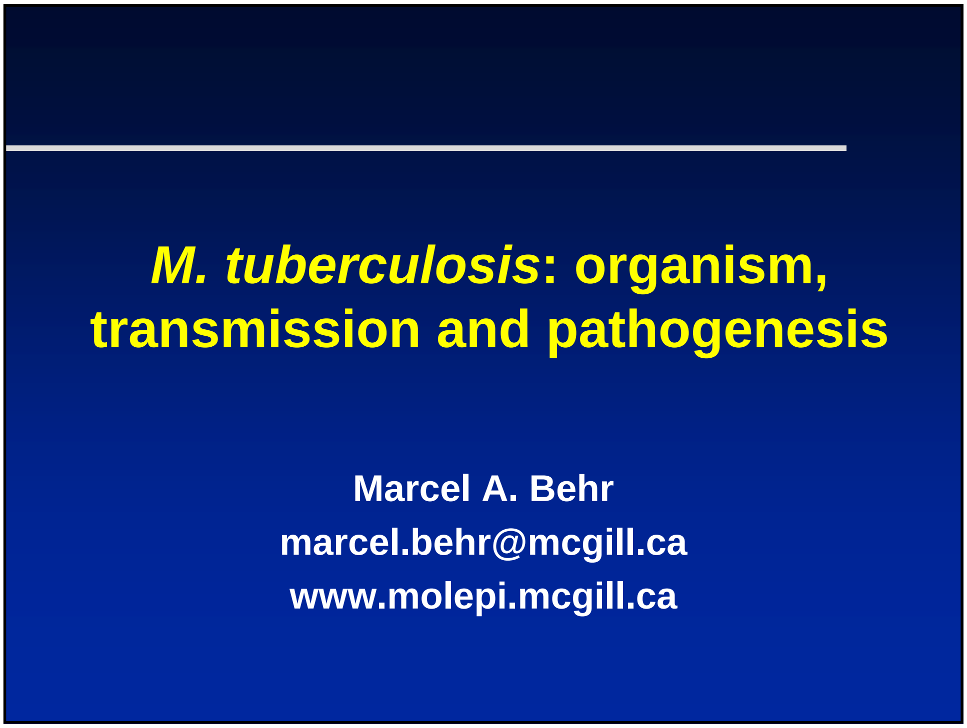

M. tuberculosis: organism, transmission and pathogenesis

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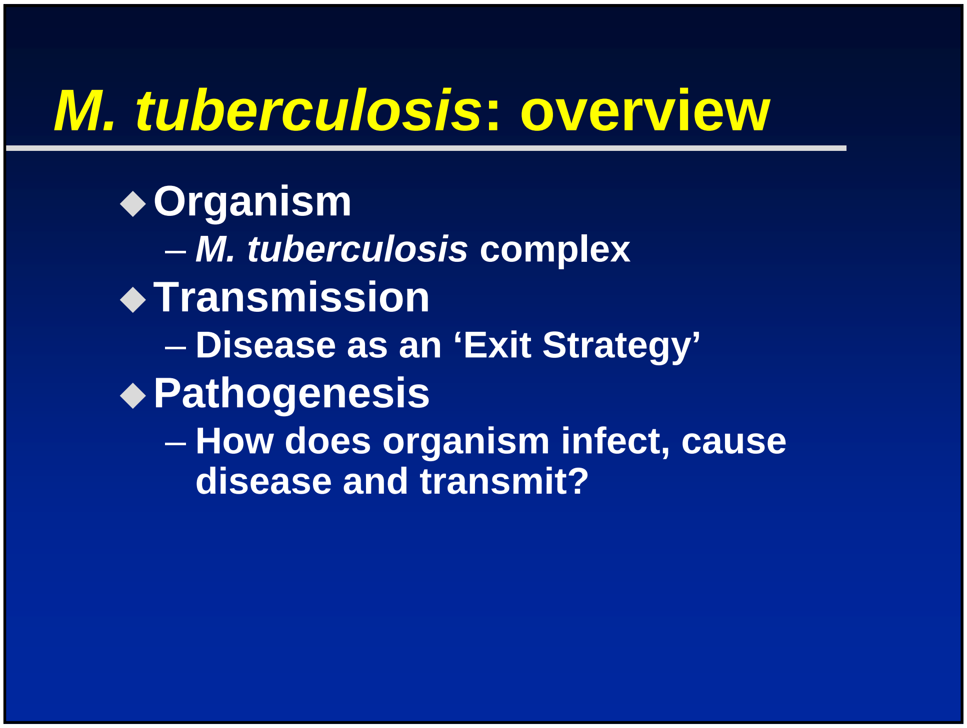
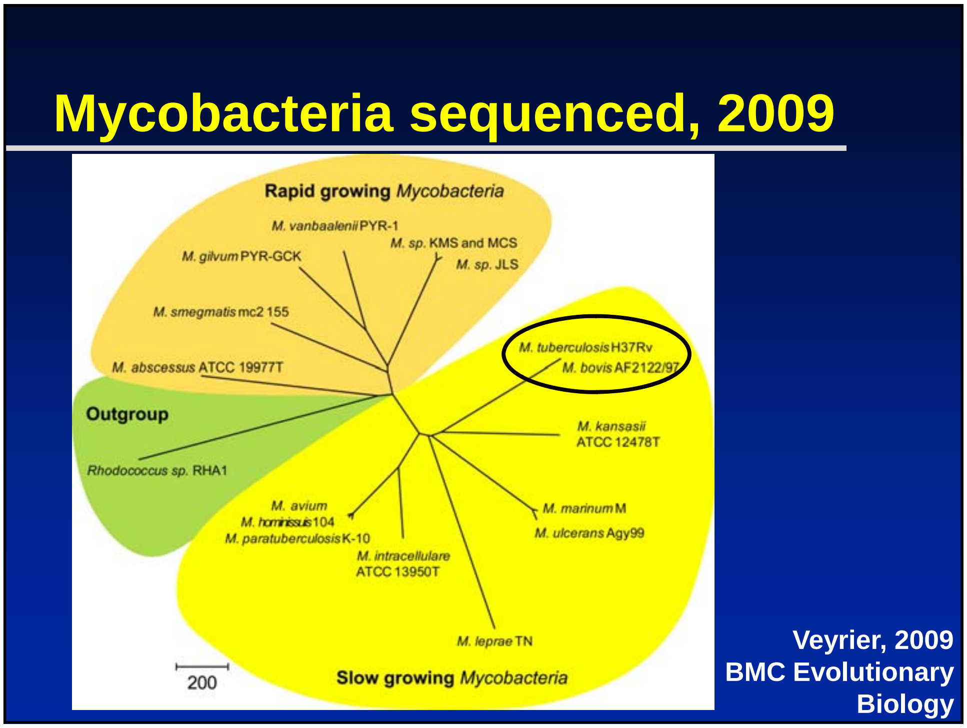


