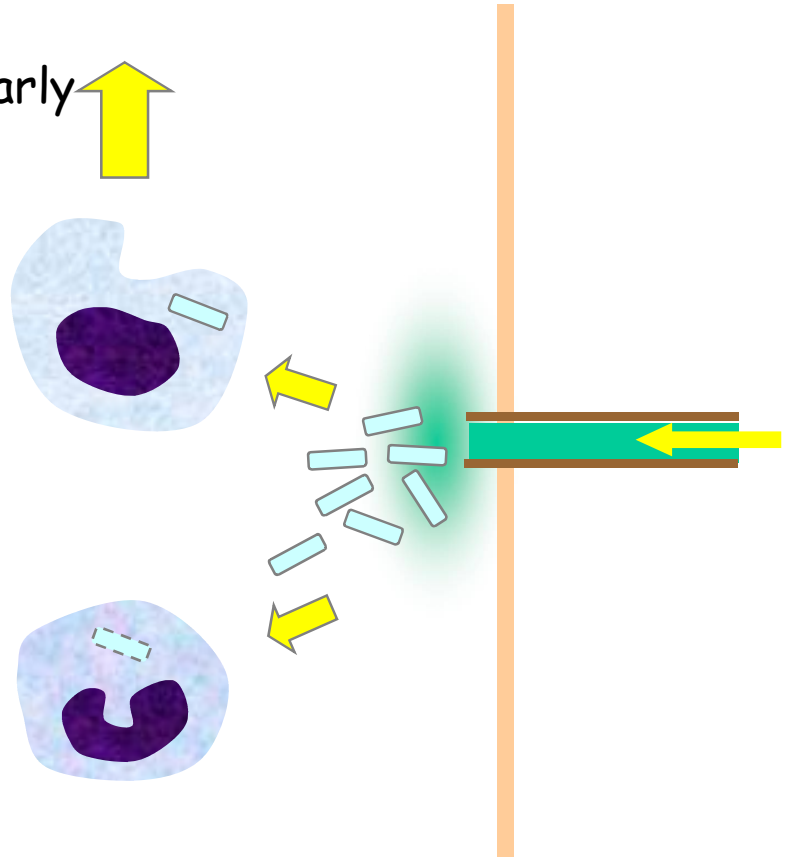
Adapted from Prof RW Titball

The fate of *Y. pestis*

Y.pestis within macrophages is trafficked to the local draining lymph node multiply extracellularly forming bubo

Y.pestis is able to survive in macrophages

Neutrophils kill ingested *Y.pestis*



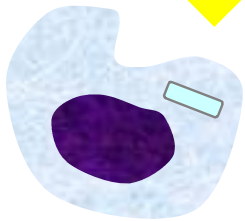
Adapted from Prof RW Titball

A brief summary of the pathogenesis of plague

(Bubo, septicaemia)

