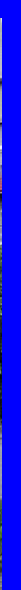


# **Tropikal Hastalıklar: Kusbakisi**

# Tropical Infections: A Bird's Eye View

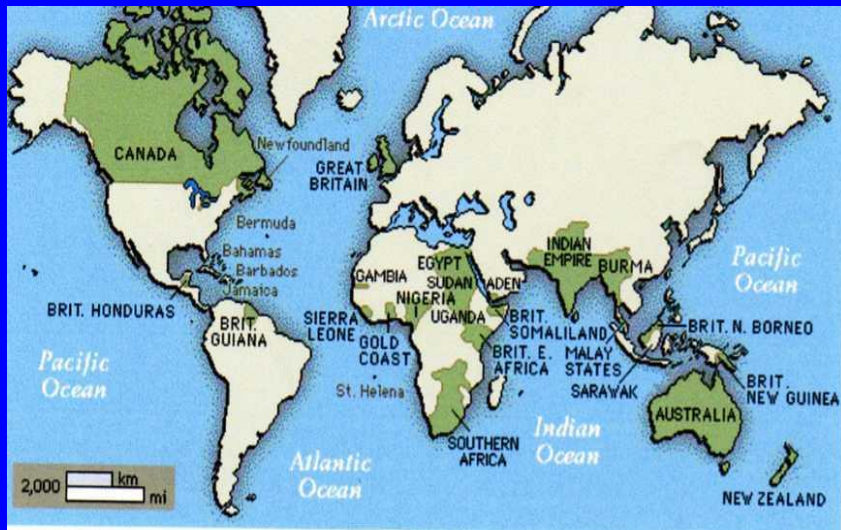
Dr. George M. Varghese MD, DNB, DTMH, FIDSA  
Department of Infectious Diseases  
Christian Medical College, Vellore, India



# Outline

- Origin of Tropical Medicine & current relevance
- Exotic tropical infections: a starter
- Recent achievements and challenges in TB
- Solving the mystery fever

# British Empire: 1900



- **British Empire - 531 million**
- Qing Empire (China) - 432 million
- Russian Empire - 176 million
- Mughal Empire (India) - 175 million
- Ming Empire (China) – 160 million
- **Ottoman Empire (Turkey) – 35 milli**

- Germ Theory & elimination of Climatic model
- Political and economic approach to empire
- Challenges of colonialism – ‘White man’s grave’
- Scientific Medicine - Tropical Medicine

# Sir Patrick Manson: Father of Tropical Medicine

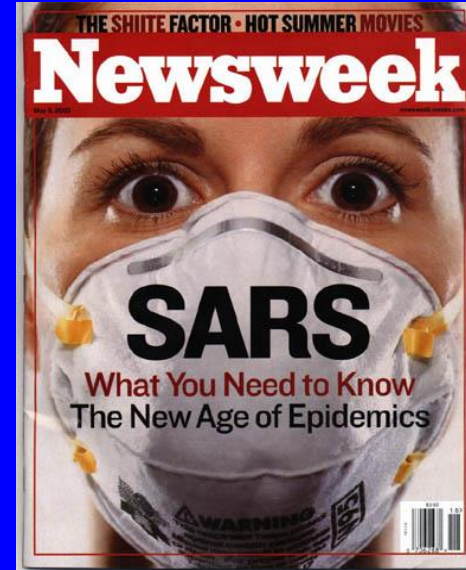


- ‘The role of the Society is to do justice to the tropical patients and to tropical diseases and hygiene’

- Sir Patrick Manson.