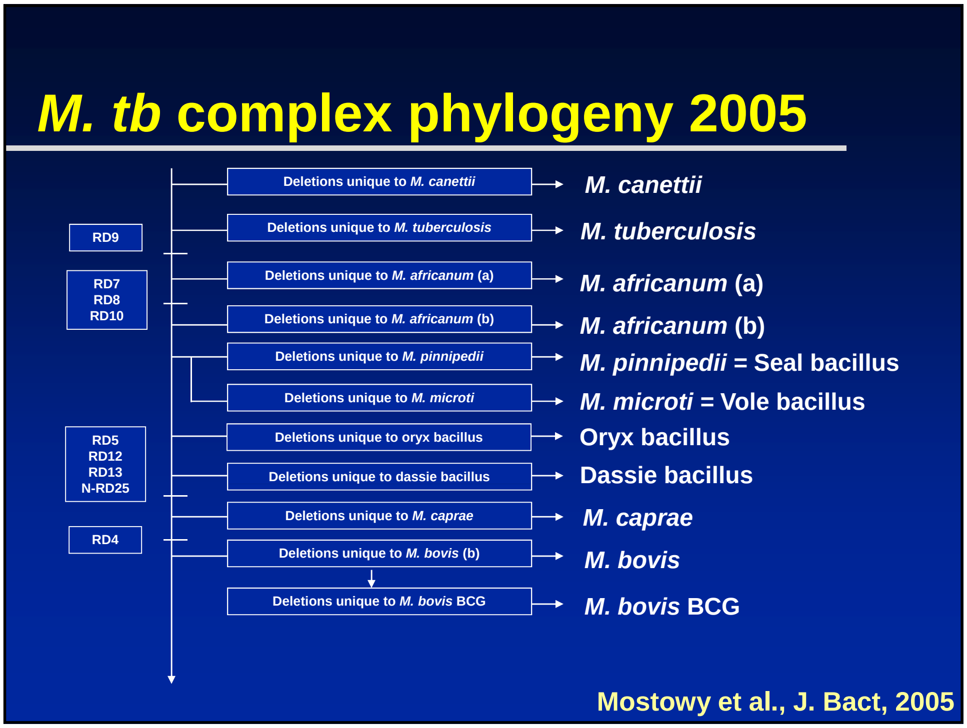
M. tuberculosis: overview

- Organism
  - *M. tuberculosis* complex
- Transmission
  - Disease as an ‘Exit Strategy’
- Pathogenesis
  - How does organism infect, cause disease and transmit?



Mycobacteria sequenced, 2009

Veyrier, 2009
BMC Evolutionary
Biology



M. tb complex phylogeny 2005

RD9

RD7
RD8
RD10

RD5
RD12
RD13
N-RD25

RD4

Deletions unique to *M. canettii* → *M. canettii*

Deletions unique to *M. tuberculosis* → *M. tuberculosis*

Deletions unique to *M. africanum* (a) → *M. africanum* (a)

Deletions unique to *M. africanum* (b) → *M. africanum* (b)

Deletions unique to *M. pinnipedii* → *M. pinnipedii* = Seal bacillus

Deletions unique to *M. microti* → *M. microti* = Vole bacillus

Deletions unique to oryx bacillus → Oryx bacillus

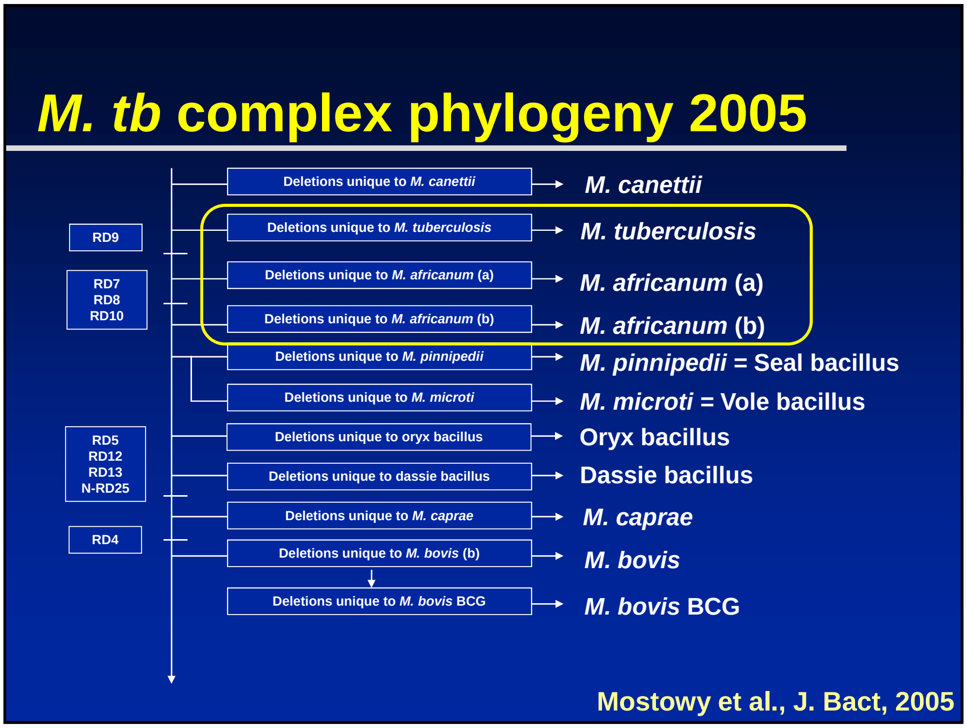
Deletions unique to dassie bacillus → Dassie bacillus