The flea phase
<37°C

The extracellular phase 1



The intracellular phase

The pneumonic phase

Adapted from Prof RW Titball

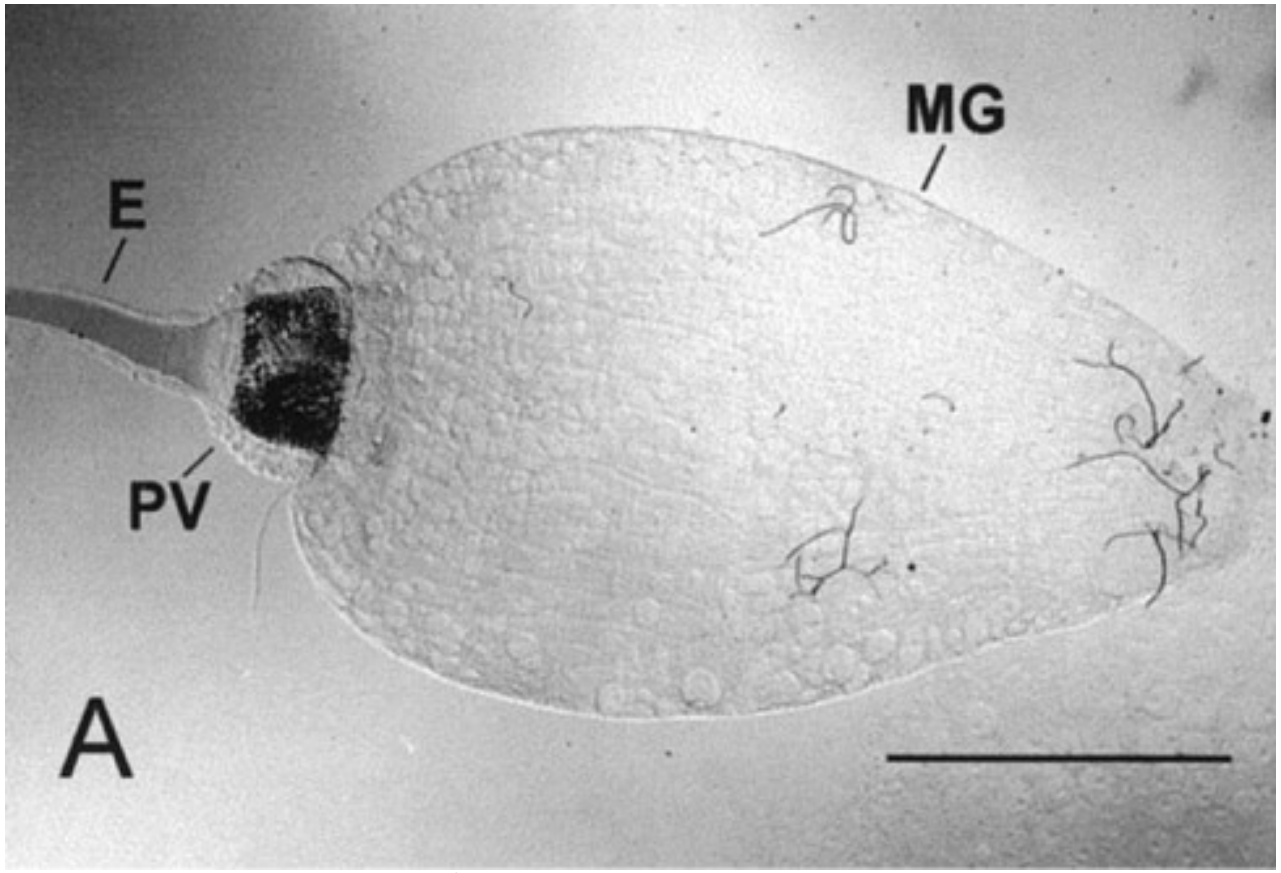
Flea infection

1: blockage + hms

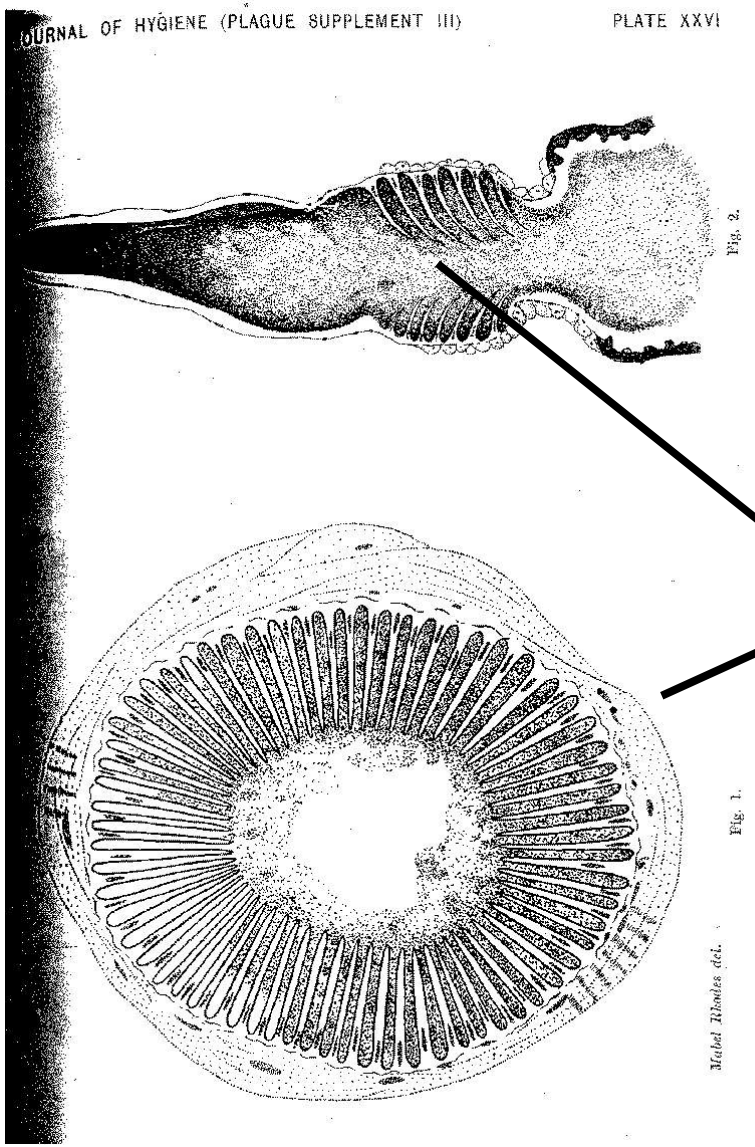
http://www3.niaid.nih.gov/labs/aboutlabs/lzp/plagueSection/

Dr BJ Hinnebusch NIAID

Flea digestive tract



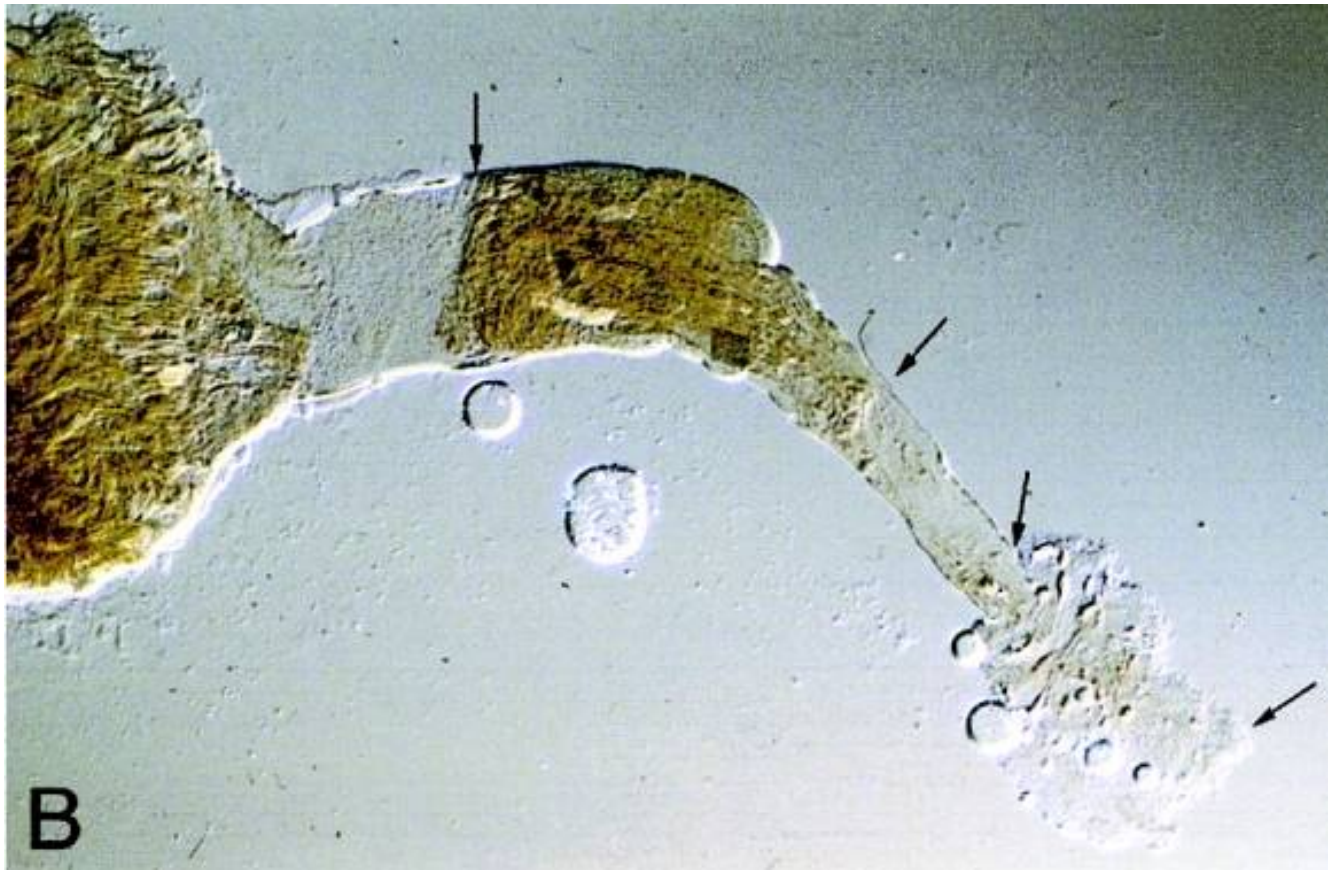
Jarrett et al J Inf Dis 2004 ; 190: 783-92