Presidential address to Society of Tropical Medicine 1907

# Challenges of Globalisation

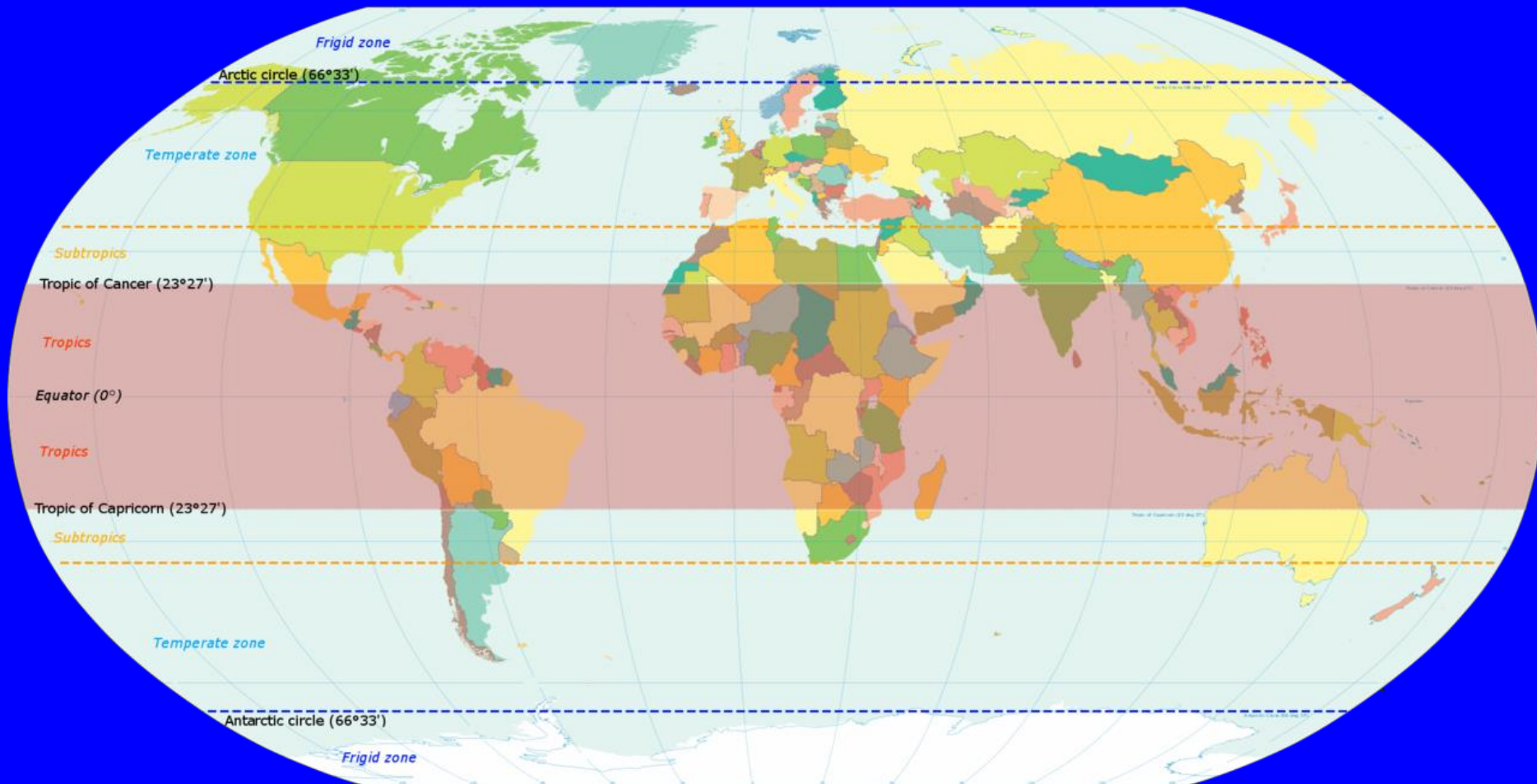


# HIV Origin



- Unusual opportunistic infections noted in 1981
- Mutated virus from Central African monkey retrovirus
- Retrospective pathology search identified oldest known AIDS patient 1957
- SIV presumably transferred to humans during the late 19th or early 20th century
- Global travel

# Tropical & Subtropical Regions



# Common Tropical Infections

- Tuberculosis
- Malaria
- Dengue infection
- Rickettsioses:
  - Scrub typhus
  - Murine typhus
- Enteric fever
  - Typhoid fever
  - Paratyphoid fever
- Leptospirosis
- Melioidosis
- Anthrax
- Helminthic infections
- Leishmaniasis
- Infective diarrhea
- Tick borne viral diseases

# An Interesting Case

- 49 year old man, a farmer
- No known co-morbid illnesses
- 3 days history of
  - Fever
  - Headache – severe
  - Dry cough
- Day of admission: 2 episodes of generalized seizures and altered sensorium

# Physical examination

- Unconscious; GCS 8/15
- Tem-101<sup>0</sup>F, HR-110/min ; BP-120/70mmHg ; RR:30/min
- No pallor / clubbing / cyanosis
- CVS:Normal
- RS: Clear
- P/A: Soft; no hepato-splenomegaly
- CNS:
  - GCS 8/15
  - Signs of meningeal irritation +
  - Pupils- equal and reacting to light
  - No focal deficits noted
  - Bilateral extensor plantars

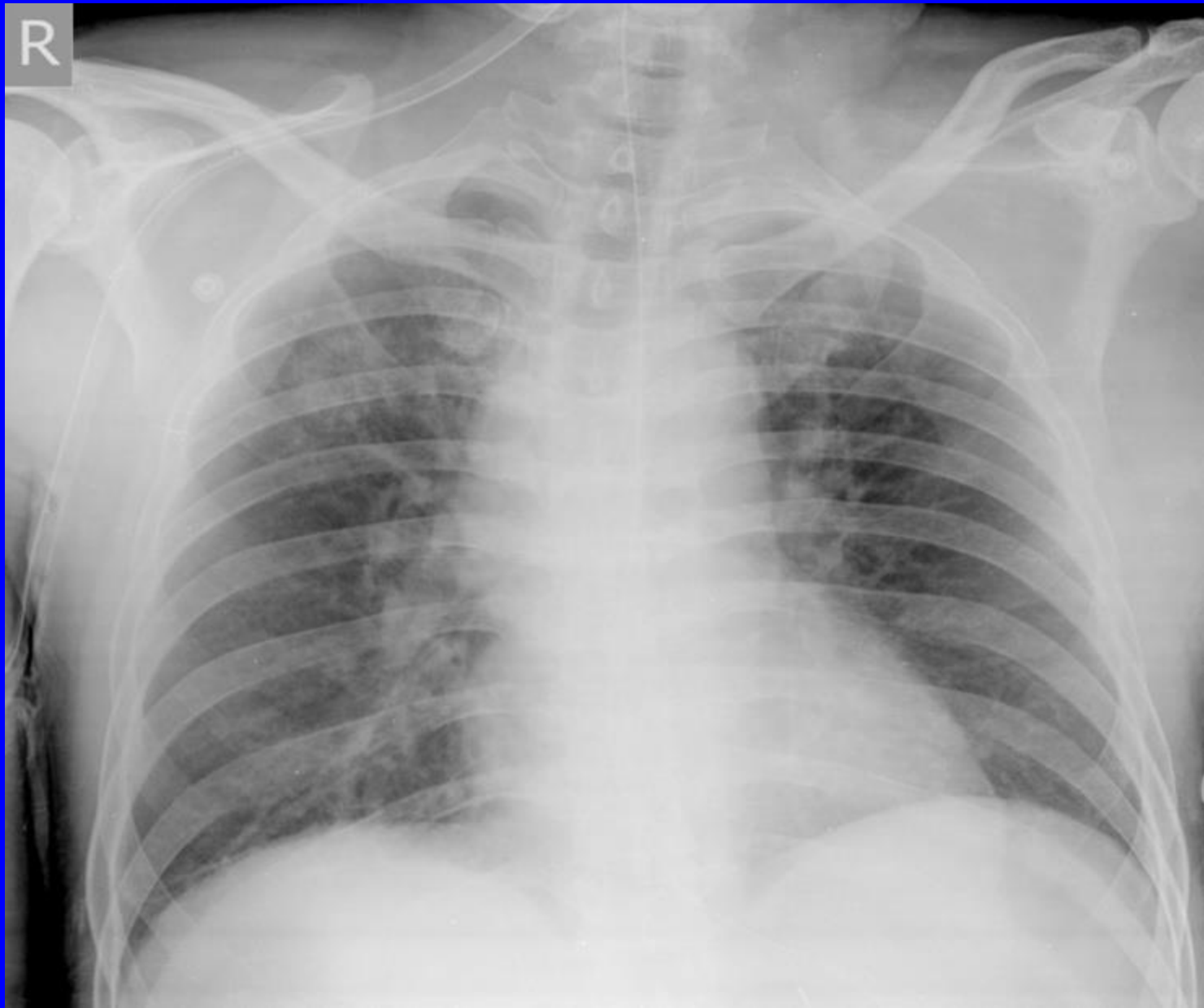
# Investigations

WBC	9500 /cumm (N 84, L7 M9)
Hb	14.7 gm/dl
<b>PLT</b>	<b>90,000/cumm</b>
Na	135 mmol/l
K	3.5 mmol/l
Cr	1.0 mg/dl

