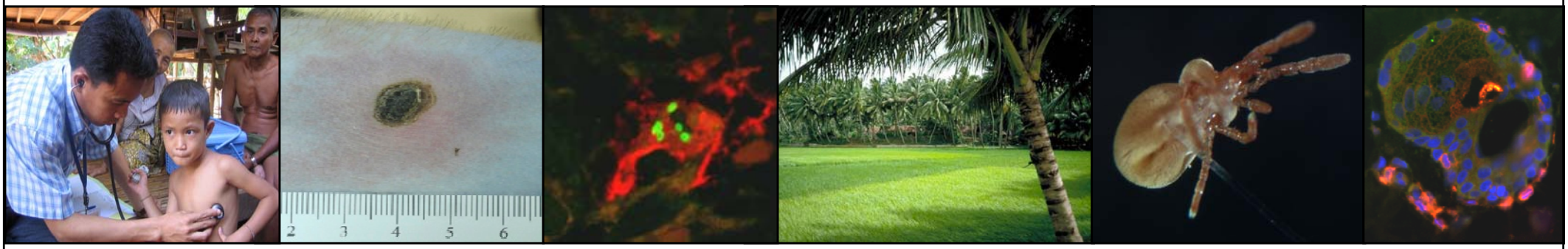
Unresolved issues related to improving the diagnosis of tropical rickettsial illnesses



