Research Collaboration between Canada and Russia on Plague

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G8 Global Partnership Against the Spread of Weapons of Mass Destruction

- At the Kananaskis Summit, G8 members, from the United States, United Kingdom, France, Germany, Italy, Japan, Russia, the European Unio and Canada, united to launch the GPP.

- The eight countries agreed to raise up to US $20 billion to support cooperation projects, in Russia.
- As Chair of the G8 for 2002, Canada reinforced its leadership on the initiative by committing up to $1 billion over 10 years, beginning in 2003.

Global Partnership Program (GPP) and Biological Non-Proliferation

Terrorism

- “Terrorism is a global threat with global effects”
- UN Secretary General Kofi Annan

Global Partnership Program and Biological Threat Reduction

"Strengthen efforts to minimize holdings of dangerous biological pathogens and toxins, based on the recognition that the threat of terrorist acquisition is reduced as the overall quantity of such items is reduced".

Global Partnership Program Functions

- Through the Global Partnership Program, Canada is:
- Working on destruction of chemical weapons
- Dismantling nuclear submarines
- Improving the security of nuclear and other radiological materials
- Redirecting expertise of FSU Scientists
- Promoting biological non-proliferation

Redirection of Expertise of FSU Scientists

National Post Report

- Canada puts up cash ($118M) to prevent nukes' proliferation

  June 26, 2009 (National Post)

  Canada has already spent about half the $1-billion it committed, helping to dismantle old Soviet nuclear submarines, "redirecting" former scientists to more peaceful endeavours and encouraging better legal controls, among other things.

Justification

- "Scientists toil in conditions that are dangerous to them, as well, lacking the equipment used by Westerners working with biothreat agents".

  (National Post)

International Science and Technology Center (ISTC) and Redirection of Expertise of FSU Scientists

International Science & Technology Center (ISTC) Governing Parties

- European Union
- United States of America
- Russian Federation
- Japan

- Canada (joined ISTC in 2004)

Other parties

- CIS
- Norway
- Republic of Korea

The International Science and Technology Center -1994

- Objectives:
- Provide Russian and CIS scientists with opportunities to re-direct their talents to peaceful activities
- Support fundamental and applied research
- Contribute to solutions of national and international problems
- Support transition to market economy
- Promote integration of Russian and CIS scientists into the world scientific community

Key Focus Areas for ISTC Projects

- Enhancing security at key biological institutes
- Integration of Russian institutions in the international healthcare and animal care research
- International accreditation and certification of Russian institutes to quality standards
- Work towards integration of disease surveillance models with a focus on Central Asia
- Development and implementation of new treatment or diagnostic methods for diseases

ISTC Project Application

- Development of S&T project with collaborators
- Project proposals are submitted to ISTC
- Projects are reviewed by ISTC
- Projects are submitted to ISTC funding parties
- Funding decision is made
- The Lead institute develops a workplan
- Workplan includes details of tasks and milestones
- A project agreement is signed
- A program manager is appointed by ISTC
- Projects are typically 2-3 years.
- Projects funded require foreign collaborators

Role of Foreign collaborator

- Assist in the development of the S&T project proposal
- Assist in the implementation of the project once funded
- Assist in the review of draft project work plans
- Joint research
- Providing advice
- Assistance in identifying appropriate sources of special materials and equipment to be used in projects.
- Guidance on the international and commercialization of the project results

Role of Foreign collaborator

- Monitor the project, review technical reports
- Keep GPP informed of progress through short quarterly emails
- Participate in on-site technical monitoring
- Participate in joint seminars, workshops, meetings, consultations
- Verify project results using independent methods and/or equipment (when necessary)
- Include assessment of the completed project as part of the final ISTC project reports

ISTC and Biological non-proliferation

- The ISTC has funded about 110 projects with a focus on infectious diseases, especially in the fields of diseases with public health implications
- Of particular importance are pathogens considered as biothreat agents.

ISTC Project 2927

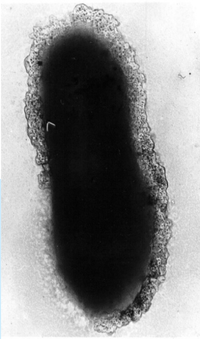
“Studies of the role of the pH 6 antigen in the virulence and immunity of Yersiniae”

ISTC Project 2927

- One of the science and technology projects funded by Canada
- Title of project is “Studies of the roles of the pH 6 antigen in the virulence and immunity of Yersiniae”
- This was a three year project at the State Research Center for Applied Microbiology (SRCAMB)-Obolensk-Moscow Region.
- Project funding was about $340,000 USD

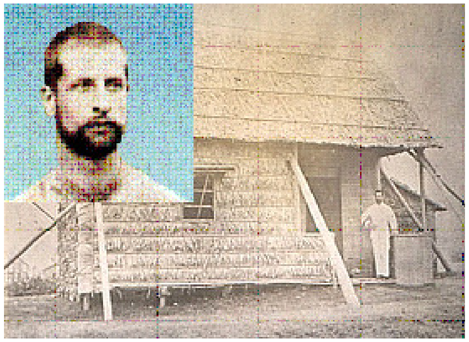
Goals of ISTC Project 2927

- The identification of mutations leading to attenuation in *Y. pestis* strain 231.
- Determine the role of pH6 antigen in virulence and immunity in *Y. pestis*.
- Elucidation of the role of pH6 antigen in adhesion
- Determine the relationship between YadA adhesin and pH6-antigen.
- Understand the evolution of adhesive function in Yersinia pathogenic for humans.



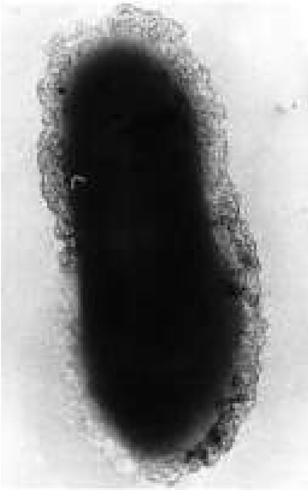
Yersinia pestis

About *Yersinia pestis*



- Named after Alexandre Yersin in 1894
- Causative agent of plague
- Gram-negative rod
- Genome size of about 4.6Mb
- Consists of three Biovars
- A clone of *Y. pseudotuberculosis*??