T. bilirub	0.7 mg/dl
D. bili	0.5 mg/dl
T. prot	6.9 g%
Alb	3.6 g%
SGOT	70U/L
SGPT	25 U/L
Alk. phos	93U/L

HIV ELISA - Negative

# Chest X-ray



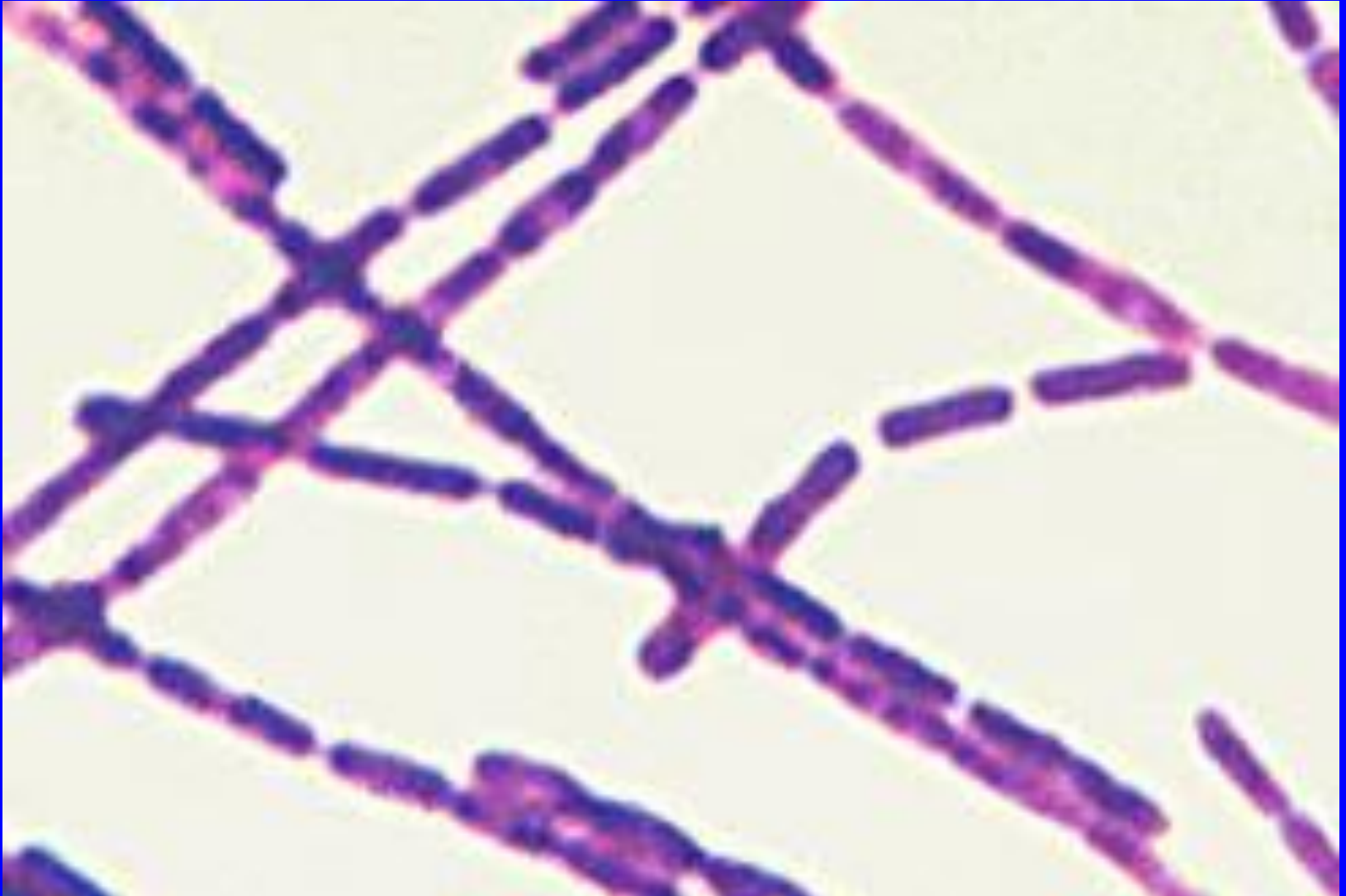
# Investigations Contd.

CSF analysis:

- WBC - 5000 cells (P80,L20); RBCs-4800 cumm  
Protein- 636 mg/dl;  
Sugar – 89 mg/dl  
RBS: 120 mg/dl