Deletions unique to *M. caprae* → *M. caprae*

Deletions unique to *M. bovis* (b) → *M. bovis*

Deletions unique to *M. bovis* BCG → *M. bovis* BCG

Mostowy et al., J. Bact, 2005



M. tb complex phylogeny 2005

RD9

RD7
RD8
RD10

RD5
RD12
RD13
N-RD25

RD4

- **Deletions unique to *M. canettii*** → ***M. canettii***
- **Deletions unique to *M. tuberculosis*** → ***M. tuberculosis***
- **Deletions unique to *M. africanum* (a)** → ***M. africanum* (a)**
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- **Deletions unique to dassie bacillus** → **Dassie bacillus**
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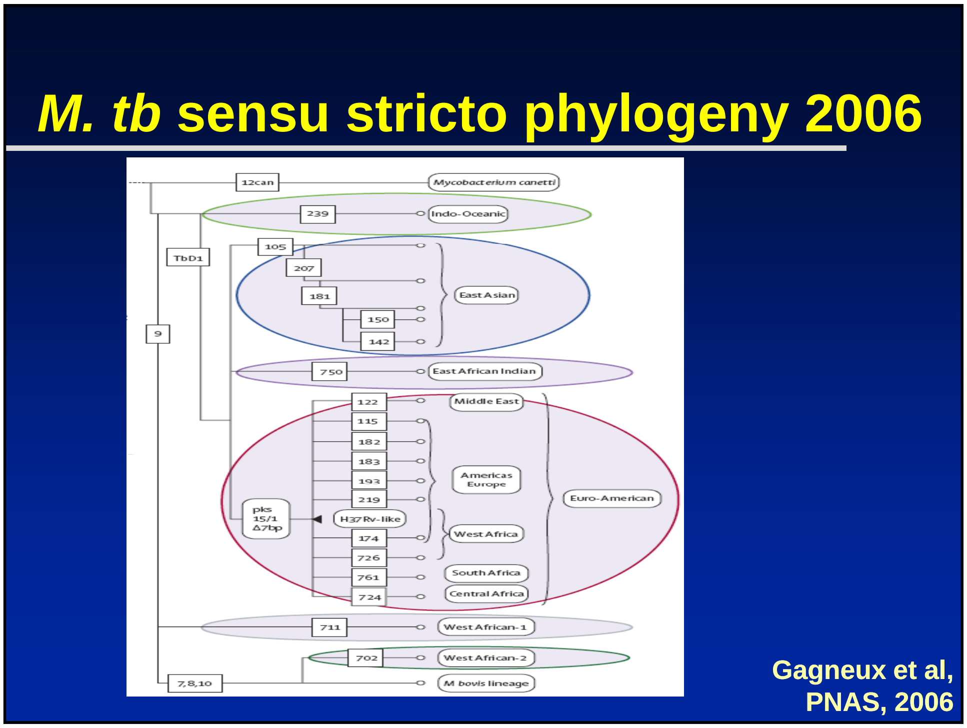
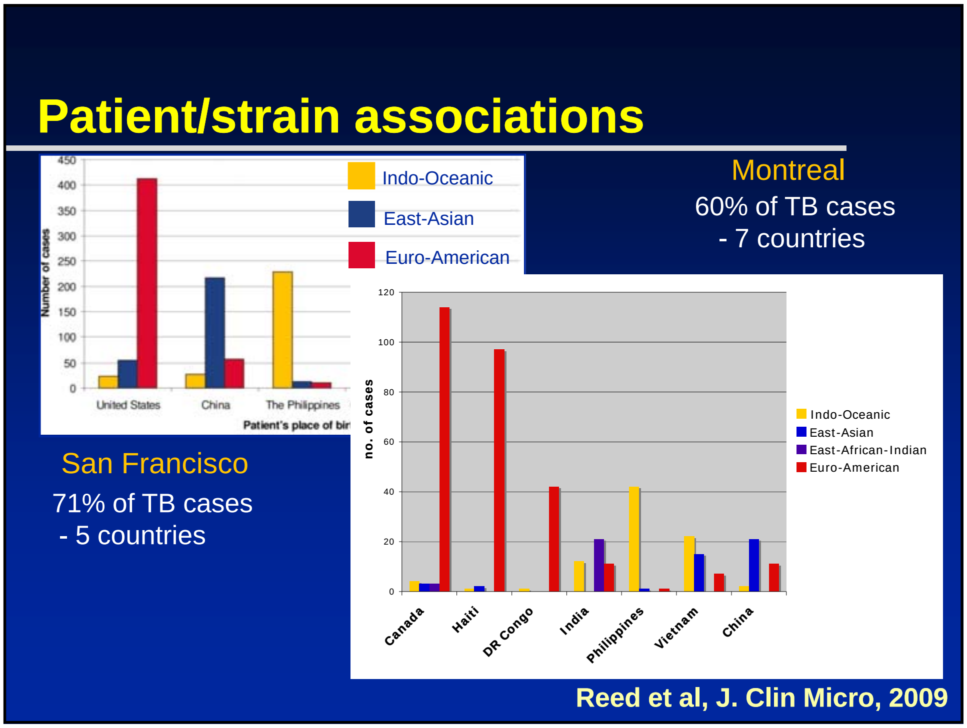
- **Deletions unique to *M. bovis* (b)** → ***M. bovis***
- **Deletions unique to *M. bovis* BCG** → ***M. bovis* BCG**