Plague is an acute contagion, considered as a rodent epizootic. It has led to several devastating pandemics killing millions of human beings.



The plague pathogen, *Yersinia pestis*, is circulating in populations of wild rodents

History of Plague

- The Libyan Plague in the 1st Century AD
- The First Pandemic or Justinian Plague- 6-8th Century
- The Black Death- 14th Century
- Modern Plague- 19th Century to present day

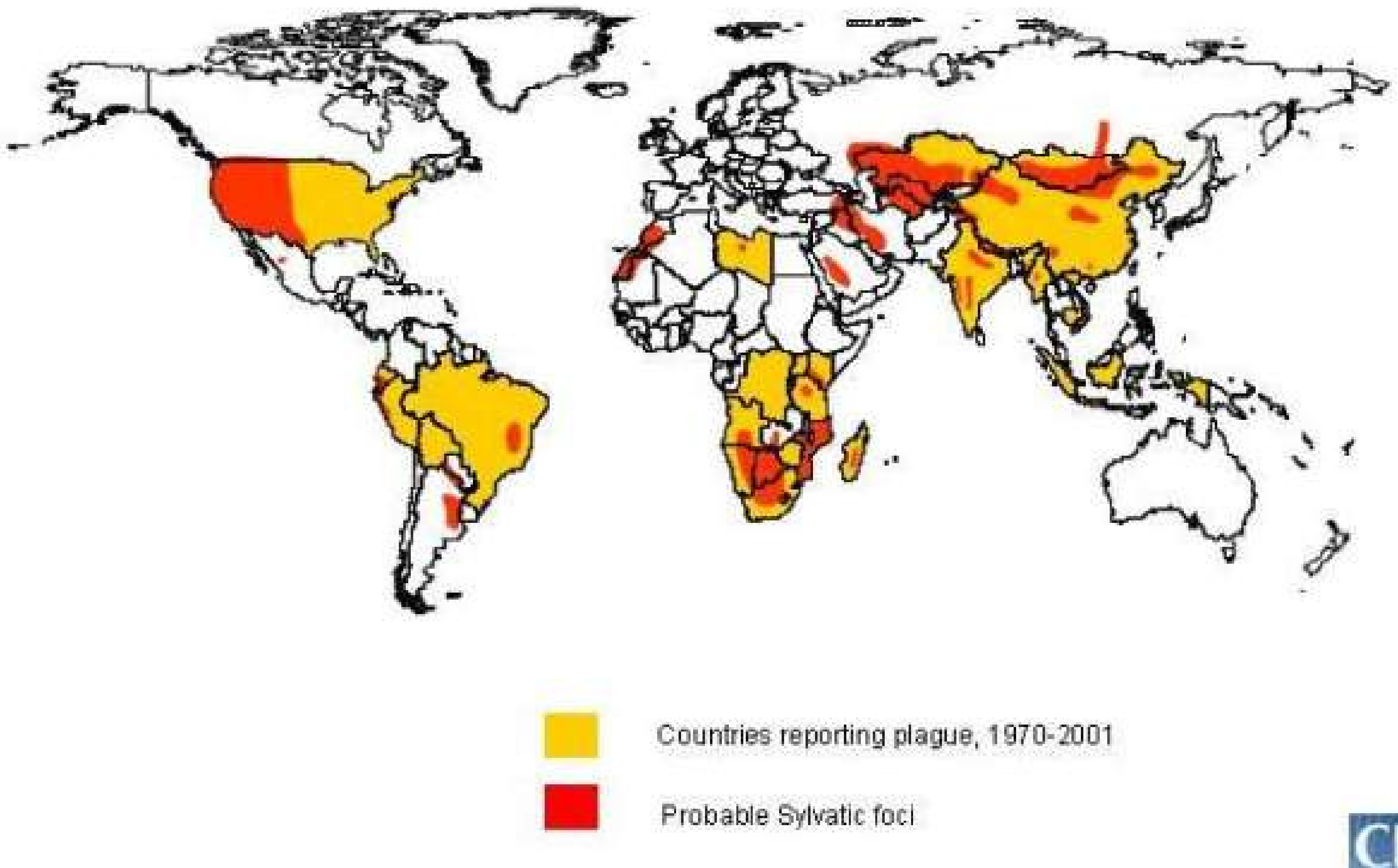
Clinical Manifestations

- Bubonic plague (3-6 days incubation period) (40-60% fatality)
- Septicemic plague (1-2 days)
- Pneumonic plague (1-2 days)

Symptoms of plague

- Sudden onset of illness
- Fever
- Chills
- Headache
- Insomnia
- Malaise and indistinct speech
- Buboes or inflammation of the lymph nodes
- Wobbly walk
- Nausea and Vomiting
- Delirious
- Violent and aggressive
- Meningitis
- Diarrhoea
- Coughing, dyspnoea and pneumonia