# CSF & Blood Culture: *Bacillus anthracis*



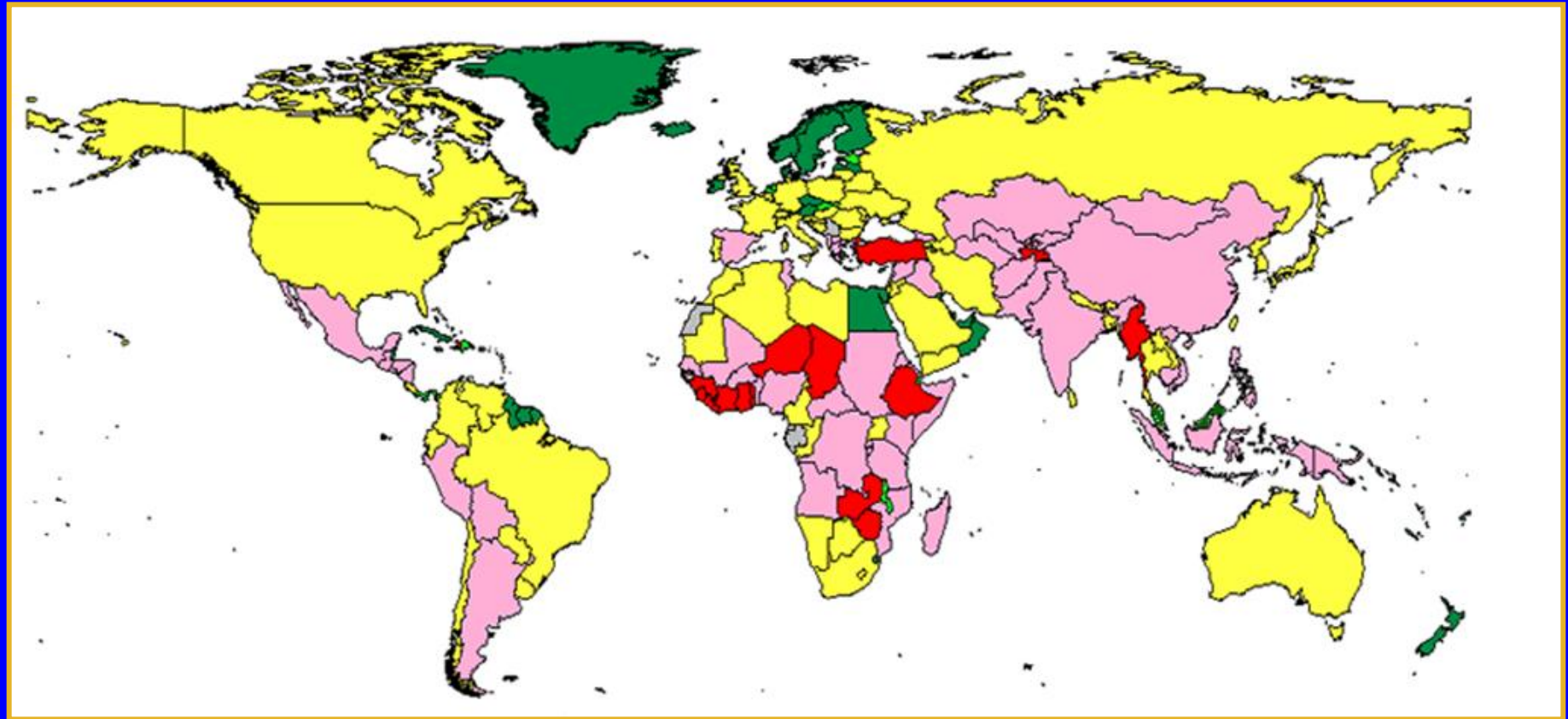
# Human Transmission

- Cutaneous
  - Contact with infected tissues, wool, hide, soil
- Inhalational
  - Tanning hides, processing wool or handling carcasses
- Gastrointestinal
  - Undercooked meat

# The Organism

- *Bacillus anthracis*
- Large, gram-positive, non-motile bacilli
- Two forms
  - Vegetative
  - Spore
- Over 1,200 strains
- Nearly worldwide distribution

# Anthrax Epidemiology

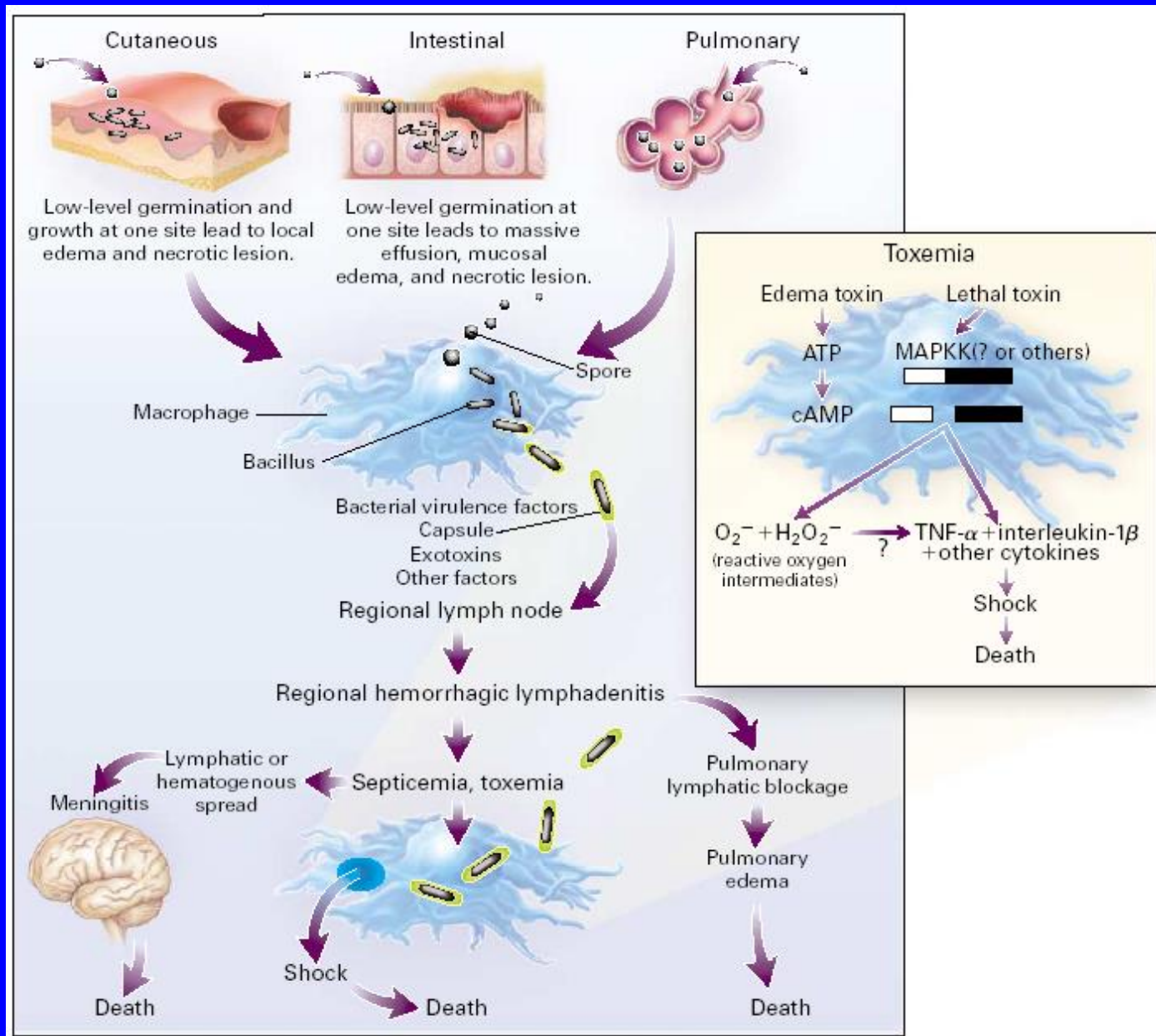


[http://www.vetmed.lsu.edu/whocc/mp\\_world.htm](http://www.vetmed.lsu.edu/whocc/mp_world.htm)