A.W. Bacot, C.J. Martin
Report on Plague
Investigations in India
1914

Proventriculus

Blockage is a biofilm requires *hms*

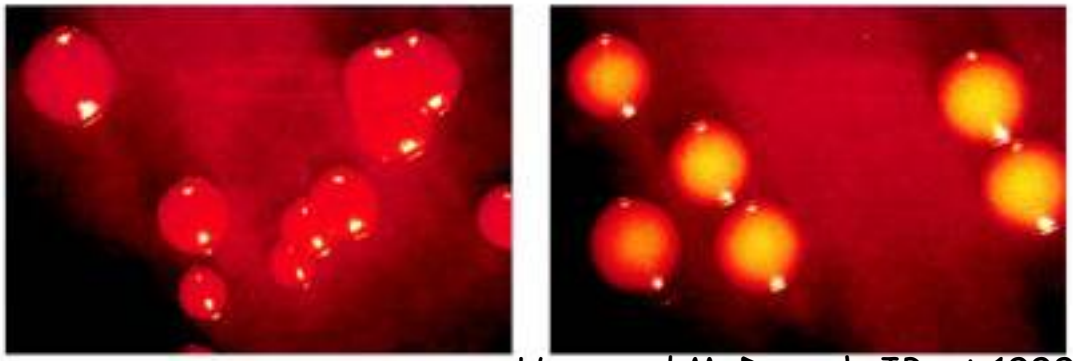


Jarrett et al J Inf Dis 2004 ; 190: 783-92

Haemin storage

Congo Red phenotype

Pigmented Non-pigmented



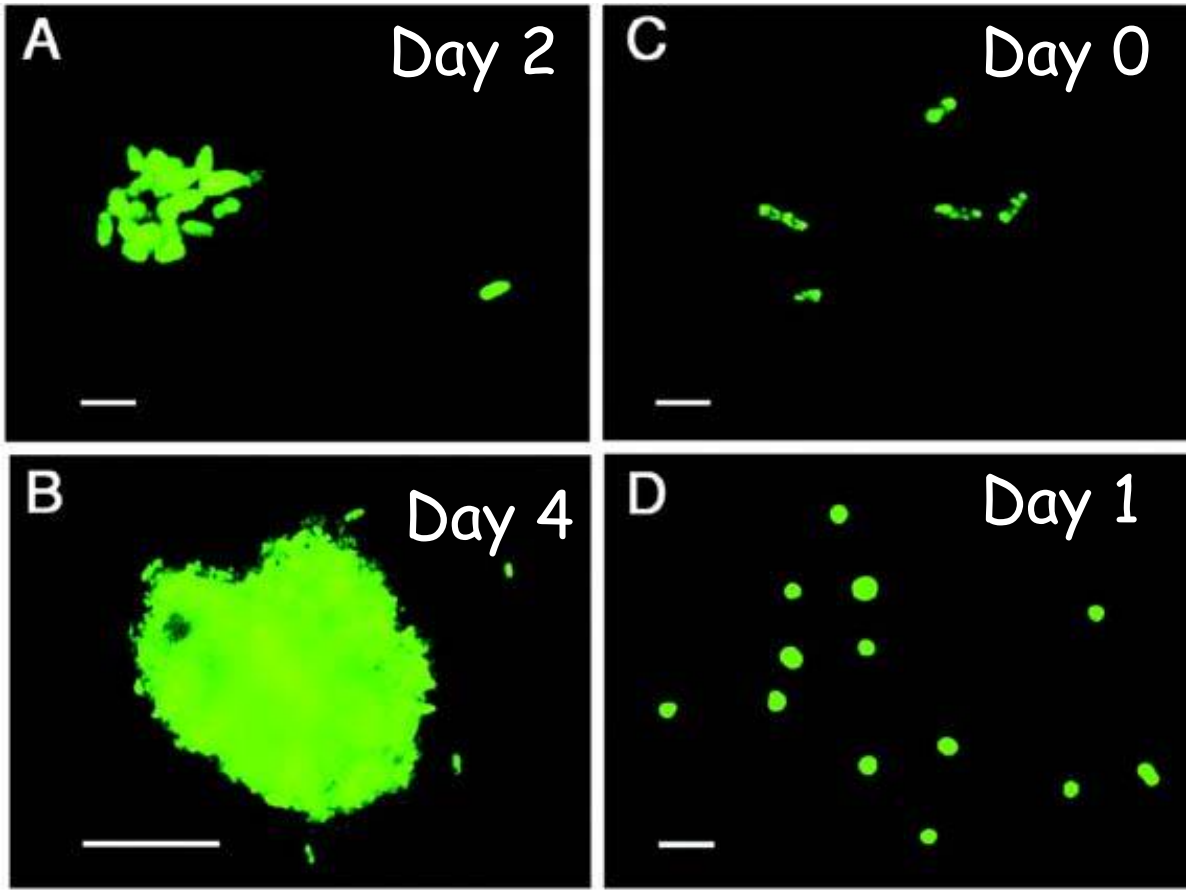
Hare and McDonagh JBact 1999

RecA independent *hms* mutation 10^{-5} in *Y. pestis*
Reduced virulence because other genes deleted
(Burrows and Bacon, Perry)

Flea Infection 2: mt

Murine toxin phospholipase D activity
stops plasma lysis Allows survival in flea midgut

IFA Assay
of dissected
flea midgut



Hinnebusch et al Science 2002;296:733-5

Arrival in mammalian host

Sebbane et al PNAS 2006

- Temperature cues shifting to 37C
- Intradermal inoculation
- Pla and yersiniabactin

Factors in spread from intradermal site

- Pla expression: plasminogen activator
  - Degrades complement
  - Adheres to laminin (extracellular matrix)
  - Essential for high virulence by SC or ID injection (Sodeinde, Goguen)
  - Essential for bubo formation
  - Pla-negative *Y. pestis* transmitted at low efficiency by fleabite can cause Septicamic plague with no bubo in mice
    - (Sebbane et al PNAS 2006)
  - (Pla important in primary pneumonic plague too)

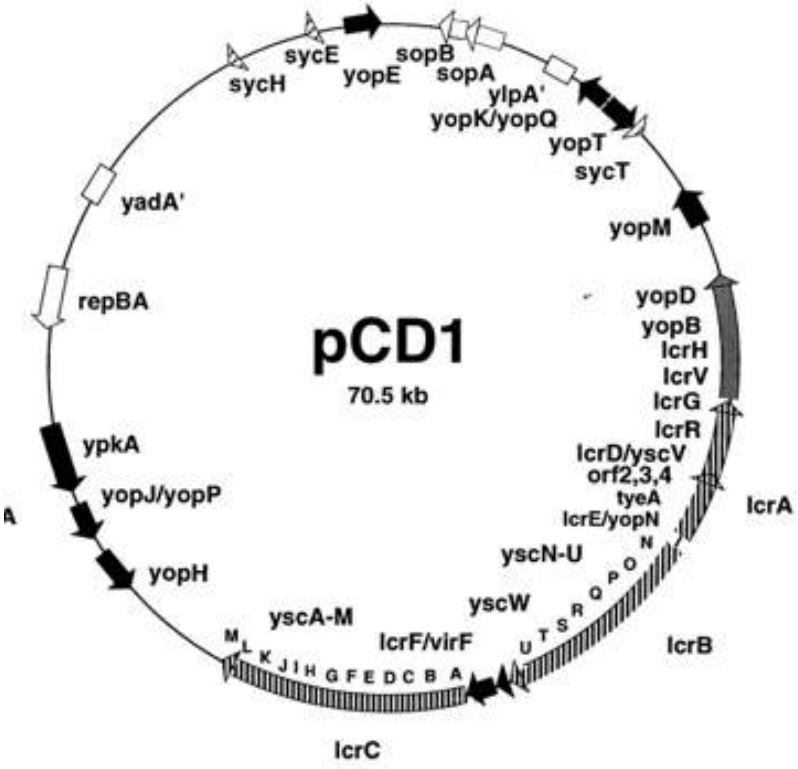
Factors important in spread from intradermal site: Yersiniabactin

- High affinity Siderophore synthesized by a multigene chromosomal operon on a "High Pathogenicity Island" (Carniel)
- If HPI deleted, greatly reduced spread from SC injection (Fetherston and Perry)
- Similar operon in *Y. enterocolitica*, *E.coli*
- Integrative and Conjugative element (ICE) horiontally self transferred and inserted in a tRNA gene (Schubert, Rakin, Heesemann)

Factors in transport to regional lymph nodes

- PhoP
  - Two component regulator required for intramacrophage survival of other pathogens e.g. *Salmonella*
    - (Titball, Oyston)