Mostowy et al., J. Bact, 2005



M. tb sensu stricto phylogeny 2006

Gagneux et al, PNAS, 2006



Patient/strain associations

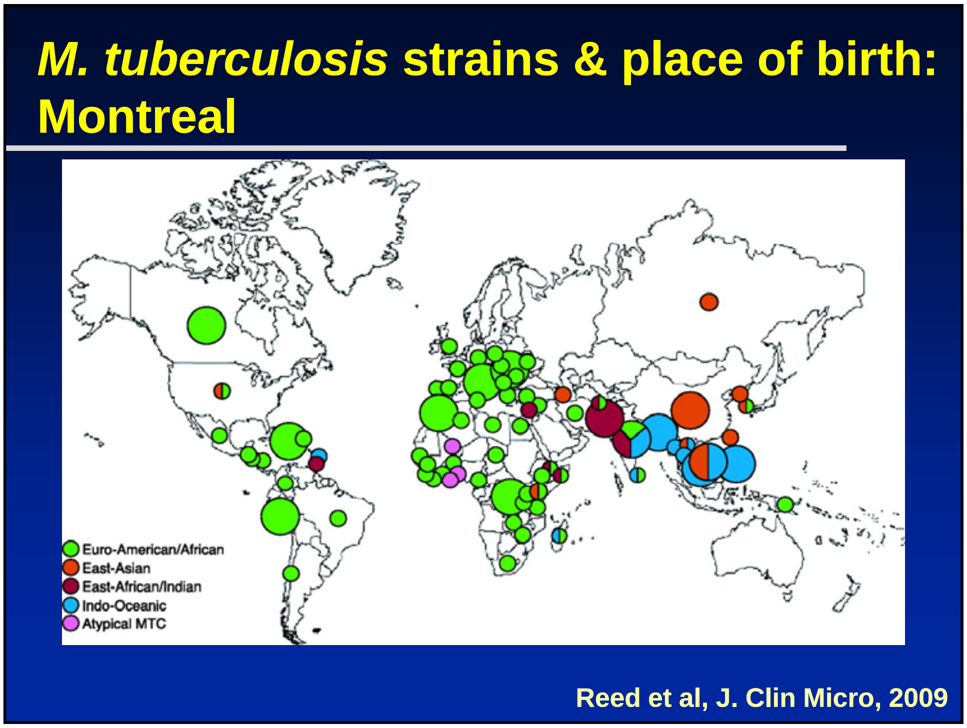
Montreal

60% of TB cases
- 7 countries

San Francisco

71% of TB cases
- 5 countries

Reed et al, J. Clin Micro, 2009



M. tuberculosis strains & place of birth: Montreal

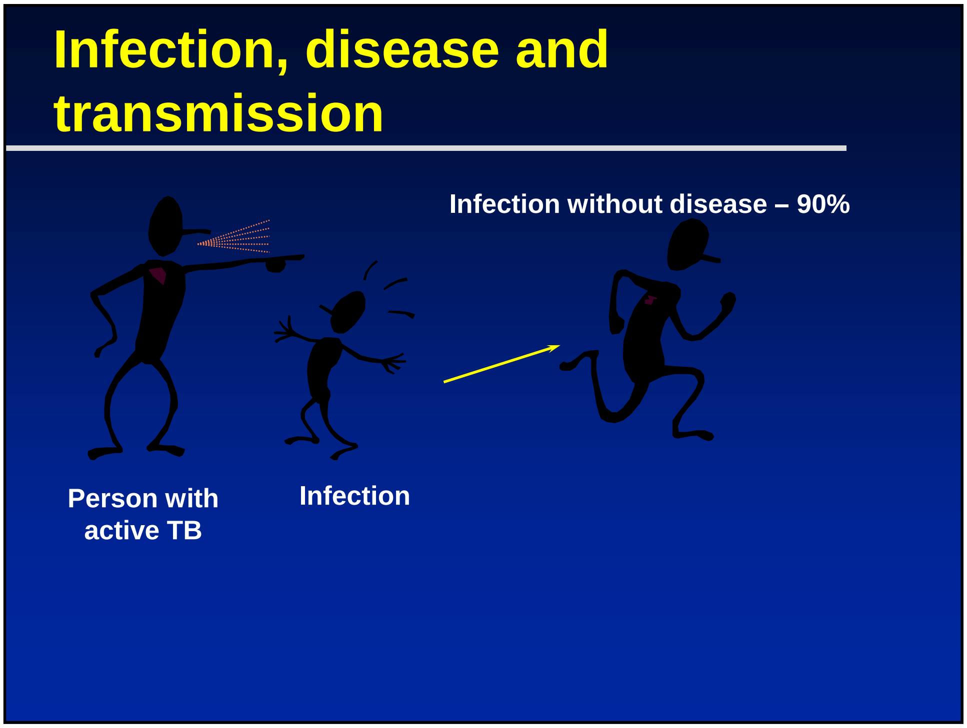
Reed et al, J. Clin Micro, 2009

Organism: Current view

- Most human TB due to *M. tuberculosis*
  - *M. bovis* rare
  - *M. africanum* geographically restricted
- Geography-associated lineages:
  - Reproducible in different settings
  - Consistent with two waves of spread:
    - » Walked out of Africa
    - » Then sailed out of Europe

Organism: Pending questions

- Are strains of *M. tuberculosis* equivalent?
  - Disease that they cause?
  - Response to treatment?
  - Ability to spread?
  - Capacity of vaccines to prevent?
- If yes, what explains clinical and epidemiologic heterogeneity?
- If no, do we need to tailor interventions, stop the ‘one size fits all’ approach?