# Inhalational Anthrax

- Incubation: 1 to 7 days
- Initial phase
  - Nonspecific (mild fever, malaise)
- Second phase
  - Severe respiratory distress
  - Dyspnoea, stridor, cyanosis, mediastinal widening, death in 24 to 36 hours
- Case fatality: 75 to 90%

# How does this kill the individual?

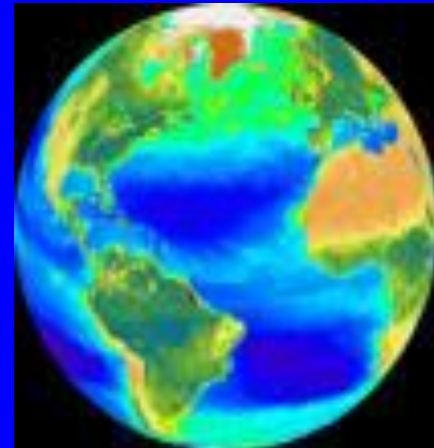


# Treatment for Systemic Anthrax with Meningitis

Preferred	Alternatives
1. A Bactericidal Agent (Fluoroquinolone)	
Ciprofloxacin 400 mg every 8H	Levofloxacin, Moxifloxacin
2. A Bactericidal Agent ( $\beta$ -lactam)	
Meropenem 2 g every 8H	Imipenem Penicillin
3. A Protein Synthesis Inhibitor	
Linezolid 600 mg every 12H	Clindamycin

# Tuberculosis – A Global Emergency

- 2 billion people are infected with *M. tb*
- 1.7 million people die/year
- Nearly half a million cases of Multi-Drug Resistant-TB/ year



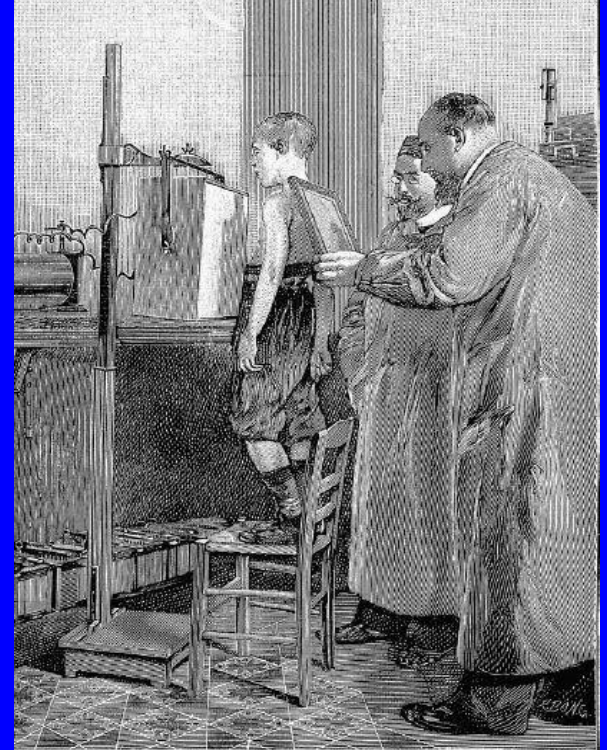
# TB Diagnosis



Microscopy  
1882



Culture  
1882



Chest X-ray  
1896

# Mycobacterial Culture

- **Manual:**

Solid egg based : L.J

Agar based Middlebrook 7H10 / 11

Liquid based : Middlebrook 7H9

- **Automated:**

BACTEC 460 TB (CO<sub>2</sub> production)

MGIT 960 (O<sub>2</sub> utilisation )

MB Bact (CO<sub>2</sub> production)



# GeneXpert® MTB/RIF Test

## Workflow

- sputum
- simple 1-step external sample prep. procedure
- time-to-result < 2 h
- throughput:  $\geq 16$  tests / day / module
- no need for biosafety cabinet
- integrated controls
- true random access

## Performance

- specific for MTB
- sensitivity better than smear, similar to culture
- detection of rif-resistance via *rpoB* gene

## Product and system design

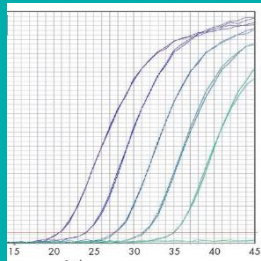
- test cartridges for GeneXpert System
- several GeneXpert modules can be combined in 1 workstation
- swap replacement of detection unit
- ~1 day technician training for non-mycobacteriologists



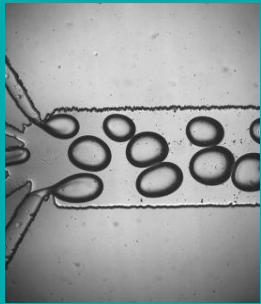
cartridge



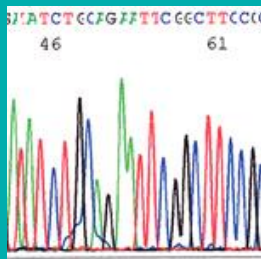
# Xpert MTB/ RIF: Integration of technologies



Real Time PCR



Microfluidics



TB DNA  
sequence

- Molecular beacons
- RT PCR
- Resistance associated mutations
- Fluorimetric probes
- Microfluidics
- Sonic bacterial lysis