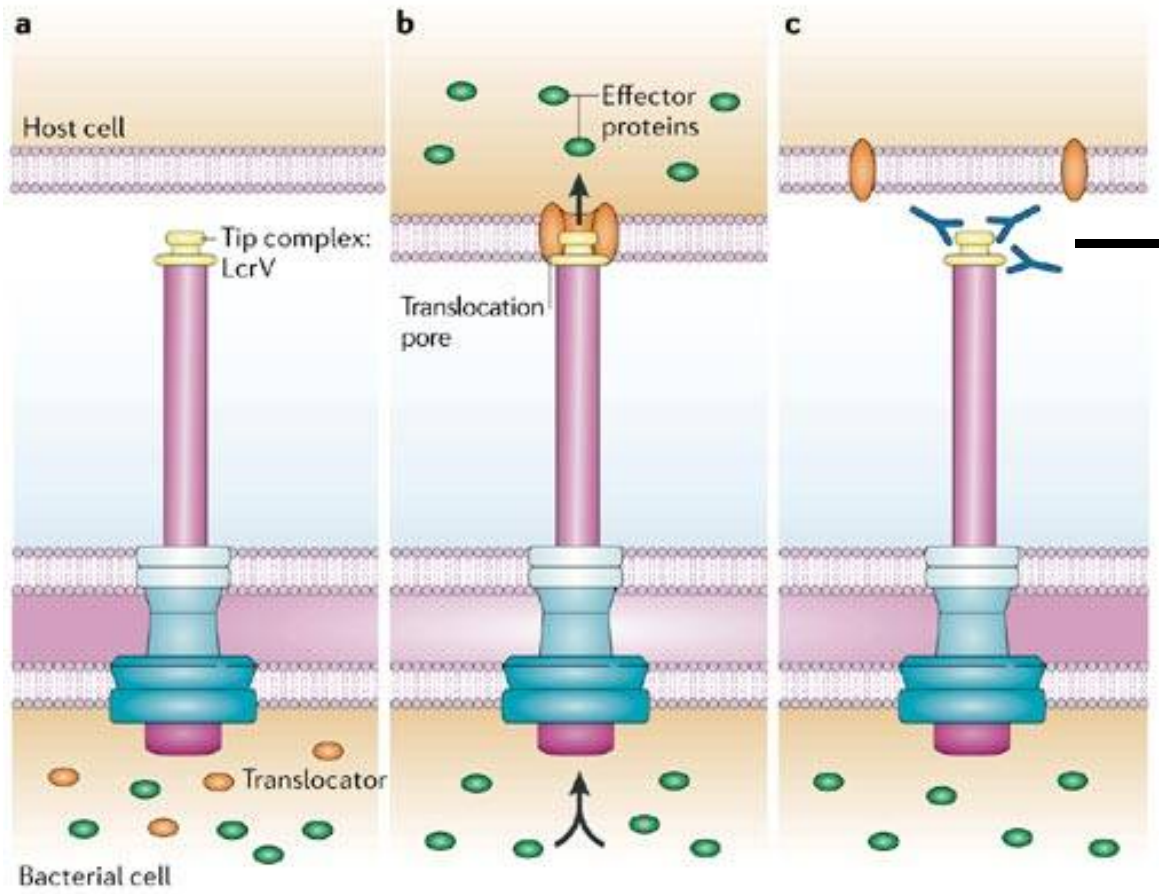
Extracellular survival in lymph nodes and spleen: pYV-located type III secretion injectisome



Cornelis. Microbiol Mol Biol Rev 1998

Wolf-Watz, Forsberg, Cornelis, Straley, Perry etc etc

- Ysc injectisome is the archetypal type III secretion system (TTSS)
- Protein export system which injects bacterial effector proteins into host cells
- Related to flagella

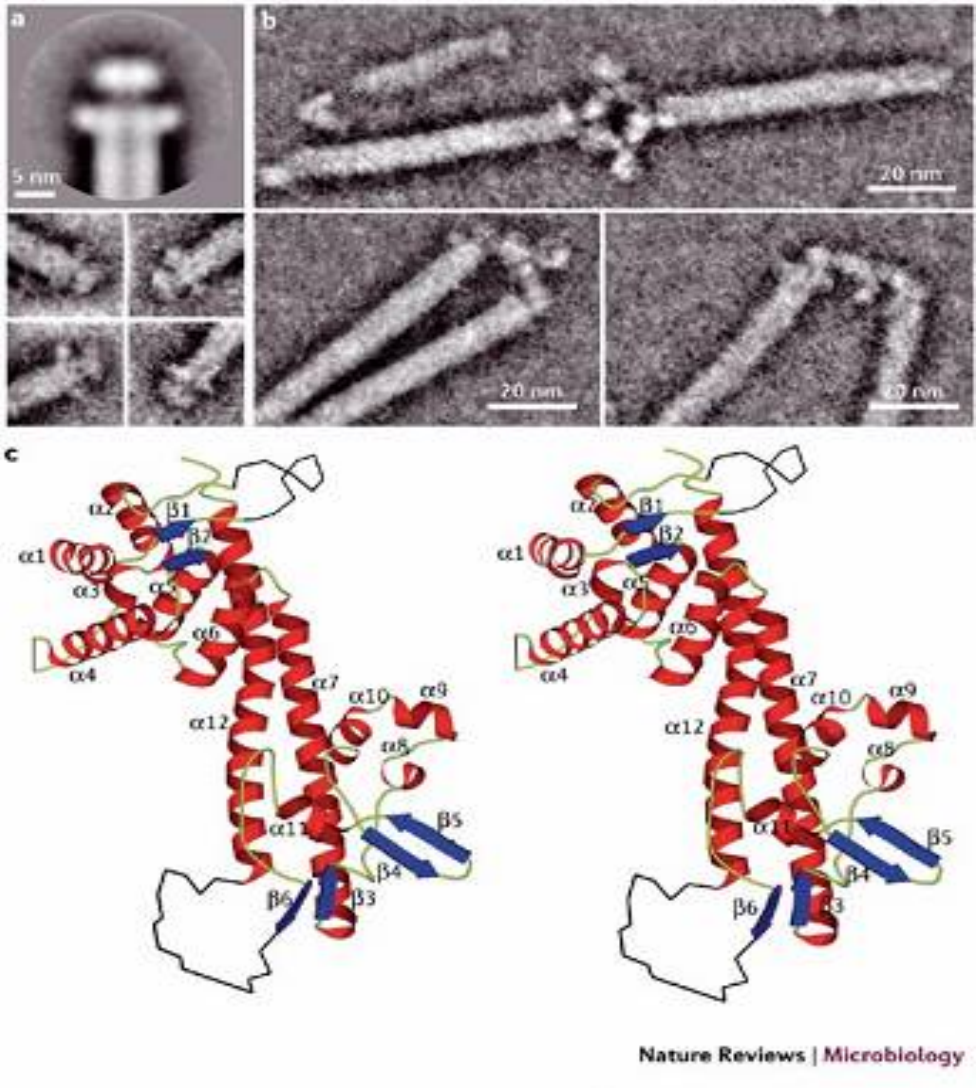


Anti LcrV Antibodies protect

Cornelis *Nature Reviews Microbiology* **4**, 811–825 (November 2006) | doi:10.1038/nrmicro1526

nature REVIEWS MICROBIOLOGY

LcrV forms
the tip of
the needle
Makes pores
By association
With YopB,
YopD to make
translocon



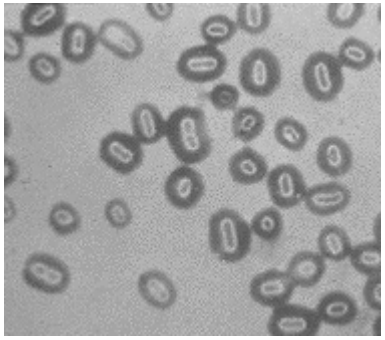
Cornelis *Nature Reviews Microbiology* **4**, 811–825 (November 2006) | doi:10.1038/nrmicro1526

nature REVIEWS MICROBIOLOGY

Effectors of Ysc injectisome

- Yop H, E, J, M, O P
- Target dendritic cells, macrophages, and neutrophils i.e. innate immunity
  - (Marketon et al Science; 309. 2005)
- Keep *Y. pestis* OUTSIDE phagocytic cells
- LcrV has anti-inflammatory, immune suppressive effects
  - (Exploitable therapeutically Foligné et al Adv Exp Med Biol 2007)