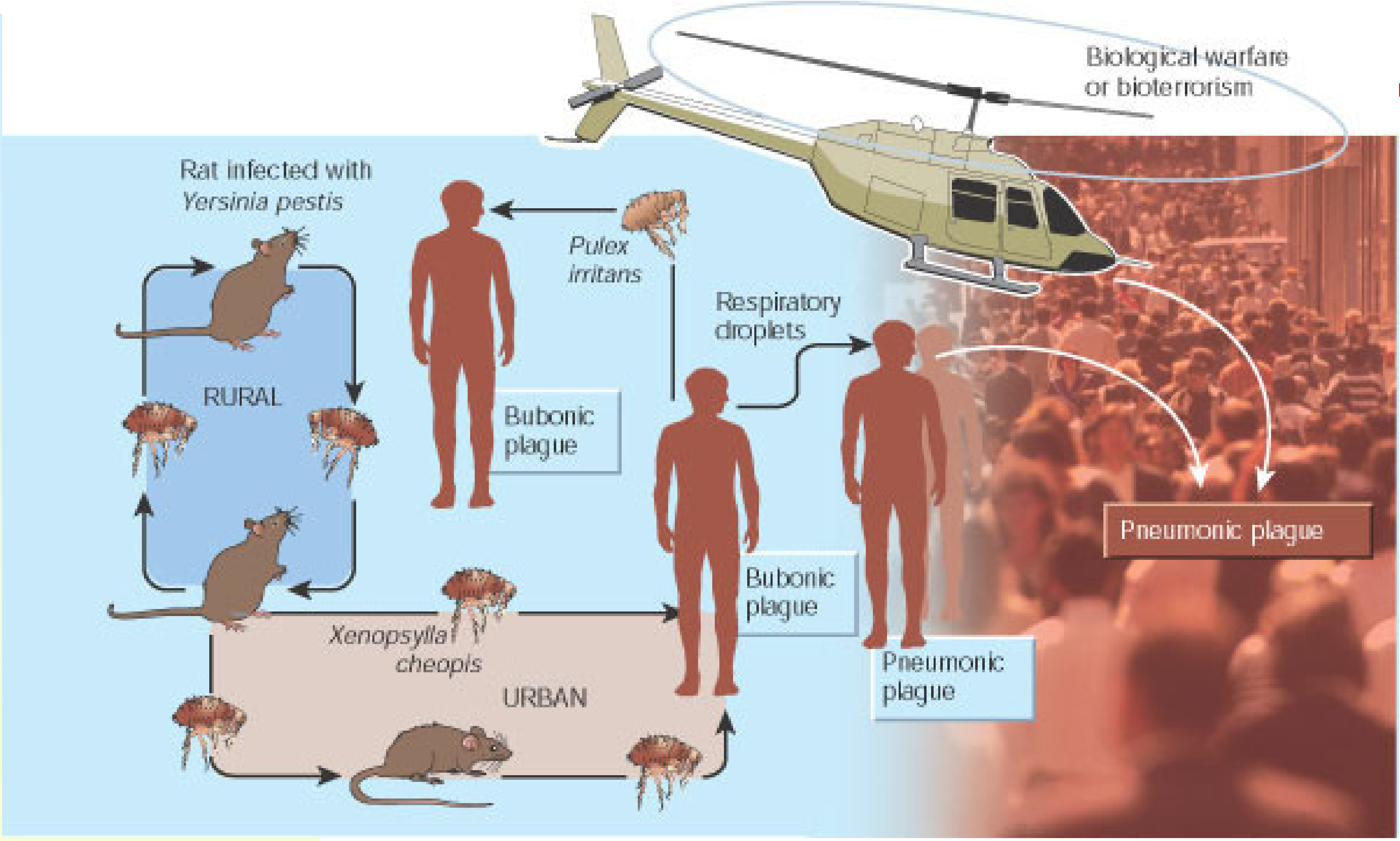
Plague foci in the world



Transmission of Plague

- Fleas bite
- Direct contact with infected samples
- Inhalation of aerosolized respiratory samples
- Consumption of insufficiently cooked meat products

How Plague is Transmitted



Stewart and Carmen Nature 2001, 413:468-470

Yersinia pestis as a bioterrorism agent

- Potential bioterrorism agent
- Aerosolization of 50kg could affect 150K killing 36K in a 5million population

- Virulence level of strain???
- Dissemination using food???

Genotypic distinctions between *Y.pestis* and *Y.pseudotuberculosis*

Determinant	*Y.pestis*	*Y.pseud.*	Size (kb)
pPCP1	+	-	10kb
pMT1	+	-	100kb
pCD1/pYV	+	+	70kb

“Studies of the role of the pH 6 antigen in the virulence and immunity of Yersiniae”

Why pH 6 antigen?

- *Y. pestis* strain 231 is a unique strain
- It is highly virulent.
- Has LD_{50} of **3** in mice
- Has LD_{50} of **4** in guinea pigs
- pH 6 is an adhesin and expressed at pH 6
- A mutation in the gene coding for pH 6 antigen leads to attenuation (LD_{50} of >10^8)

About the pH6 Antigen

*Initially described by Ben-Efraim *et al.* in 1961

*Antigen synthesized by *Y. pestis* at the temperature close to body temperature of mammals (35-41ºC)

*Synthesized at acidic pH values close to pH of abscesses or phagolysosomes in macrophages

About the pH6 Antigen

-molecular weight of 15 kDa

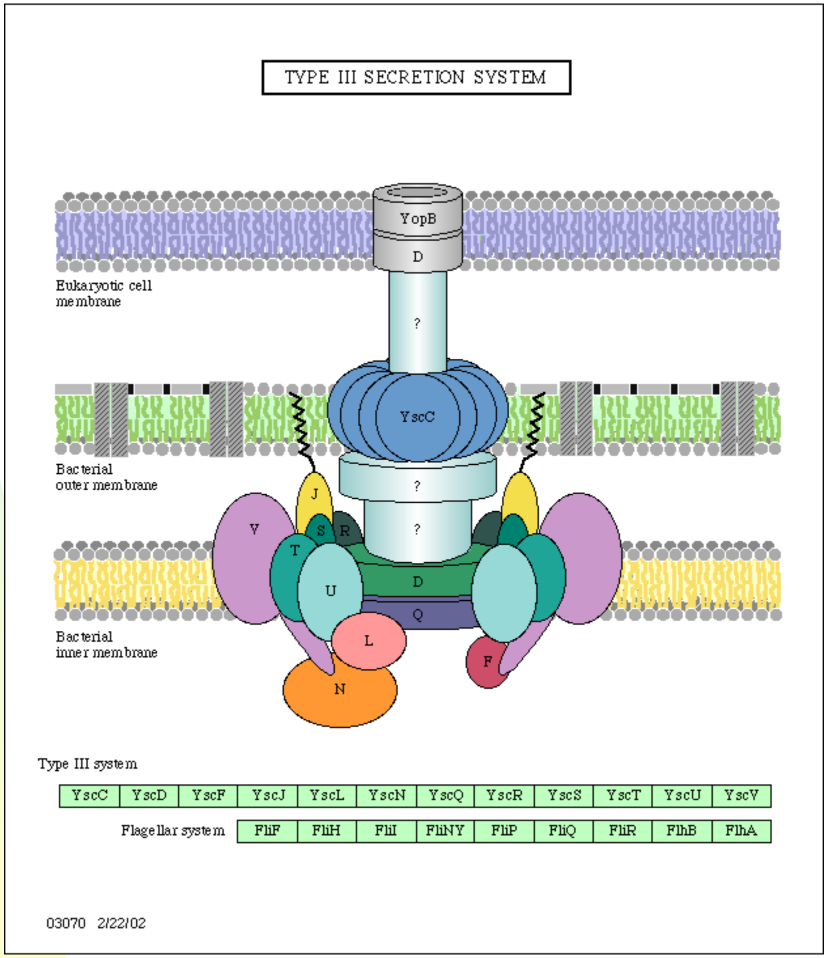
-assembles at the surface of *Y. pestis* and *Y. pseudotuberculosis* into homopolymer macromolecular complexes,