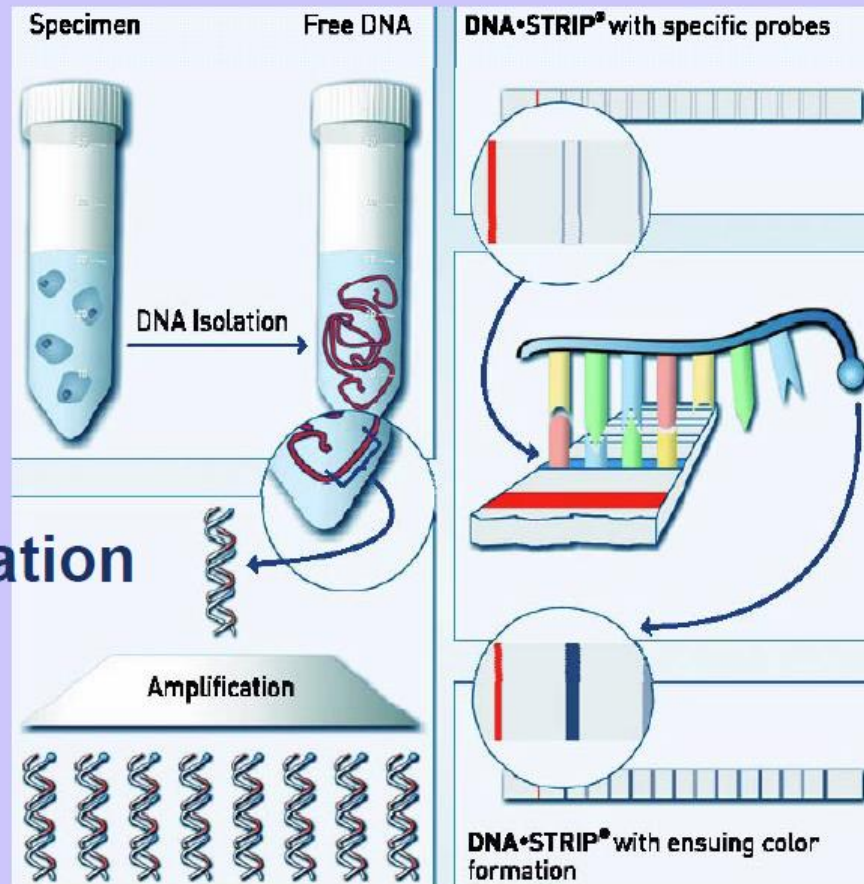


**Table 3.** Sensitivity and Specificity of the MTB/RIF Test for the Detection of Rifampin and Multidrug Resistance, as Compared with Phenotypic Drug-Susceptibility Testing Alone and in Combination with Sequencing of Discrepant Cases, According to Site.\*

Site and Total	Phenotypic Drug-Susceptibility Testing†		Phenotypic Drug-Susceptibility Testing and Discrepant Resolution by Sequencing†	
	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance
Lima, Peru — no./total no. (%)	16/16 (100.0)	190/193 (98.4)	19/19 (100.0)	190/190 (100.0)
Baku, Azerbaijan — no./total no. (%)	47/49 (95.9)	90/94 (95.7)	51/52 (98.1)	90/90 (100.0)
Cape Town, South Africa — no./total no. (%)	15/16 (93.8)	126/126 (100.0)	15/15 (100.0)	126/126 (100.0)
Durban, South Africa — no./total no. (%)	3/3 (100.0)	38/38 (100.0)	3/3 (100.0)	38/38 (100.0)
Mumbai, India — no./total no. (%)	119/121 (98.3)	61/64 (95.3)	121/122 (99.2)	62/62 (100.0)
Total for rifampin resistance				
Correct — no./total no. (%)	200/205 (97.6)	505/515 (98.1)	209/211 (99.1)	506/506 (100.0)
95% CI — %	94.4–99.0	96.5–98.9	96.6–99.7	99.2–100.0
Total for multidrug resistance				
Correct — no. /total no. (%)	195/200 (97.5)		197/199 (99.0)	
95% CI — %	94.3–98.9		96.4–99.7	