Extracellular survival: F1-capsular antigen



Adapted from Prof RW Titball

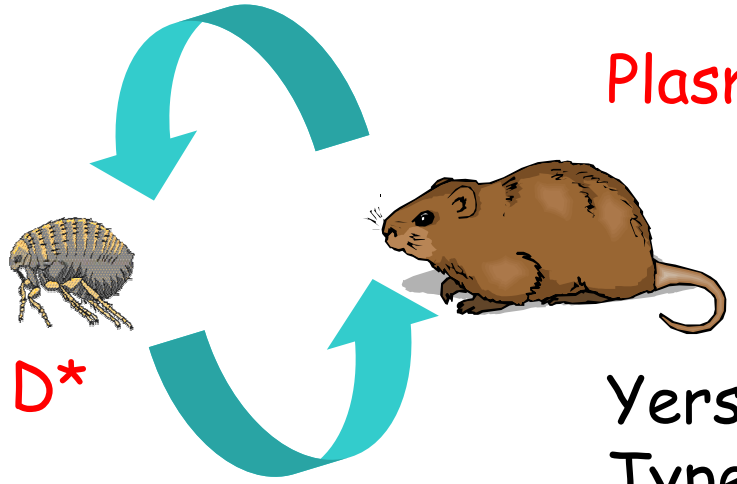
- Encoded by the *caf* operon on pFra plasmid
- 15.5 kD caf1M monomer assembles on the bacterial cell surface

Y. pestis:
Genetic basis of two host life styles

Expressed 26°C

Pigmentation
hms

Phospholipase D*
ymt



Expressed 37°C

Plasminogen activator *pla**

Yersiniabactin *irp2**
Type III secretion pYV*
F1 Capsular antigen *caf1**

* Located on plasmid or otherwise mobile element
Not in *Y. pseudotuberculosis*

Bubonic plague

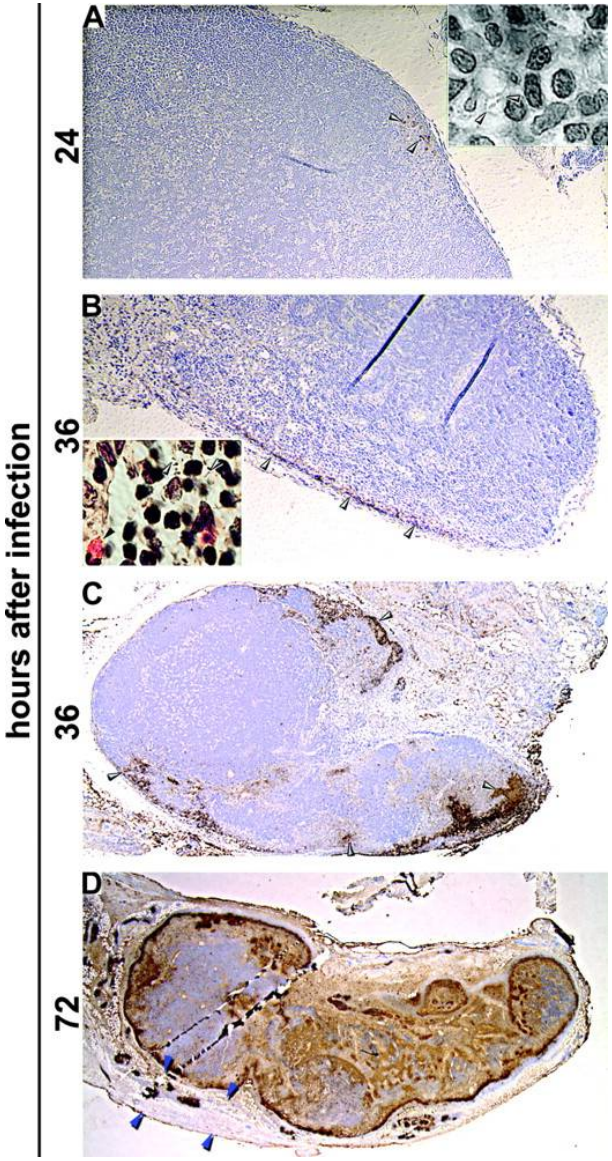
2-6 days incubation
Fever, malaise
Tense, tender , swelling (bubo)
Necrotic focus at regional lymph node
(Cervical, axillary, inguinal)

Inguinal and axillary buboes

Ruptured inguinal bubo
Image ID 2046
http://phil.cdc.gov/phil/details.asp

Axillary bubo
Image ID 2045
http://phil.cdc.gov/phil/details.asp

Intradermal injection in mice



Y. pestis (stained brown) appears peripherally extracellularly in marginal sinus 24-36 hours with few polymorphs

Bacteria infiltrate the cortex, with polymorphs 36 hours

Bacteria, necrotic polymorphs and fibrin replace normal node architecture, gelatinous nodal capsule (blue arrows) contains bacteria

American Journal of Pathology. 2005;166:1427-1439. Sebbane et al

Pneumonic plague

- Secondary pneumonic plague
  - Consequence of bacteraemic spread to lungs from flea-transmitted plague
- Primary pneumonic plague
  - Consequence of inhaled droplets (family, nosocomial spread) bacterial growth initial antiinflammatory/later inflammatory reaction
    - (Latham et al PNAS 2005)
  - Consequence of inhaled aerosol (bioweapon)
  - 100% fatal untreated, 50% fatal treated
- Key sign - cough with bloody sputum

Extracellular *Y. pestis* in lungs

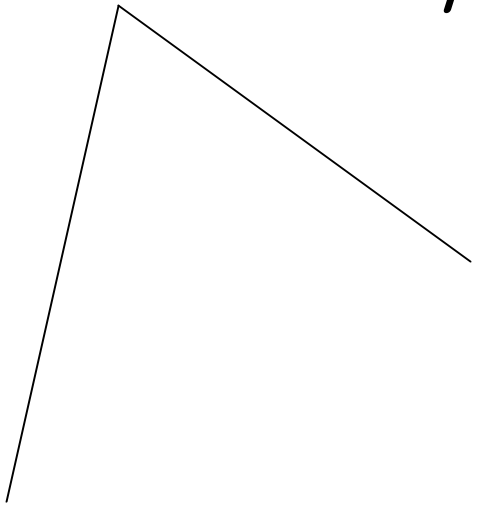
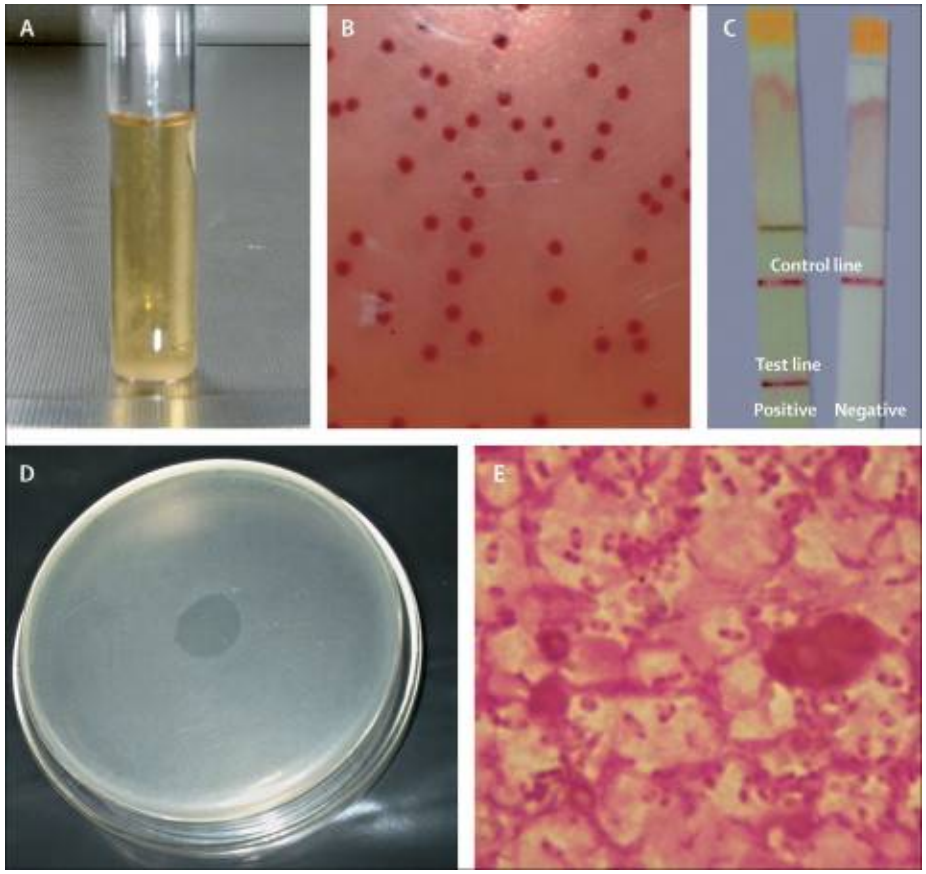