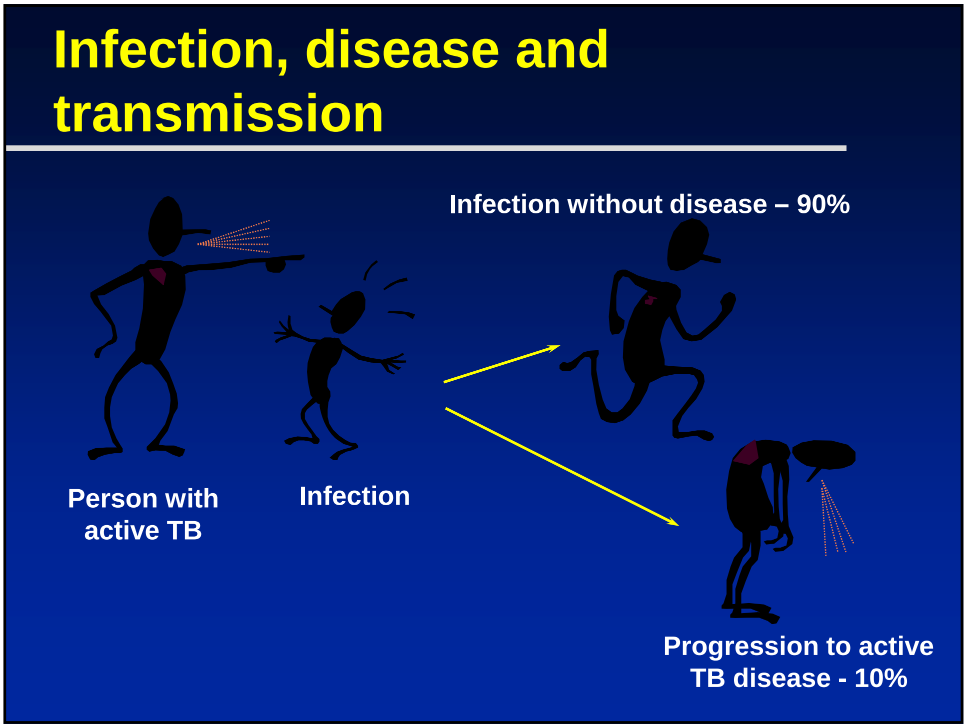


Infection, disease and transmission

Infection without disease – 90%

Person with
active TB

Infection



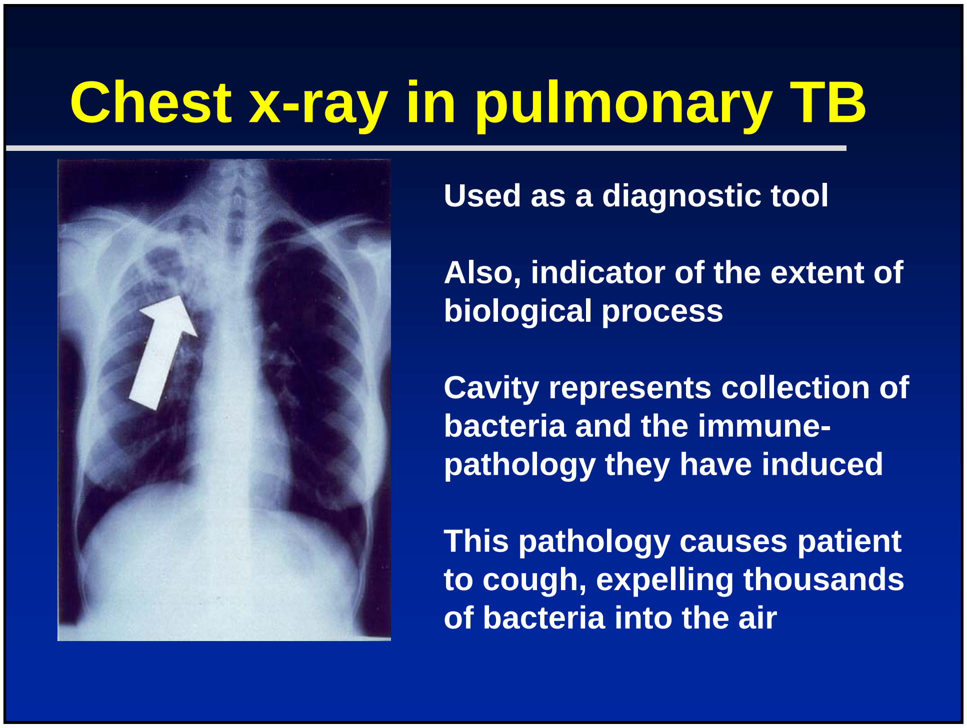
Infection, disease and transmission

Infection without disease – 90%

Person with
active TB

Infection

Progression to active
TB disease - 10%



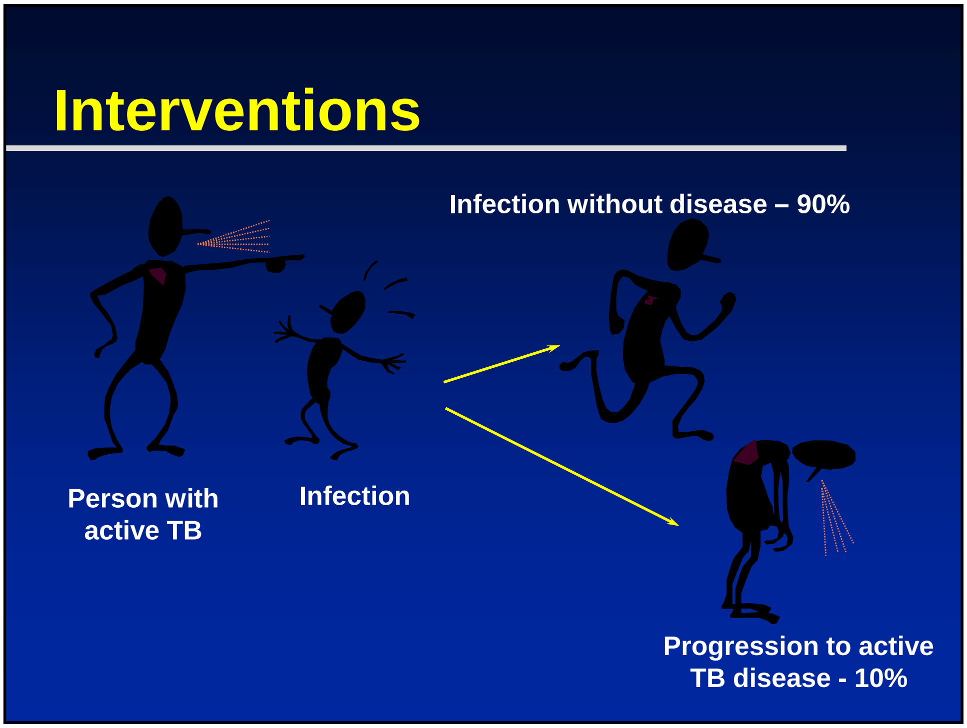
Chest x-ray in pulmonary TB

Used as a diagnostic tool

Also, indicator of the extent of biological process

Cavity represents collection of bacteria and the immune-pathology they have induced

This pathology causes patient to cough, expelling thousands of bacteria into the air