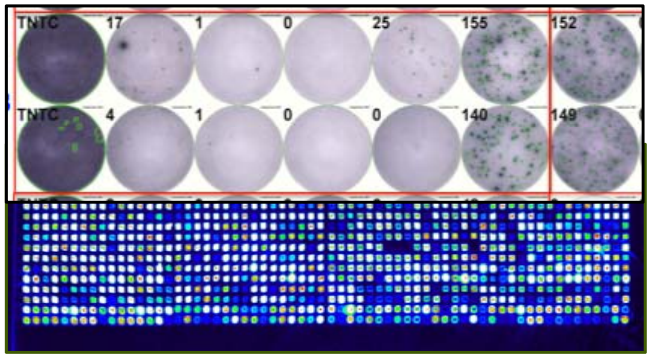
Daniel H. Paris

JITMM

December 2013

Mahidol Oxford

WELLCOME TRUST · MAHIDOL UNIVERSITY-OXFORD
TROPICAL MEDICINE RESEARCH PROGRAMME



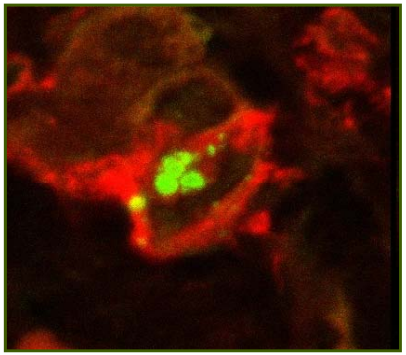
Immunology / Ag discovery

Clinical Research

Entomology

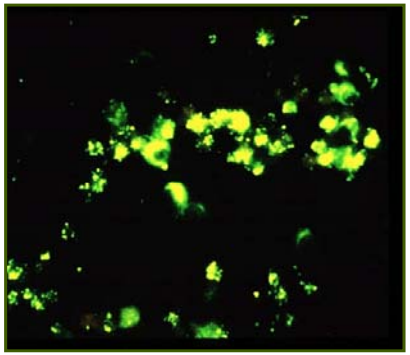
Ecology

Rickettsial Research



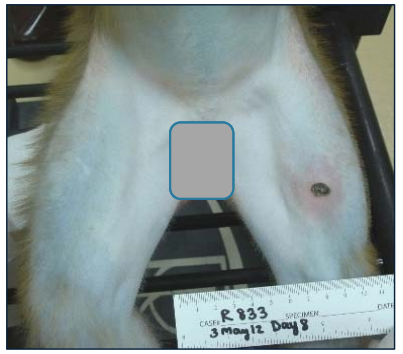
Pathophysiology

Pathogen Discovery



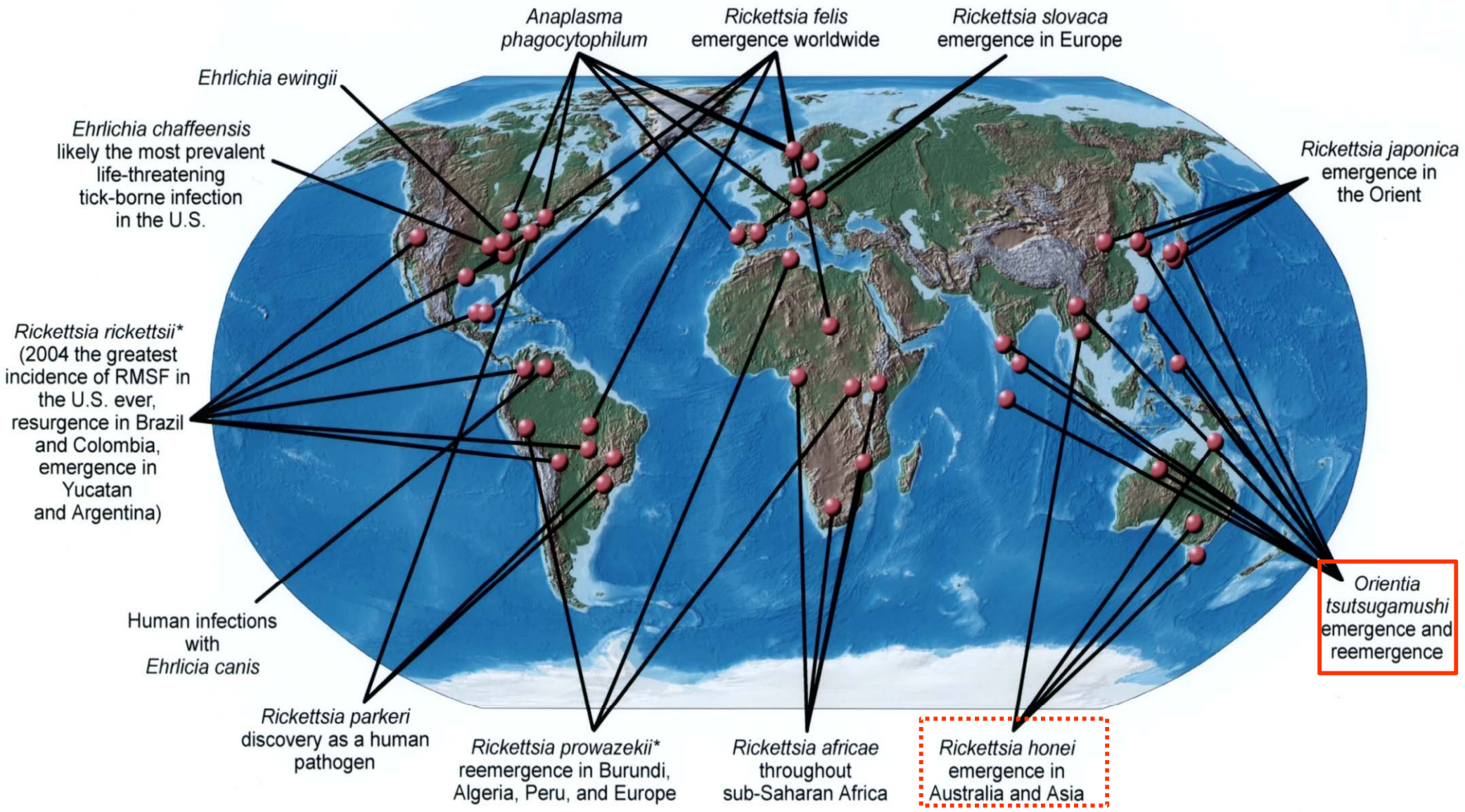