-adhesion pili [Vodop'ianov, 1985, 1990, Lindler, 1993, Pizarro-Cerda, 2006, Thanassi, 1998]

-forms a capsule-like structure similar to the *Y. pestis* F1 capsular antigen [Cherepanov, 1991, 1998].

prevents phagocytosis [Stepanshina et al., 1993, Huang et al., 2004],

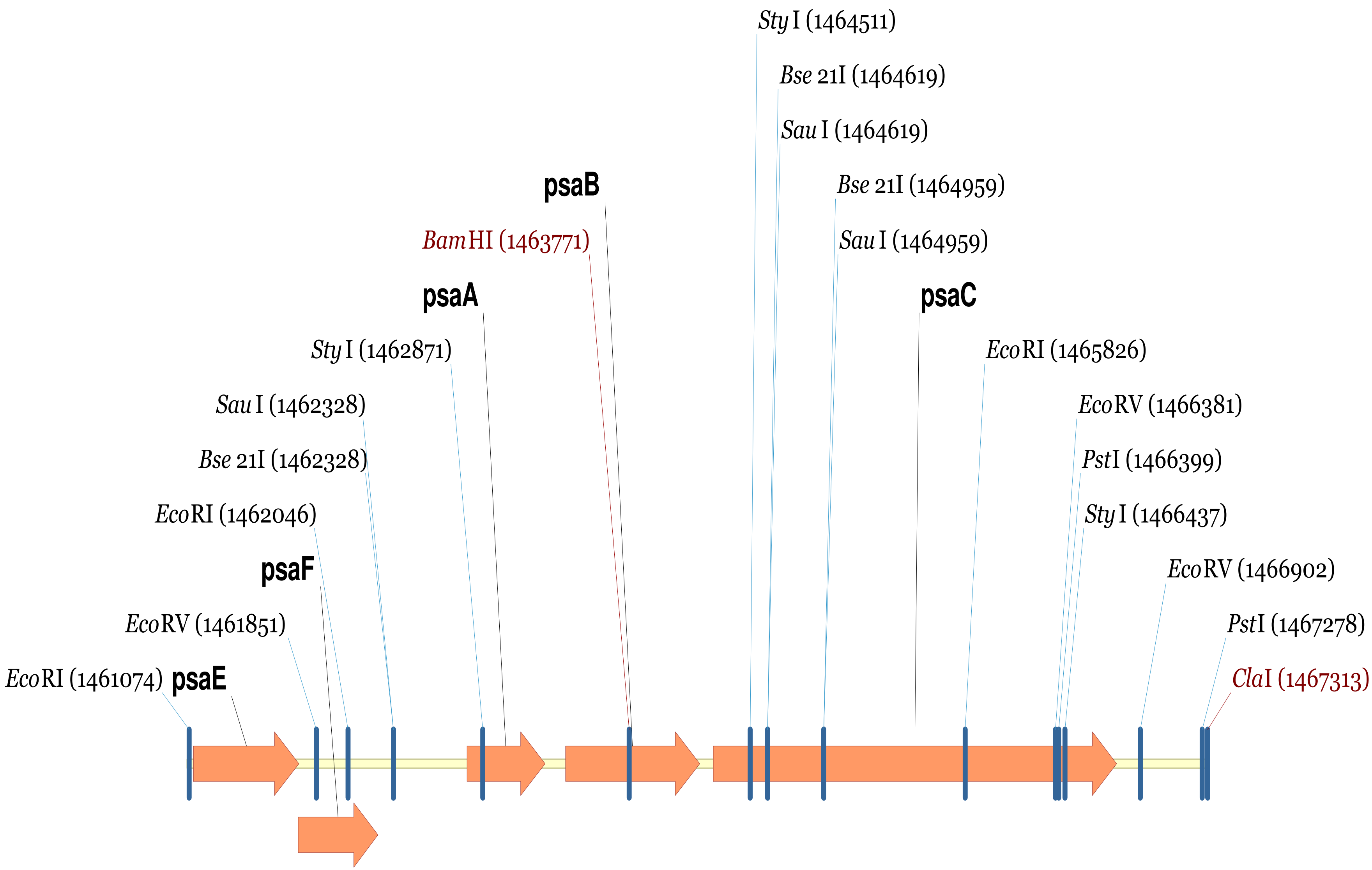
Type III Secretion System



Project tasks

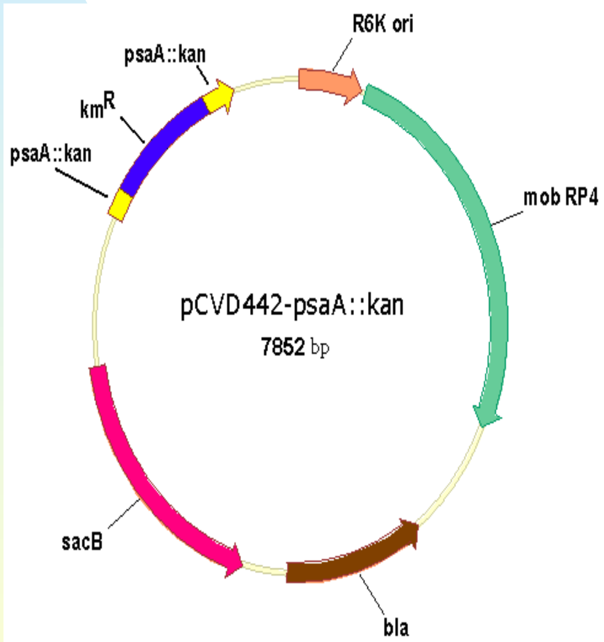
- Generate psa knockout mutants
- Studies of the biological properties of *Y. pestis* psa knockout mutants.
- Studies of pH6-antigen interaction with YadA in *Y. pestis*

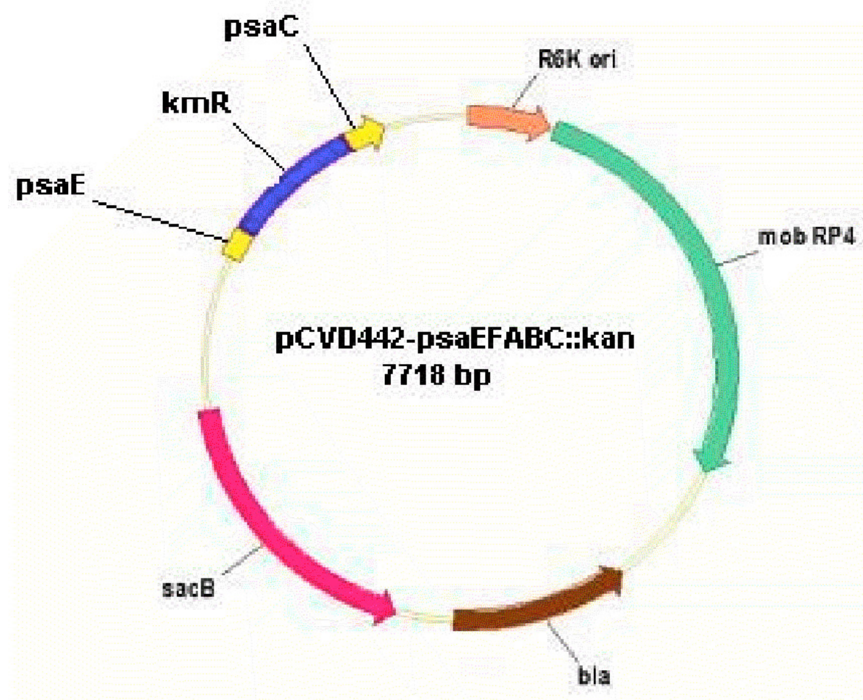
psa operon



Sty I (1464511)
Bse 21I (1464619)
Sau I (1464619)
Bse 21I (1464959)
Sau I (1464959)
psaB
Bam HI (1463771)
psaA
psaC
Sty I (1462871)
Eco RI (1465826)
Sau I (1462328)
Eco RV (1466381)
Bse 21I (1462328)
Pst I (1466399)
Eco RI (1462046)
Sty I (1466437)
psaF
Eco RV (1466902)
Eco RV (1461851)
Pst I (1467278)
Eco RI (1461074)
psaE
Cla I (1467313)