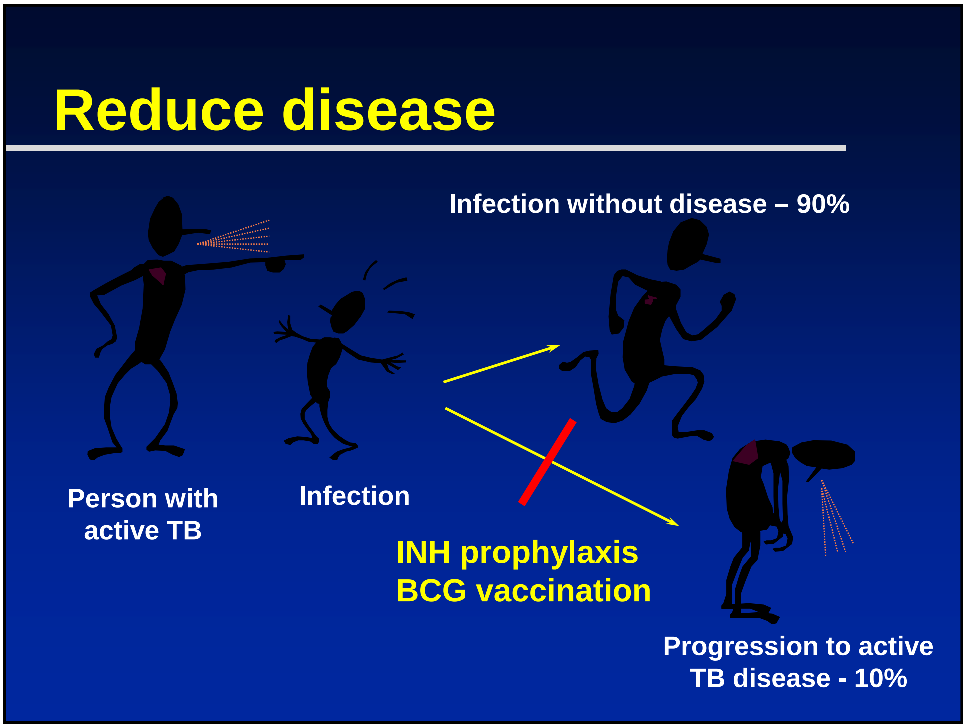
Interventions

Person with active TB

Infection

Infection without disease – 90%

Progression to active TB disease - 10%



Reduce disease

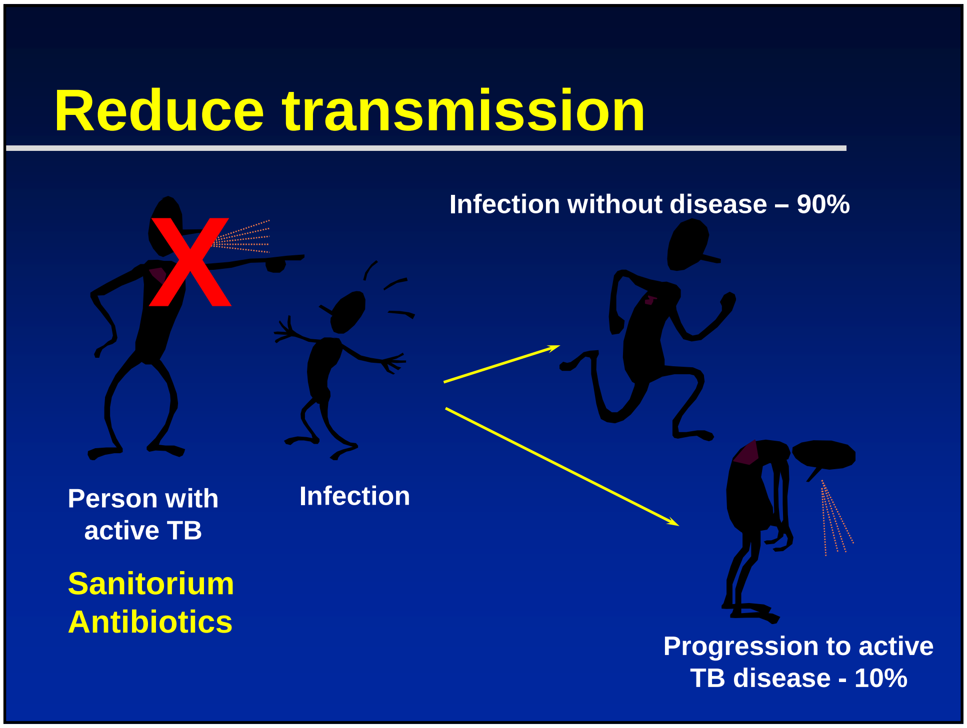
Infection without disease – 90%

Person with
active TB

Infection

INH prophylaxis
BCG vaccination

Progression to active
TB disease - 10%



Reduce transmission

Infection without disease – 90%

Person with
active TB

Infection

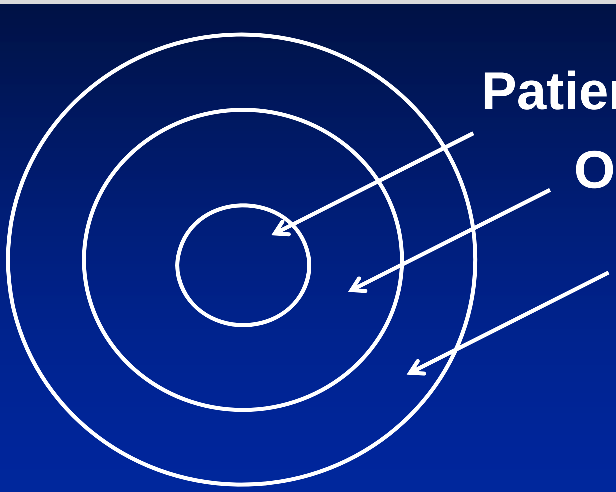
Sanitorium
Antibiotics

Progression to active
TB disease - 10%

Measuring transmission

- Pre-1992, transmission inferred
  - E.g. household contacts have higher rate of TB than others
- Post-1992, direct documentation by DNA-fingerprinting methods
- Goal:
  - Use bacterial genome as a tool against the pathogen, through strain comparisons

Molecular epidemiology



Patient

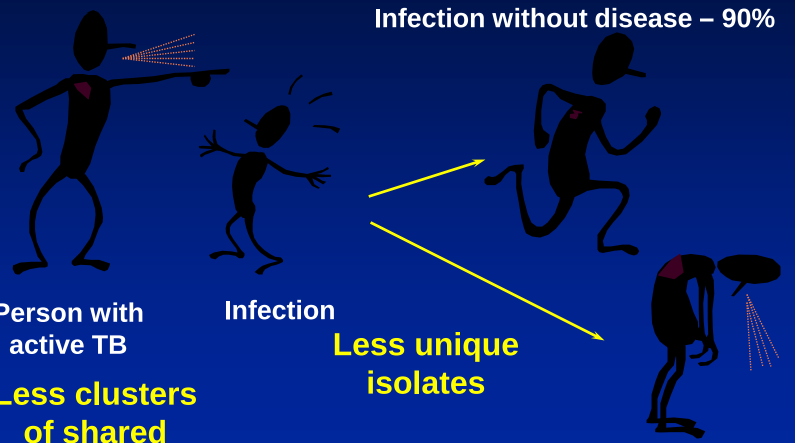
Outbreak

Population

Molecular epidemiology questions

- Patient: TB recurrence
  - If new strain, can infer re-infection
- Outbreak: Extent
  - Are all people in a suspect outbreak infected by the same strain?
- Population: Ongoing spread
  - What proportion of TB cases in defined jurisdiction represent transmission?

Interventions and molecular epidemiology



Infection without disease – 90%

Person with active TB

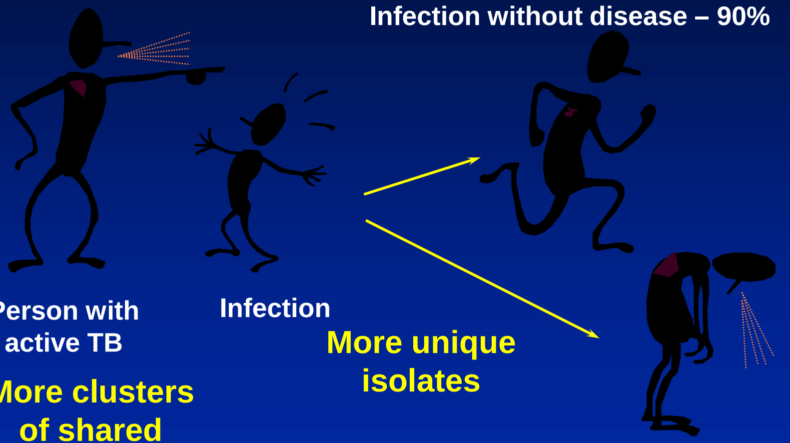
Infection

Less unique isolates

Less clusters of shared isolates

Progression to active TB disease - 10%

HIV/AIDS and molecular epidemiology



Infection without disease – 90%

Person with
active TB

Infection

More unique
isolates

More clusters
of shared
isolates

Progression to active
TB disease - 10%

Transmission: Current view

- We can now document transmission with great accuracy
- Degree of TB due to reactivation vs. transmission varies considerably between settings
  - Between countries, e.g. Montreal vs. Capetown
  - Within countries, e.g. Montreal vs. Nunavik

Transmission: pending questions

- Do all strains transmit equally?
- Do all hosts transmit equally?
- Can we immunize to prevent progression to disease, and hence contagion?
- If we cannot modify hosts or their strains, how best can we modify the environment?

Clinical States to Pathogenesis

- Tuberculous infection = latent infection = LTBI
  - Clinically latent, either because bacteria latent or bacterial replication = death
  - Tuberculin / IGRA positive
- Tuberculosis = TB
  - Clinical disease
  - Culture positive = contagious

GET IN
STAY IN

GET OUT
SPREAD

Clinical States to Pathogenesis

- Tuberculous infection = latent infection = LTBI
  - Clinically latent, either because bacteria latent or bacterial replication = death
  - Tuberculin / IGRA positive
- Tuberculosis = TB
  - Clinical disease
  - Culture positive = contagious

Bacterial survival

?

Bacterial pathogenesis

Bacterial factors:

- What are bacterial factors that permit infection and persistence?
- What are bacterial factors that provoke disease, to continue transmission cycle?
- How do you measure their effect?

Defining virulence

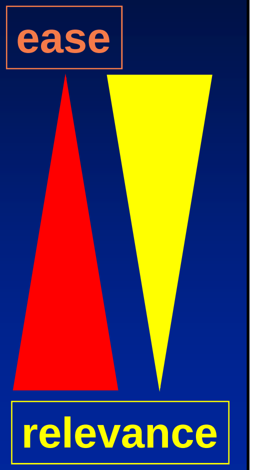
- *M. tuberculosis* more virulent for humans than cows
- *M. bovis* more virulent for cows than humans
- Which one is ‘more virulent’?
  - *M. bovis* more virulent in mouse, rabbit
  - Does this matter?