Image 741 Dr Marshall Fox
http://phil.cdc.gov/phil/details.asp

Multilobar involvement: Plague pneumonia

http://phil.cdc.gov/phil/details.asp
Image ID 4136. Dr Jack Poland

Conventional Laboratory Diagnosis



Picture courtesy Dr L Rahalison, Institut Pasteur de Madagascar

Category 3 pathogen

Grows on ordinary media (broth, CIN)

May ID as *Y. pseudotuberculosis*

Reference laboratory: Phage.

F1 ELISA Serodiagnosis

Bioterrorism: preliminary culture in local laboratory, referral to specialist laboratory

- USA Sentinel laboraries - referral ro State Public Health Laboratories
- Sentinel Guidelines ASM/CDC
  - http://www.asm.org/Admin/Index.asp?downloadid=2178
- Many rapid diagnostic tests being developed - no market leader

Treatment

- Streptomycin, 1g IM twice daily
- Gentamicin, 5 mg/kg IM or IV once daily
- or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV three times daily
  - Mwengee Clin Infect Dis 2006
- Plasmid-mediated multi drug resistance reported in Madagascar
  - Galimand NEJM 1997, Guiyoule EID 2001

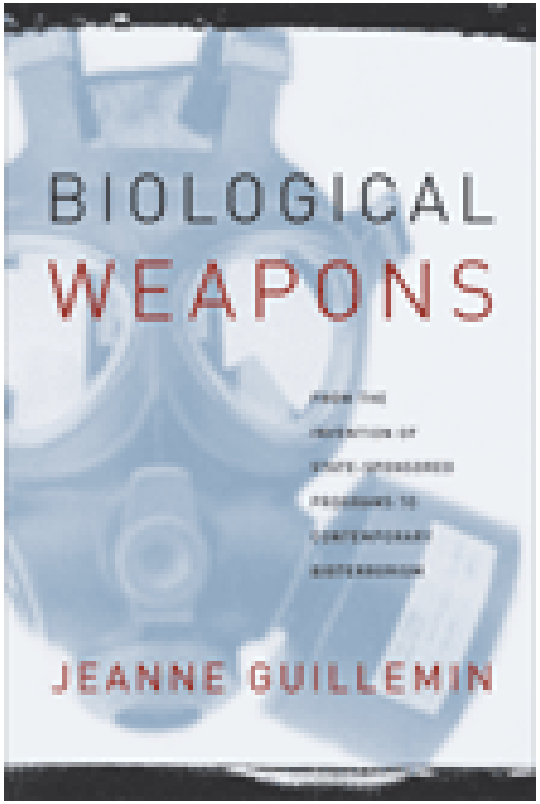
Plague control

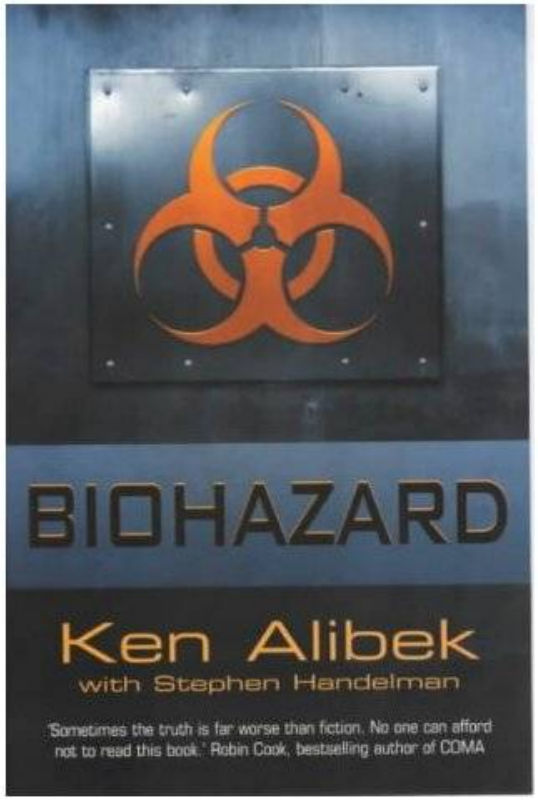
- Zoonosis: vector and flea control in endemic areas

Vaccine

- Previous vaccines
  - Killed whole cell vaccines modestly protective against bubonic plague, not against respiratory challenge in animals
  - Live "attenuated" EV76 strain
- Subunit vaccine containing LcrV and F1 antigens protective in animals against respiratory challenge
- Separate USA/UK LcrV/F1 subunit vaccines in Phase 2 trials (Titball, Williamson)

Biological warfare related activity involving *Y. pestis* by nation states





Japan: alleged use 1930s, WWII in China
USSR: alleged Cold War Weapon Production system (**USA/UK** aerosol animal experiments)

Y. pestis and "reverse public health"

- High mortality by aerosol in primates
- Readily engineered (antibiotic resistance, vaccine evasion)
- Fearful reputation inducing panic, major disruption from handful of cases in non-endemic area e.g. India 1993
- Stability issues cf *Bacillus anthracis* spores in any aerosol delivery system
- Doubtful environmental persistence vs *B. anthracis*
- Subunit vaccine in trials

Panel: **Recommendations for care of people with plague**

- Implement droplet precautions for patients with suspected cases of pneumonic plague until they have received effective antibiotic treatment for 48 h
- Wear disposable surgical masks to help reduce the risk of transmission from large respiratory droplets
- Use standard isolation precautions for non-pneumonic plague patients
- Monitor body temperature of potentially exposed individuals
- Consider postexposure chemoprophylaxis for people who have been in unprotected close contact (defined as coming within 2 m)[11] with a person with pneumonic plague who has not had antibiotic treatment for at least 48 h. (Doxycycline, ciprofloxacin, chloramphenicol, or co-trimoxazole can be used as prophylactics)
- Isolation of asymptomatic people who have had close contact with infected individuals is not recommended

Kool JL. Clin Infect Dis 2005; 40:1166-72
Building on: Lien Te Wu J Hyg 1913; 13:237-90