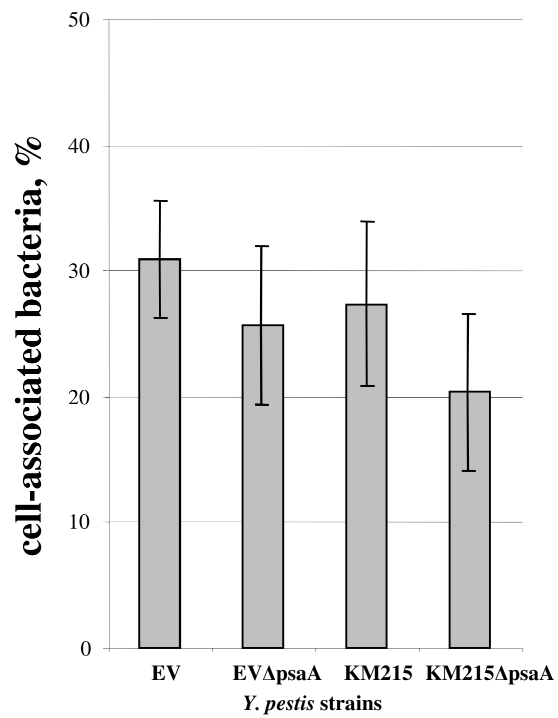
Generation of psa Mutants



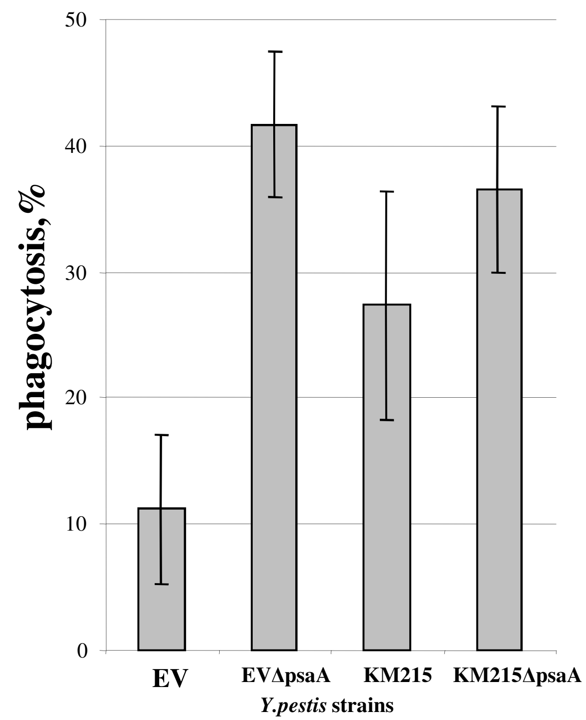


Adherence and Phagocytosis of Macrophage-like cells

Adherence



Phagocytosis

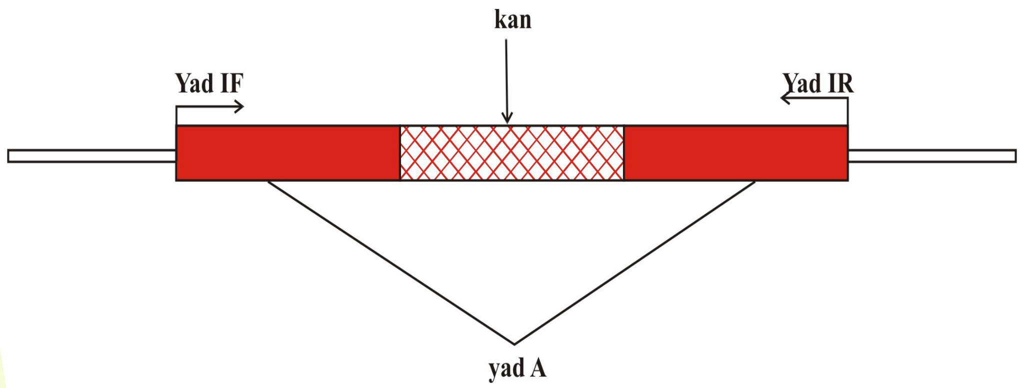


	EV	EVΔpsaA	KM215	KM215ΔpsaA	EV	EVΔpsaA	KM215	KM215ΔpsaA
1 **F1**	+	+	−	−	+	+	−	−
2. ***Psa***	+	−	+	−	+	−	+	−

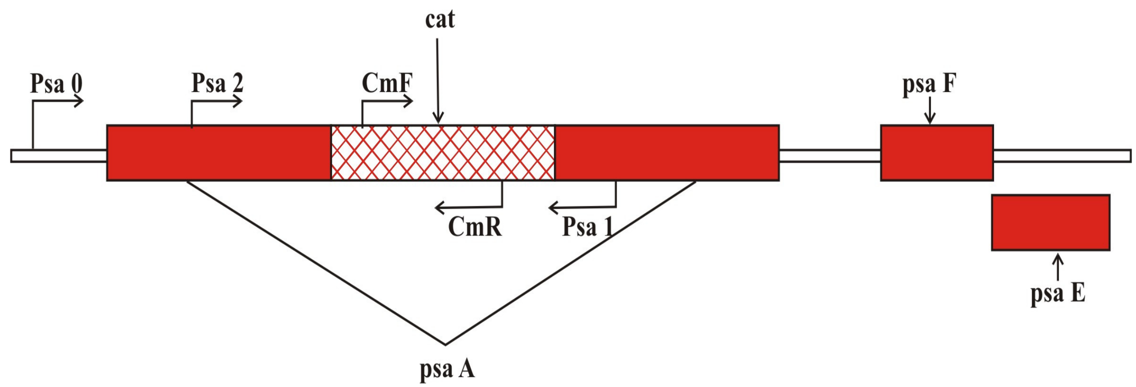
LD_{50} and average survival time in mice infected subcutaneously with *Y. pestis* strains

Y. pestis strain	pH 6	Naïve mice		Immune mice	
		LD_{50}, CFU*	▪Mean time to death	LD_{50}, CFU	▪Mean time to death