Human vs. animal studies

- Human data most relevant
  - Not amenable to experimentation
    - No human TB challenge
    - Human BCG challenge OK
  - Therefore, limited to observation
    - Confounded by known & unknown host and environmental variables
- Human data to generate hypotheses
- Animal models to test hypotheses

Models to study TB

- Monkeys closest disease to humans
  - expensive, also ethics issues
- Rabbits close in pathology
  - get cavitary lung lesions
- Mice easier, immunology reagents
  - help with primary infection
- Cell culture easiest
  - Only one cell, therefore immunologically simplified



Bacterial factors: e.g. 1 - disease

- *M. tuberculosis* causes disease in ~ 1 / 10 infected
- BCG vaccine causes disease in ~ 1 / 100,000 immunized
- Why?

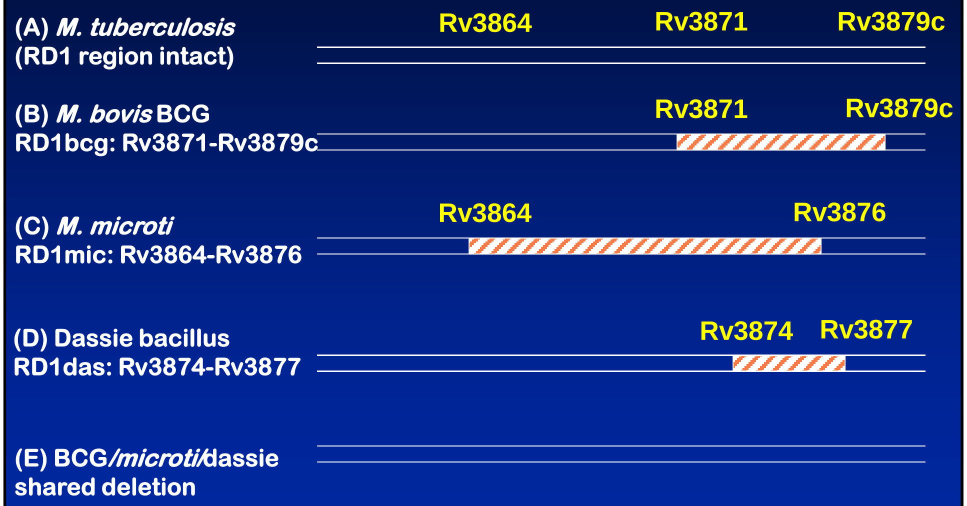
Microarray reveals polygenic regions missing from BCG strains

Regions in TB, not in BCG	# deletions	# ORFs
Variable among *M. tuberculosis* isolates	4	43
Present in *M. tuberculosis*, missing from *M. bovis*	7	48
Present in *M. bovis* isolates, missing from all BCG strains	1	9
Variably deleted among BCG strains	4	29
Total:	16	129

RD1

Behr et al., 1999

Region of Difference 1 (RD1): Three attenuated strains



(A) ***M. tuberculosis***
(RD1 region intact)

(B) *M. bovis* BCG
RD1bcg: Rv3871-Rv3879c

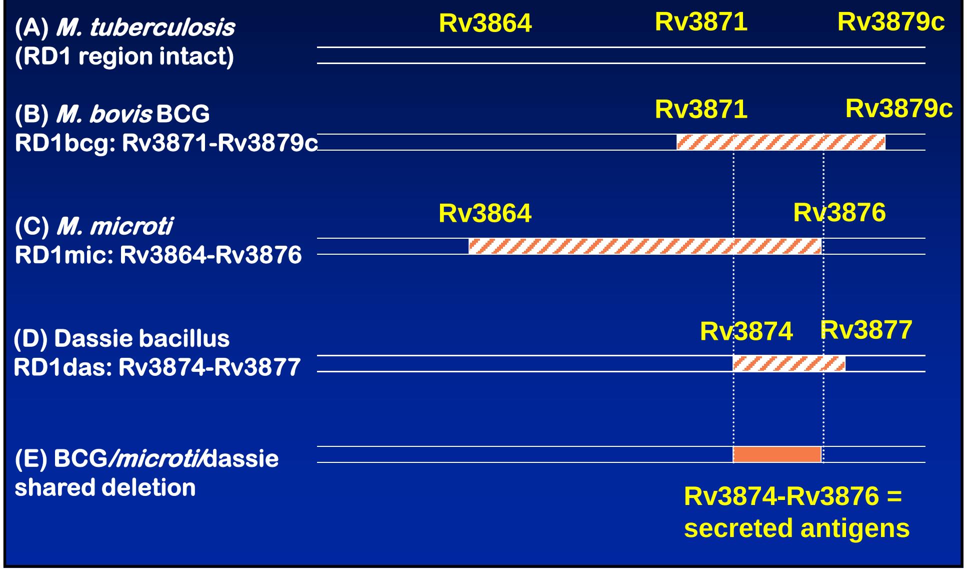
(C) *M. microti*
RD1mic: Rv3864-Rv3876

(D) Dassie bacillus
RD1das: Rv3874-Rv3877

(E) BCG/*microti*/dassie
shared deletion

Mostowy et al., 2004

RD1 region in attenuated strains



(A) ***M. tuberculosis***
(RD1 region intact)

Rv3864 Rv3871 Rv3879c

(B) *M. bovis* BCG
RD1bcg: Rv3871-Rv3879c

Rv3871 Rv3879c

(C) *M. microti*
RD1mic: Rv3864-Rv3876

Rv3864 Rv3876

(D) Dassie bacillus
RD1das: Rv3874-Rv3877

Rv3874 Rv3877

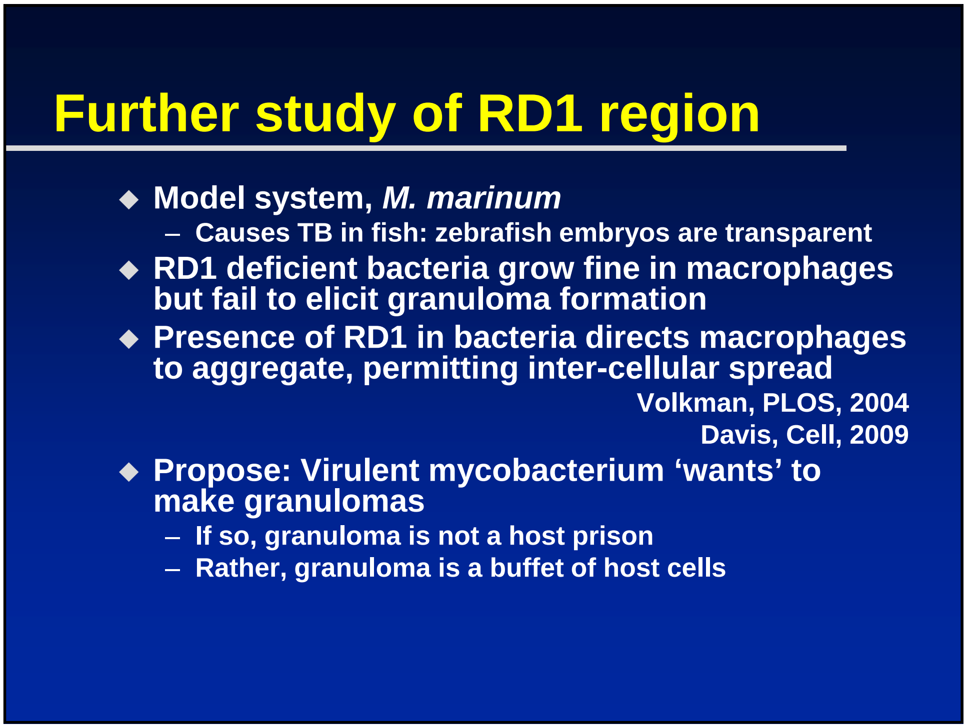
(E) BCG/*microti*/dassie
shared deletion