# Genotypic tests : Hain Line Probe Assay (LPA)

## 1) DNA Extraction



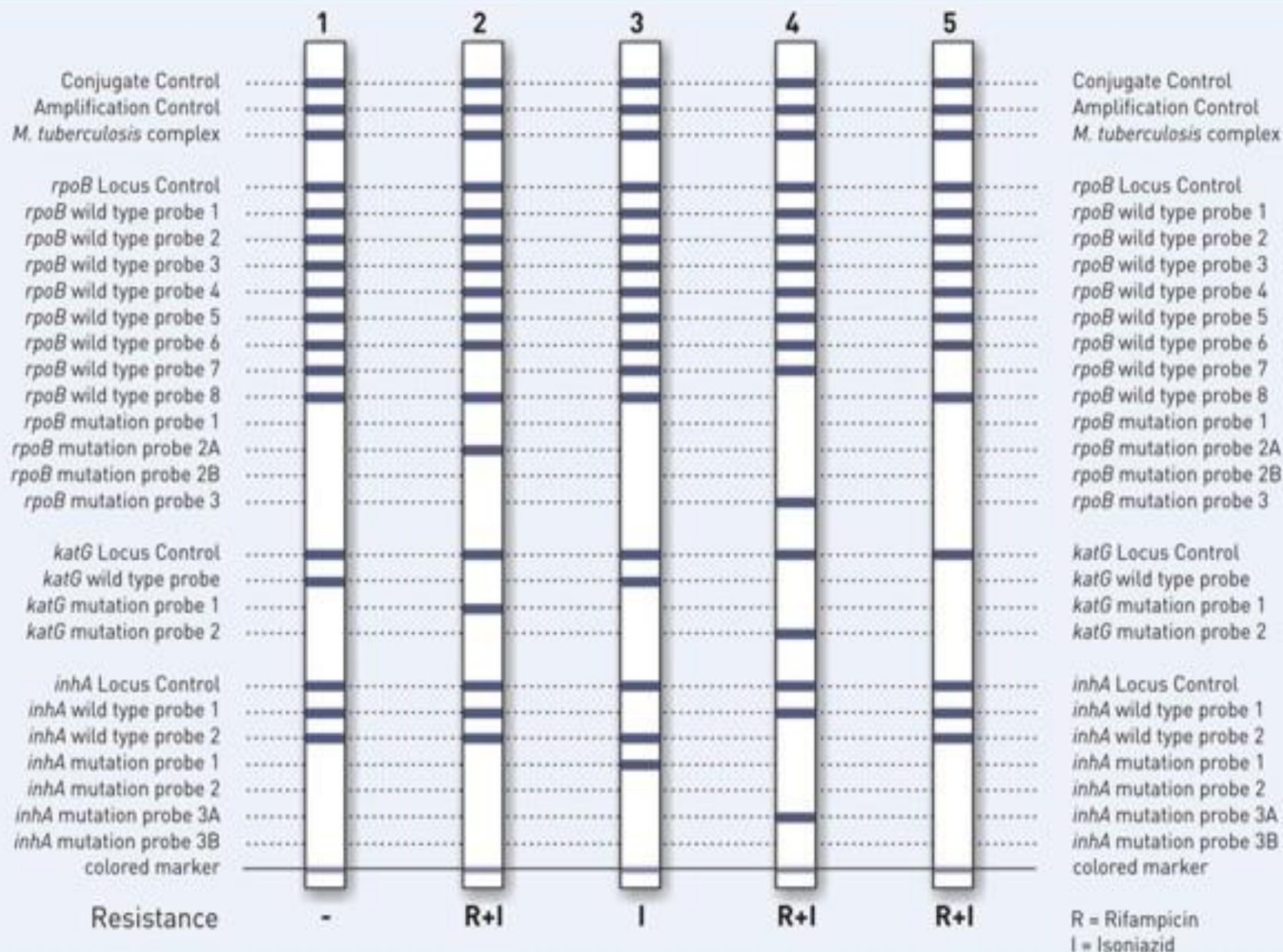
## 3) Hybridization




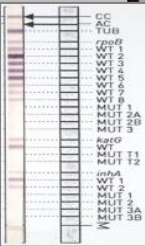


## 4) Evaluation

## 2) DNA Amplification by PCR





# WHO Endorsements in TB Diagnostics

	Year	Technology	Turnaround time	Sensitivity gain
	<b>Before 2007</b>	ZN microscopy Solid Culture	2 days 30-60 days	Baseline
	<b>2007</b>	Liquid Culture Rapid speciation	15-30 days	+10% compared to LJ
	<b>2008</b>	Line Probe Assay	2-4 days	At this time for S+ only
	<b>2009</b>	LED-based FM	1-2 days	+ 10% compared to ZN
	<b>2010</b>	Integrated NAAT (TB, Rif)	90 minutes	+ 40% compared to ZN

# Hand held Portable devices in development



**TrueLab NAAT by Molbio  
Diagnostics Private Ltd., India**



**Genedrive technology by  
Epistem Ltd, Manchester , UK**

# Unusual Acute Febrile Illness in 90s

- Acute febrile illness with multiple organ involvement

WBC	7100
Hb	15.4
PLT	30000
SGOT	164
SGPT	216
Alk. ph	156

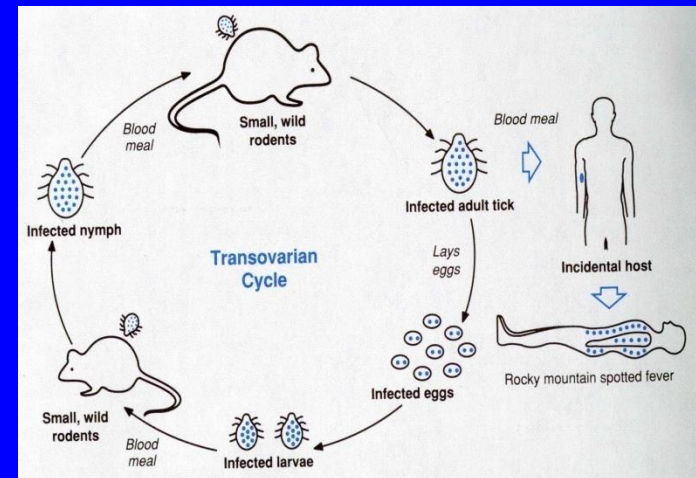
CSF: WBC – 65; (P 2, L 98)  
Protein – 103; Glucose – 56



# Scrub Typhus



- A mite-borne, zoonotic bacterial infection
- Causative agent: *Orientia tsutsugamushi* (*Rickettsia tsutsugamushi*)
- Three major serotypes – Kato, Karp & Gilliam
- Manifests as fever and multiorgan involvement.
- Vector: chiggers (larva of trombiculid mite - *Leptotrombidium*)
- Reservoir: chiggers & rats
  - Normal cycle: rat to mite to rat
  - Transovarian transmission
- Humans incidentally infected



# When should scrub typhus be suspected?

- Undifferentiated febrile illness with:
  - Pathognomonic eschar
  - Evidence of multisystem involvement, especially with:
    - Transaminase elevation
    - Thrombocytopenia
    - Leukocytosis

# Organ Involvement & Complications

- ARDS - 44%
- Respiratory involvement (breathlessness / pneumonitis) in >60%
- Hepatic involvement >80%
- Refractory shock – 25%
- Aseptic meningitis or meningoencephalitis– 19%
- Renal dysfunction – 13%
- MODS – 38%

## ORIGINAL ARTICLE

# Single-nucleotide polymorphisms in Toll-like receptor (TLR)-2, TLR4 and heat shock protein 70 genes and susceptibility to scrub typhus

Jeshina Janardhanan<sup>1</sup>, Sherry Joseph Martin<sup>1</sup>, Elisabeth Astrup<sup>2,3</sup>, R Veeramanikandan<sup>4</sup>, Pål Aukrust<sup>2,3,5</sup>, Ooriapadickal C Abraham<sup>1</sup> and George M Varghese<sup>1</sup>

- 137 scrub typhus patients and 134 controls
- PCR restriction fragment length polymorphism
- Significant heterozygous TLR4 - Asp299Gly among cases

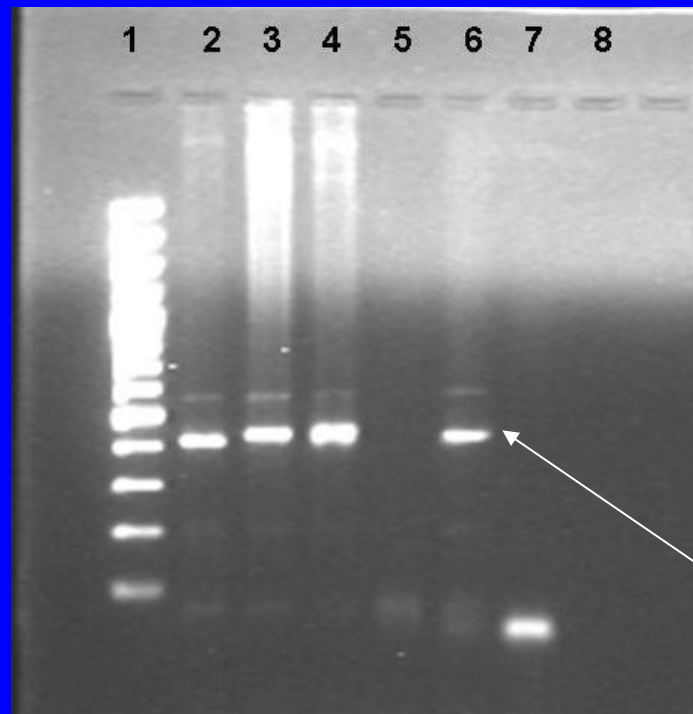
# Cytokine Network in Scrub Typhus: High Levels of Interleukin-8 Are Associated with Disease Severity and Mortality