231	+	1 (1-2)	5,7 ± 1,48	2 (1-10)	5,9 ± 1,56
231Δ*psaEFABC*	–	2 (1-5)	5,5 ± 1,10	1 (1-2)	5,9 ± 1,56
231Δ*psaEFABC*pJG428	+	3 (1-10)	4,9 ± 1,15	ND	ND
231Δ*psaEFABC*pIG924	+	3 (1-10)	5,5 ± 1,80	2 (1-7)	5,8 ± 1,37
I-1996	+	1 (1-2)	6,2 ± 1,56	4 (1-15)	6,4 ± 1,19
I-1996Δ*psaA*	–	4 (1-16)	5,7 ± 1,10	ND	ND
I-1996Δ*psaEFABC*	–	3 (1-13)	6,1 ± 1,36	1 (1-2)	6,1 ± 1,36
I-1996Δ*psaEFABC*pIG924	+	1 (1-5)	7,1 ± 2,21	1 (1-5)	7,2 ± 2,12

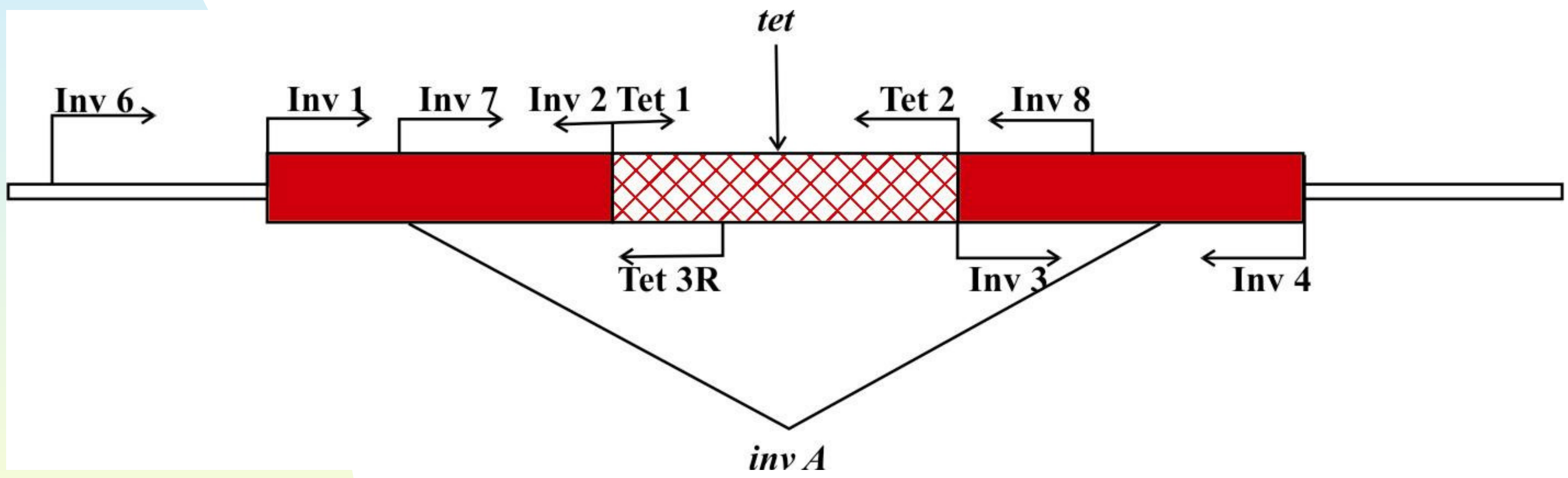
The map of the *Y. pseudotuberculosis* chromosome fragment containing the *yadA* gene