Rv3874-Rv3876 =
secreted antigens

RD1 deletion analysis: results

- **Deletion of RD1 from *M. tuberculosis* results in**
  - **Decreased growth in macrophages**
  - **Decreased destruction of macrophages**
  - **Decreased growth in lungs**
  - **Decreased spread from lungs to spleen**
  - **Decreased pathology in host**
- **RD1 region lost during derivation of BCG in part responsible for attenuation**

Lewis, JID, 2003

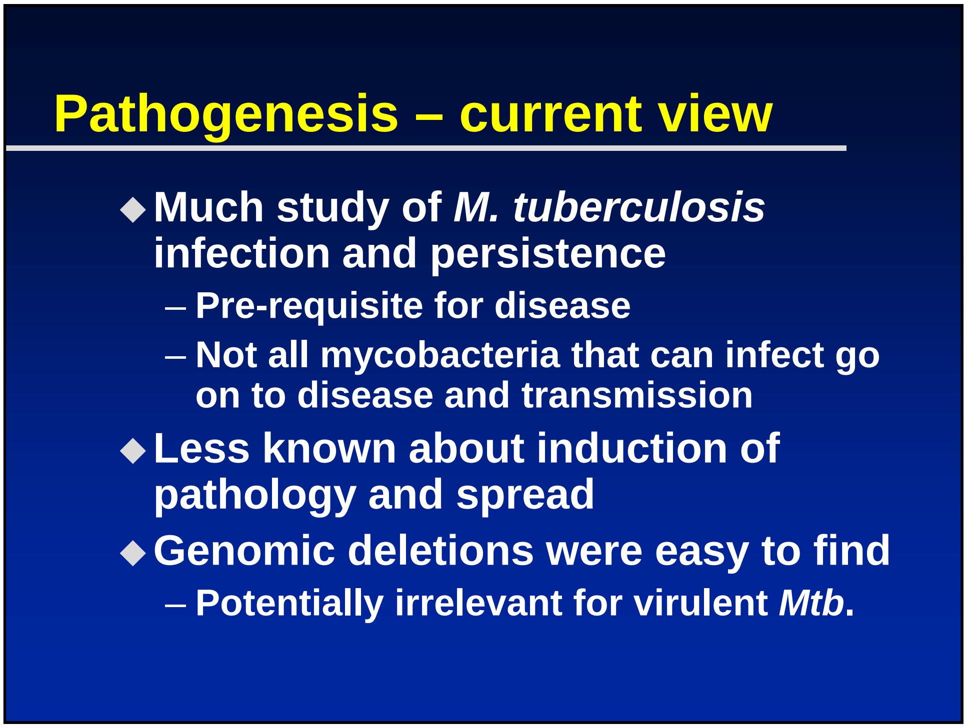


Further study of RD1 region

- Model system, *M. marinum*
  - Causes TB in fish: zebrafish embryos are transparent
- RD1 deficient bacteria grow fine in macrophages but fail to elicit granuloma formation
- Presence of RD1 in bacteria directs macrophages to aggregate, permitting inter-cellular spread

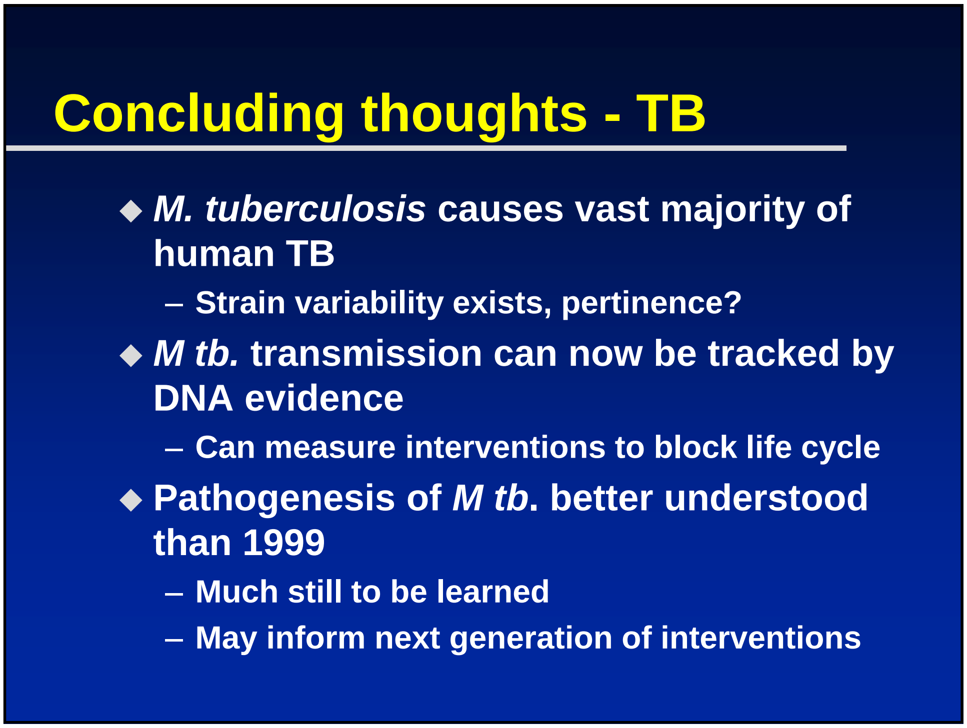
Volkman, PLOS, 2004
Davis, Cell, 2009

- Propose: Virulent mycobacterium ‘wants’ to make granulomas
  - If so, granuloma is not a host prison
  - Rather, granuloma is a buffet of host cells



Pathogenesis – current view

- Much study of *M. tuberculosis* infection and persistence
  - Pre-requisite for disease
  - Not all mycobacteria that can infect go on to disease and transmission
- Less known about induction of pathology and spread
- Genomic deletions were easy to find
  - Potentially irrelevant for virulent *Mtb*.



Concluding thoughts - TB

- *M. tuberculosis* causes vast majority of human TB
  - Strain variability exists, pertinence?
- *M tb.* transmission can now be tracked by DNA evidence
  - Can measure interventions to block life cycle
- Pathogenesis of *M tb*. better understood than 1999
  - Much still to be learned
  - May inform next generation of interventions

Questions?

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