Elisabeth Astrup<sup>1,2\*</sup>, Jeshina Janardhanan<sup>3</sup>, Kari Otterdal<sup>2,4</sup>, Thor Ueland<sup>2,4</sup>, John A. J. Prakash<sup>5</sup>, Tove Lekva<sup>2,4,6</sup>, Øystein A. Strand<sup>7</sup>, O. C. Abraham<sup>3</sup>, Kurien Thomas<sup>8</sup>, Jan Kristian Damås<sup>9,10</sup>, Prasad Mathews<sup>8</sup>, Dilip Mathai<sup>8</sup>, Pål Aukrust<sup>2,4,11</sup>, George M. Varghese<sup>3</sup>

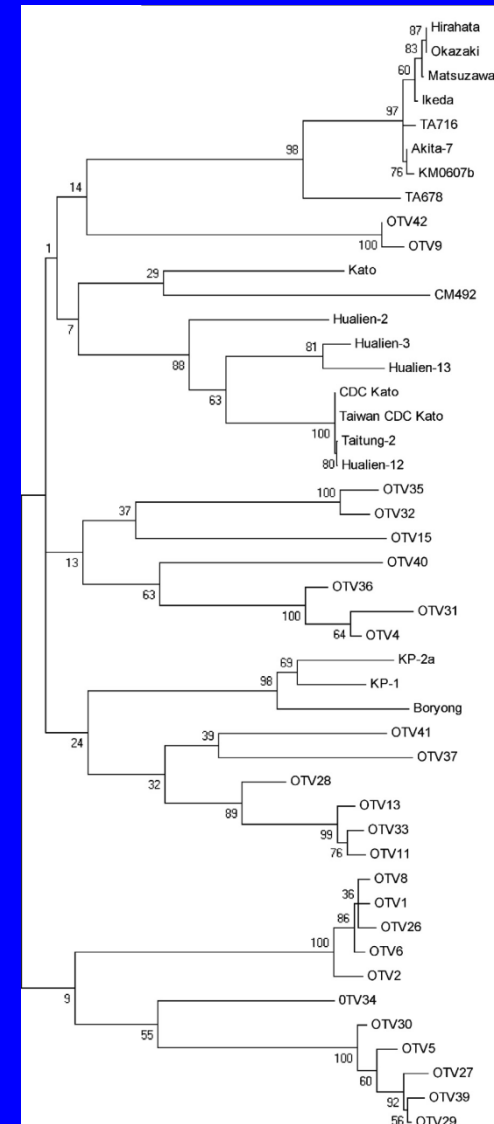
- Cytokine profile and their relation to disease severity and clinical outcome
- 129 ST patients compared to 31 healthy controls and 31 infectious disease controls
- IL-8, monocyte chemoattractant peptide-1 and macrophage inflammatory protein-1b were associated with disease severity and mortality
- Platelet-derived mediators – RANTES decreased

# Molecular Detection & Sequencing of *O. tsutsugamushi*

- Kato-like – 61%
- Karp like – 28%
- Gilliam – 2%



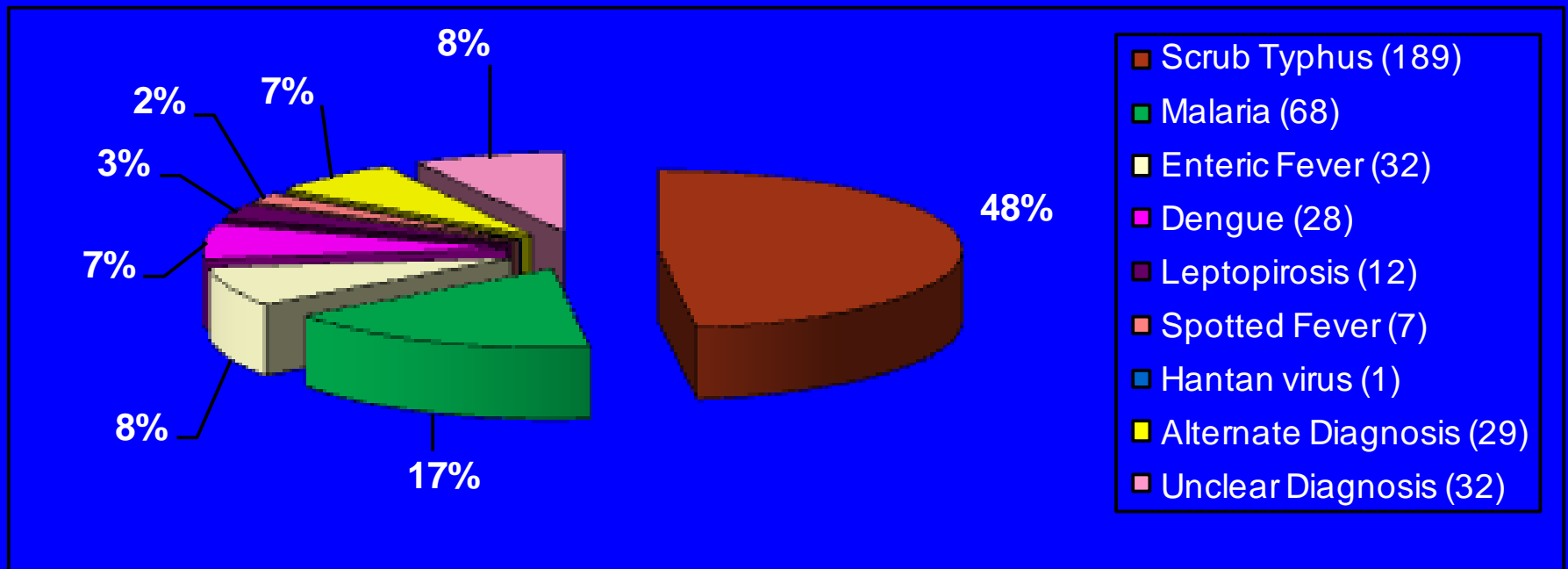
56 kDa



Varghese GM et al. Emerg Infect Dis. 2015

# Acute Febrile Illness – CMC Vellore

(398 patients)



# Summary

- The dramatic emergence of tropical infections underscores the ease with which pathogens can move between countries and continents via today's modern transportation
- These requires more than local geographic considerations with more universal etiology of infectious agent, reservoir, and vector
- It is an intellectually challenging and rapidly changing area of Infectious Diseases

**Thank You**