The map of the *Y. pseudotuberculosis* chromosome fragment containing *psaA* gene



Physical map of *Y. pseudotuberculosis* chromosomal fragment carrying inactivated *inv*A



The lethality of mice infected with the isogenic strains of *Y. pseudotuberculosis*.

Strain phenotype	LD_{50} (cfu)	SLD
$Inv^{+}Yad^{-}Psa^{-}$	5×10^{5}	10^{4}
$Inv^{+}Yad^{-}Psa^{+}$	5×10^{5}	10^{4}
$Inv^{+}Yad^{+}Psa^{-}$	10^{4}*	ND*
$Inv^{-}Yad^{+}Psa^{+}$	10^{4}*	ND*
$Inv^{-}Yad^{+}Psa^{-}$	10^{4}*	ND*
$Inv^{-}Yad^{-}Psa^{+}$	10^{6}	10^{5}
$Inv^{-}Yad^{-}Psa^{-}$	5×10^{6}	5×10^{5}
$Inv^{+}Yad^{+}Psa^{+}$	10^{4}	Lower 10^{3}

Effect of Inv, Yad, and Psa on the invasion of Hep2 cells.

Strain phenotype	Number of bacteria added to mono layers	Number of intracellular bacteria
$Inv^{+}Yad^{-}Psa^{-}$	7×10^{7}	1×10^{6}
$Inv^{-}Yad^{+}Psa^{-}$	9×10^{7}	9×10^{3}
$Inv^{-}Yad^{-}Psa^{+}$	6×10^{7}	6×10^{3}
$Inv^{-}Yad^{-}Psa^{-}$	6×10^{7}	3×10^{3}
$Inv^{+}Yad^{+}Psa^{+}$	6×10^{7}	1×10^{6}

Conclusion

- A mutation in the psa operon may be compensated for by the regulation of other genes associated with adhesion in *Y. pestis*
- pH 6 antigen is not a suitable molecular target for treatment or immunoprophylaxis of bubonic or pneumonic plague.

Publications

- The subcutaneous inoculation of pH 6 antigen mutants of *Yersinia pestis* does not affect virulence and immune response in mice
  Journal of Medical Microbiology (2009), 58, 26–36.

- A comparative study of the role of the *yadA, invA,* and *psaA* genes in the pathogenicity of *Yersinia pseudotuberculosis.* Molecular Genetics, Microbiology and
  Virology, 2008, Vol. 23, No. 4, pp. 168–177

- Cytotoxic, mitogenic and adhesive activities of *Yersinia pestis* pH 6 antigen
  Manuscript in revision

Treatment of plague: promising alternatives to antibiotics

J. Med. Microbiol. 2006, 55, 1461-1475. (Review paper)

By Dr. Andrey Anisimov and Dr. Kingsley Amoako

Areas covered

- Overview
- Pathogenesis and virulence factors
- Methods of treatment
- Promising alternative treatments
- Conclusion

Antimicrobial Resistance

- Natural isolates of plague are susceptible to antimicrobials such as Tet. Gent. Chlor. Strept. Doxycyc. Cipro. etc.
- A natural resistant strain isolated in Madagascar in 1995-17/95 strain
- Was found to be resistant to all the recommended antibiotics for plague
- Plasmid conjugates with high frequency to other *Y. pestis* strains

Virulence

Adherence
Invasion
Evasion

Pathogenesis and virulence factors of Plague

Bacterial factor	Function or activity	Reported decrease of virulence in knock-outs (by order of magnitude)		
		Dramatic (> 4)	Moderate (4-2)	Low (2-0)
Pathogenicity factors				
Low-calcium response (LCR) stimulon (bacterial virulence-associated type III secretion (T3S) system; *Yersinia* outer membrane proteins (Yop) virulon) including:	System permitting extracellular yersiniae to counteract the non-specific immune response on account of an impairment of phagocytic and signalling activity of macrophages and induction of apoptosis of phagocytic cells (delivery of toxic bacterial proteins, Yops, from extracellularly located bacteria into the eukaryotic cell cytosol)	+	–	–
LcrV	Yop translocator; inhibition of chemotaxis of neutrophils; suppression of the synthesis of γ-interferon and tumor necrosis factor α - cytokines necessary for nonspecific activation of professional phagocytes and the formation of productive granulomas, on account of a stimulation of the production of interleukin 10 - a repressor of the cytokines indicated above; inhibition of the lipopolysaccharide (LPS) induced production of IL-1β in macrophages; competition with LPS for toll-like receptor 2 binding	+	–	–
YopD	Yop translocator	ND	ND	ND
YopE	Antiphagocytic; actin depolymerization; inactivation of Rho proteins	+	–	–
YopH	Antiphagocytic; protein tyrosine phosphatase; induction of apoptosis; inhibition of lymphocyte proliferation	+	–	–
YopM	Disruption of the interaction of thrombin with thrombocytes and hindering of their aggregation, essential for the formation of a blood clot	+	–	–