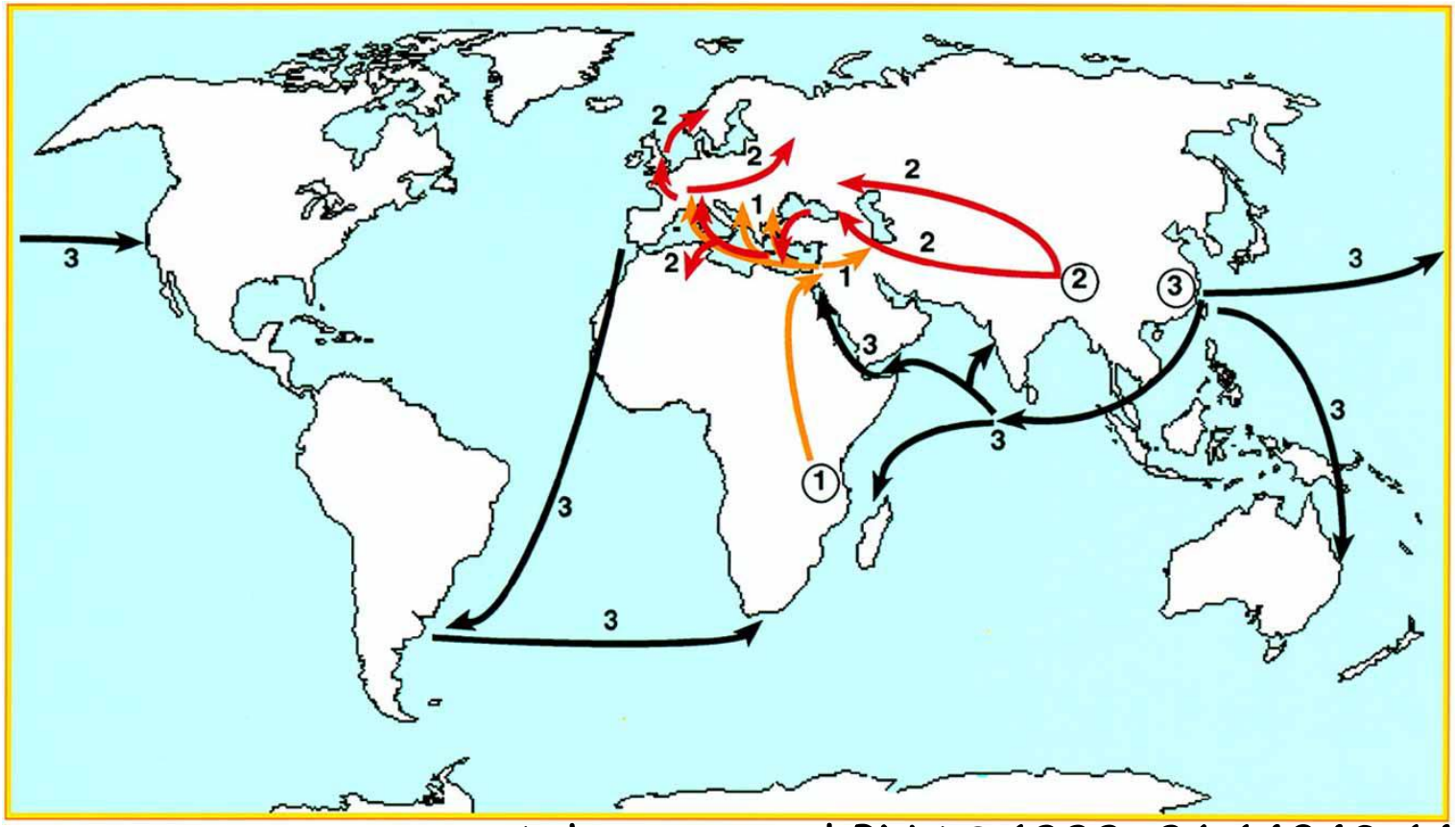
Yersinia pestis emergence hypotheses: Three biovars of *Y. pestis*

	Antiqua (1st pandemic)	Medievalis (2nd pandemic)	Orientalis (3RD pand.)
Glycerol	+	+	—
Nitrate	+	—	+
Distribution	Central Africa Asia	Iraq Turkey Russia	Worldwide

Devignat: Hypothesis linking Biovars and Pandemics 1951(Bull WHO)

Three pandemics of plague

1: 541-767 2: 1346-1720 3: 1850-

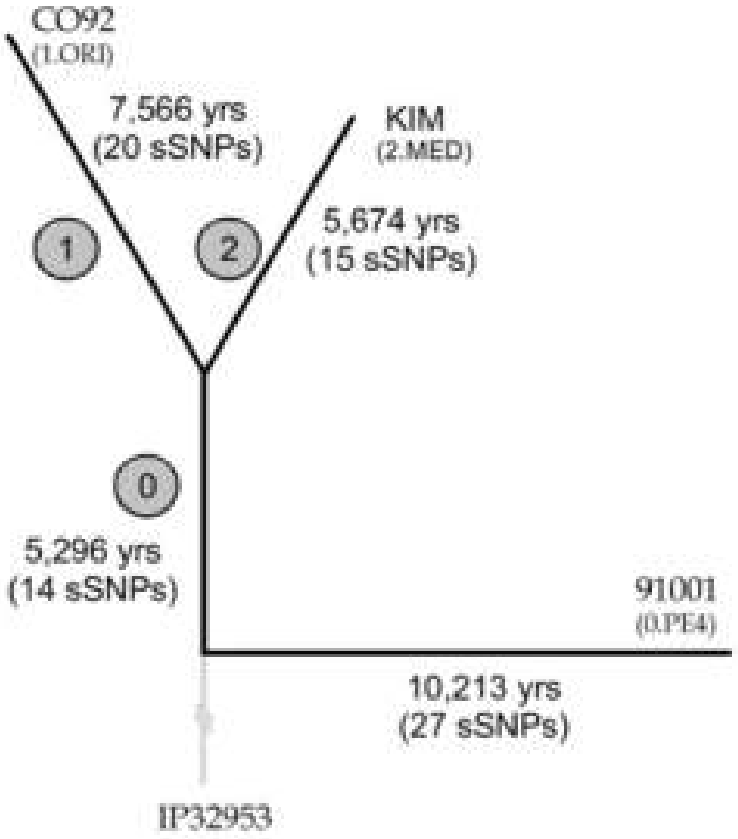


Achtman et al PNAS 1999; 96:14043-14048

Multi-genomic comparison

- *Y. pestis* KIM
  - Deng et al JBact 2002; 184:4601-11
- *Y. pseudotuberculosis* IP 33953
  - LPS locus resembling *Y. pestis*
  - Chain, Carniel et al PNAS 2004;101:13826-31
- *Y. pestis* 91001 (avirulent to humans)
  - Song et al DNA Res 2004; 11:179-97

Molecular clock and 76 Genome-Wide SNPs



Current *Y. pestis* strains split @6500 years ago
(no biovar -pandemic assn apart from Orientalis)

Worldwide spread of strains On Branch 0 which split >10,000 years ago

Widest distribution of strains in China ? Origin
Ancient DNA evidence ???