YopP/YopJ	Downregulation of the inflammatory response of macrophages, epithelial and endothelial cells by blocking the mitogen-activated protein kinase and nuclear factor-κB pathways	–	–	+
YopT	Antiphagocytic; inactivation of Rho proteins; the depolymerization of actin stress fibres	ND	ND	ND
YpkA/YopO	Antiphagocytic; inactivation of Rho proteins; autophosphorylating serine/threonine kinase	ND	ND	ND
YscF	Formation of the external needle of the T3S system; participation in virulence protein secretion, in translocation of virulence proteins across eukaryotic membranes and in the cell contact- and calcium-dependent regulation of T3S	ND	ND	ND
Plasminogen activator (Pla)	Spreading factor that promotes generalization of infection, possesses proteolytic properties and determines the fibrinolytic (37°C) and plasmocoagulase (28°C) activities of the plague pathogen; post-translational degradation of Yop proteins; adhesive and invasive activity	+	+	+ [a]
pH 6 antigen (PsaA)	Antiphagocytic; adhesive activity	+	+	–
Capsular antigen fraction I (F1; Caf1)	Antiphagocytic; adhesive activity	+	+	+
Ail	Adhesive activity; resistance to the bactericidal action of normal sera	–	–	–
Yersinia "murine" toxin (Ymt)	Development of toxic shock (lethal effect on mice and rats); reinforcement of endotoxic shock in mammals	–	–	+
LPS	Development of endotoxic shock; resistance to the action of bactericidal cationic peptides and normal sera; adhesive activity	+	–	–

Factors responsible for nutrient requirements and "housekeeping" genes				
Yersiniabactin transport and biosynthesis	Siderophore-dependent iron transport system	+	−	−

[a] More than one "+" indicates the existence of conflicting data reported by different researchers

ND, Not Determined

Methods of plague treatment

Vaccine Development

- Live attenuated strain from Russia (EV strain) originally from Madagascar
- Formalin-fixed virulent strain from USA (195/P) originally from India. Currently manufactured only in Australia
- Porton Down vaccine in Britain

- Protection is delayed for at least one week after immunization-very crucial period???

Methods of plague treatment

- Mysticism and superstition, eg. magic, talisman, blood, dung etc.
- Antibiotic prophylaxis and therapy (MDR?)
- Immunotherapy (LcrV, F1 Capsular, YscF) Protection?
- Phage therapy (specificity, resistance, genetics?)
- Bacteriocin therapy eg. colicins, lantibiotics, halocins, nisin A, mutacin B-Ny266. (Toxicity?)
- Symptomatic treatment. Recovery?
- Inhibitors of virulence factors

Inhibitors of virulence factors

- Plague has restricted tropism for oligosaccharides
- Could prevent adherence to host cells/surfaces eg. terminal di- or trisaccharides of oligosaccharides
- Adherence factors such as pH6 antigen, F1 (Caf1), Pla and S-Layer proteins
- Inhibition of the Type III secretion weaponry
- Inhibition of siderophore/yersiniabactin biosynthesis
- Quorum sensing and the two-component regulatory systems

The use of Receptor mimics

- Plague adheres more efficiently to alveolar epithelial cells
- Compounds such as tunicamycin reduced the attachment to human epithelial cell lines by 55-65%
- Compounds such as salicyladehydes can also inhibit the type 3 secretion system.
- Compounds such as aryl sulfamoyl adenosine derivatives inhibits yersiniabactin biosynthesis

Inhibitors of virulence factors

- Unlikely to affect host cells
- Unlikely to be cross-resistant to existing therapies
- Does not induce resistance themselves
- Inhibit the establishment of infection or alter its course rather than viability
- Potential to attenuate pathogen to the advantage of the host cell

Summary/Conclusion

- Plague causes high mortality
- Possible use as a bioterrorism agent
- Recent increase in MDR is a concern
- Novel treatment methods such as inhibitors of virulence factors should be explored
- Receptor mimics such as short chain oligosaccharides should be explored
- Single or combination of multiple treatment methods should be considered
- Further research needed for the receptor mimics

Canada and FSU Collaboration?

Canada and FSU Collaboration?

FSU scientists are well trained in good techniques to minimize aerosols (controlling hazard at source) and we can learn from their practices and procedures.

Canada and Russian/CIS Collaboration

- **Unique expertise in working safely with dangerous endemic diseases (e.g. anthrax, brucella, plague, CCHF, FMD, Sheep pox)**

- **No lab-acquired infections despite working with these pathogens on open-bench (no biosafety cabinets, no secondary containment systems).**

New ISTC Project in Kyrgyz Republic

- Title: Investigation of plague epidemiology in Kyrgyzstan
- Duration: Three years
- Partners: Karakol Department of Quarantine and Dangerous Infections,
  Issyk-Kul State University

New ISTC Project in Kyrgyz Republic

- Title: Prevention of Spread of Dangerous Infectious Diseases in Kyrgyzstan
- Duration: Three years
- Partners: National Academy of Sciences of the Kyrgyz Republic,

  Anti-plague station of the Kyrgyz Republic,

  Sanitary and Epidemiological control center

Benefits to Canada

- Prevent the spread of bioweapons technology
- Provides a more secured world/Canada
- Helps in sharing the expertise of FSU scientists
- Helps Canadian scientists in their preparedness for any potential BT event
- Possibility of exchange of information/ strains/genetic materials????
- Whatever the risk is today, it will be greater tomorrow.
- We do not have to be vulnerable. Much can be done to reduce dangers of bioterrorism by focusing on its global dimensions.

Is The Threat of Bioterrorism Real?

- "We need to replace complacency with a new sense of urgency"

Donna Shalala

Secretary of Health and Human Services (1999)

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- Hosted by the SRCAMB and ISTC
- Others