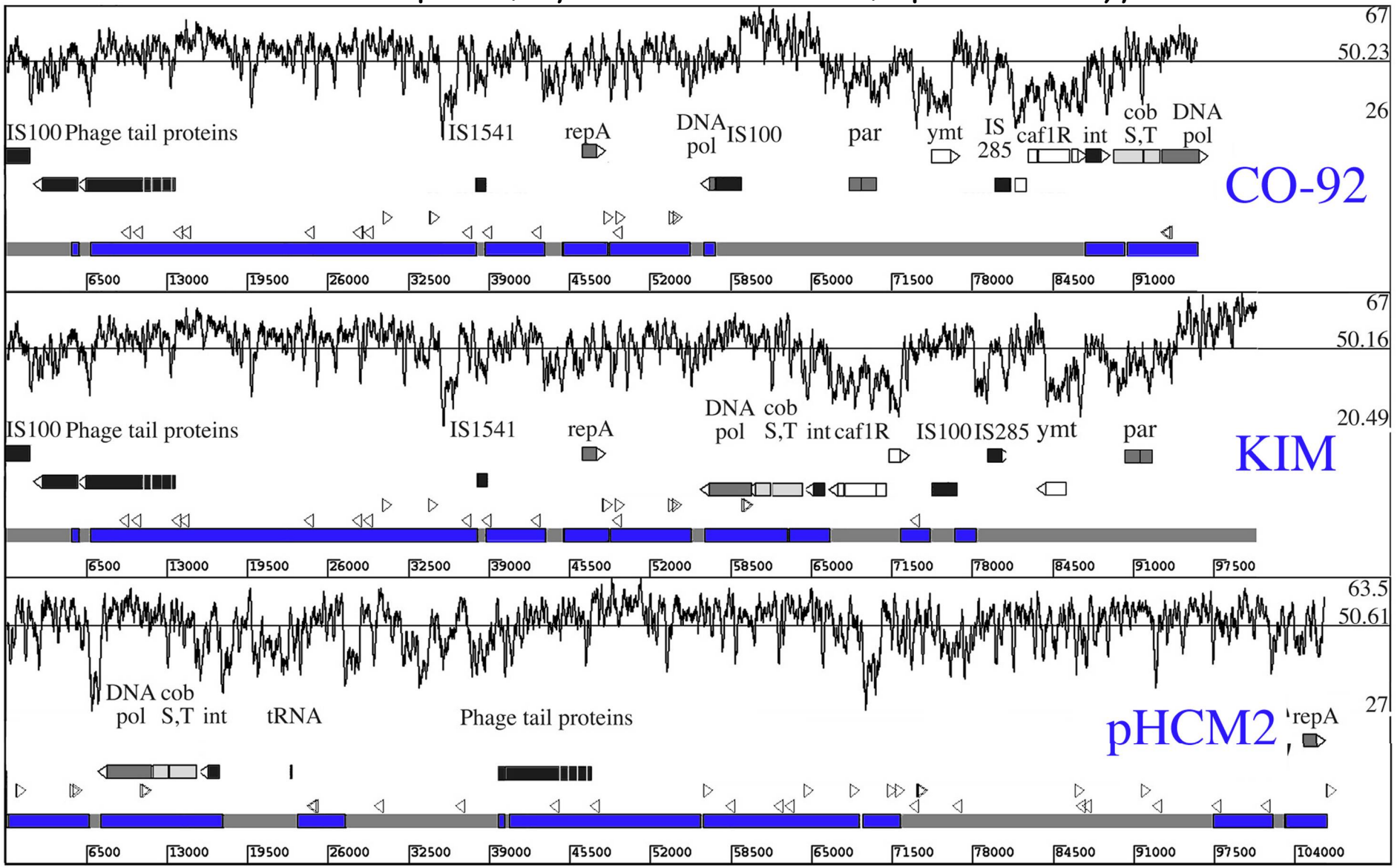
Achtman et al PNAS 2004; 101:17837-42

Virulence factors plasmid encoded

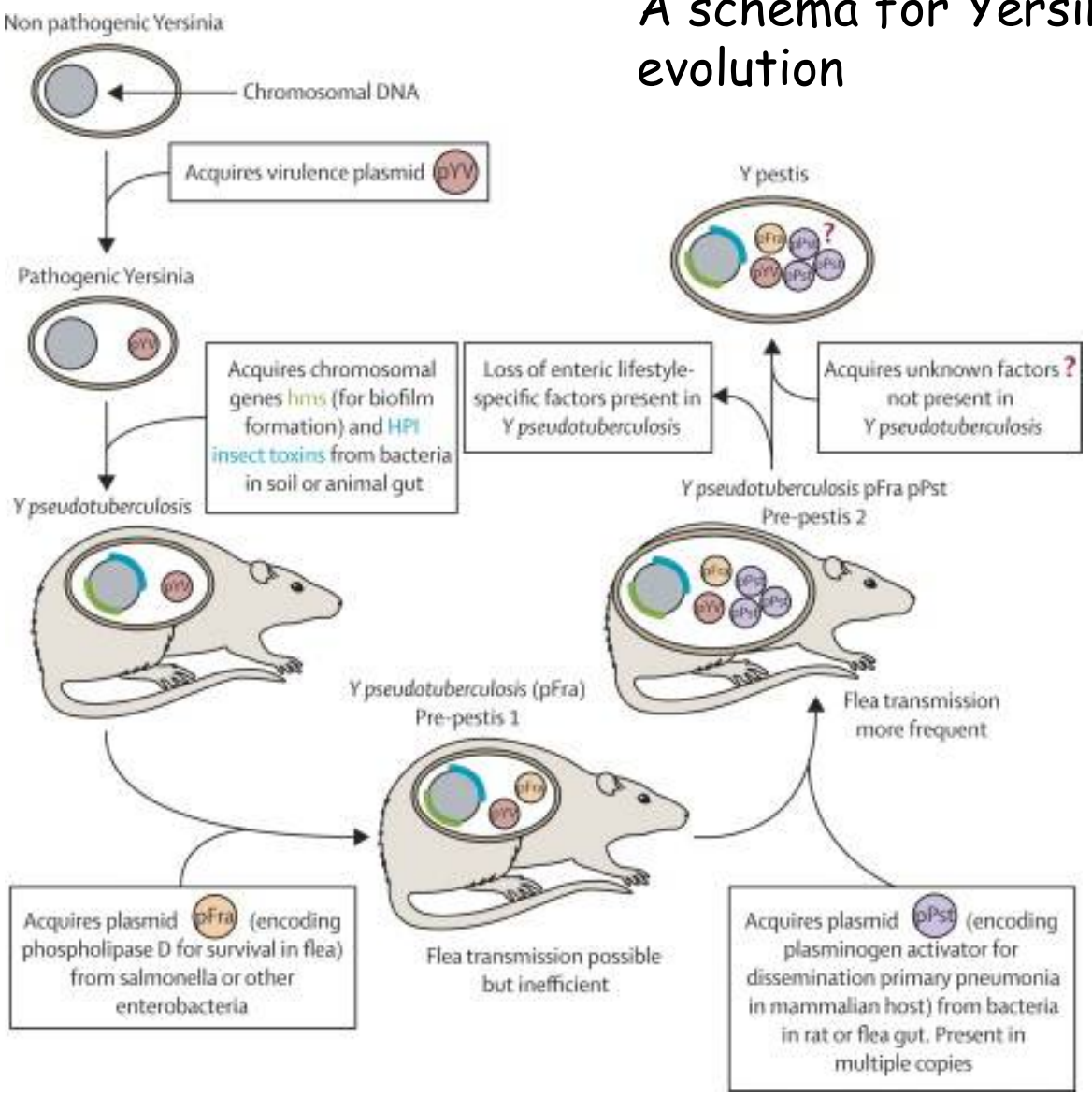
- *Y. pestis* exchanges plasmids with other bacteria
- Drug resistance in Madagascar
- Over 50% of pFra has been found in *S.enterica* Serovar Typhi

  Prentice JBact 2001

Areas in blue identical pFra (*Y. pestis* CO-92, KIM)+ pHCM2 *S. typhi*



A schema for Yersinia evolution



B.W. Wren. Nat Rev Microbiol. 2003 Oct;1(1):55-64 (similar schema from E. Carniel, J Hinnebusch)

Summary

- *Y. pestis* is a clone of an enteric pathogen *Y. pseudotuberculosis*
  - Minor changes in terms of plasmid specified DNA have allowed it to infect fleas, be transmitted by respiratory route
  - Although not contiguous with known pandemics, SNP data shows an origin 10-20,000 years ago is conceivable
- Many virulence factors are known and rational subunit vaccines have been developed
  - Bioterrorism concerns from historic reputation, past investigation as bioweapons agent

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- LSHTM
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- Institut Pasteur
- E. Carniel