Diagnostics

Epidemiology



NHP / vaccine models

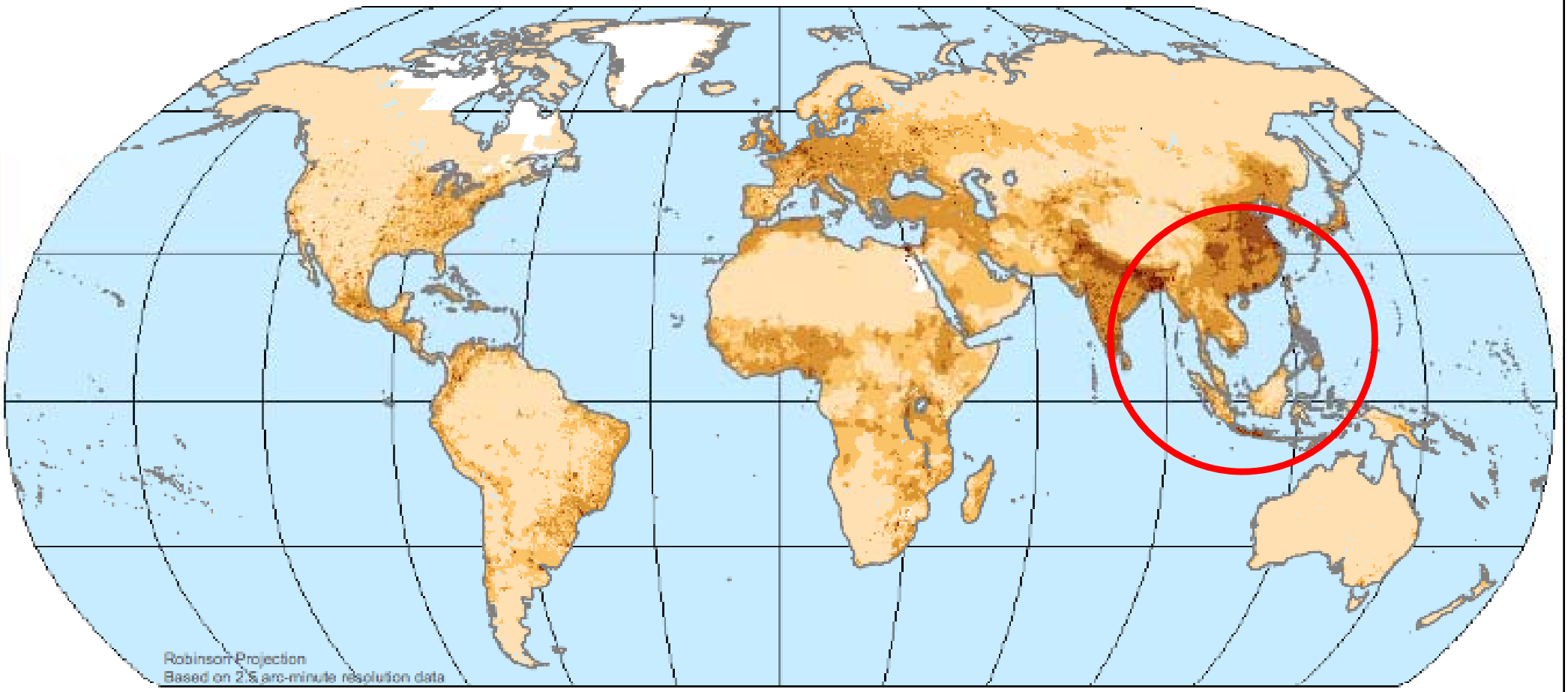
Emerging rickettsial infections



*By criteria of low dose aerosol infectivity, high case fatality rate, high disease:asymptomatic infection ratio, availability in nature, and potential for genetic engineering of complete antibiotic resistance, *R. rickettsii* and *R. prowazekii* should be category A agents, and *R. typhi* and *R. conorii* category B agents.

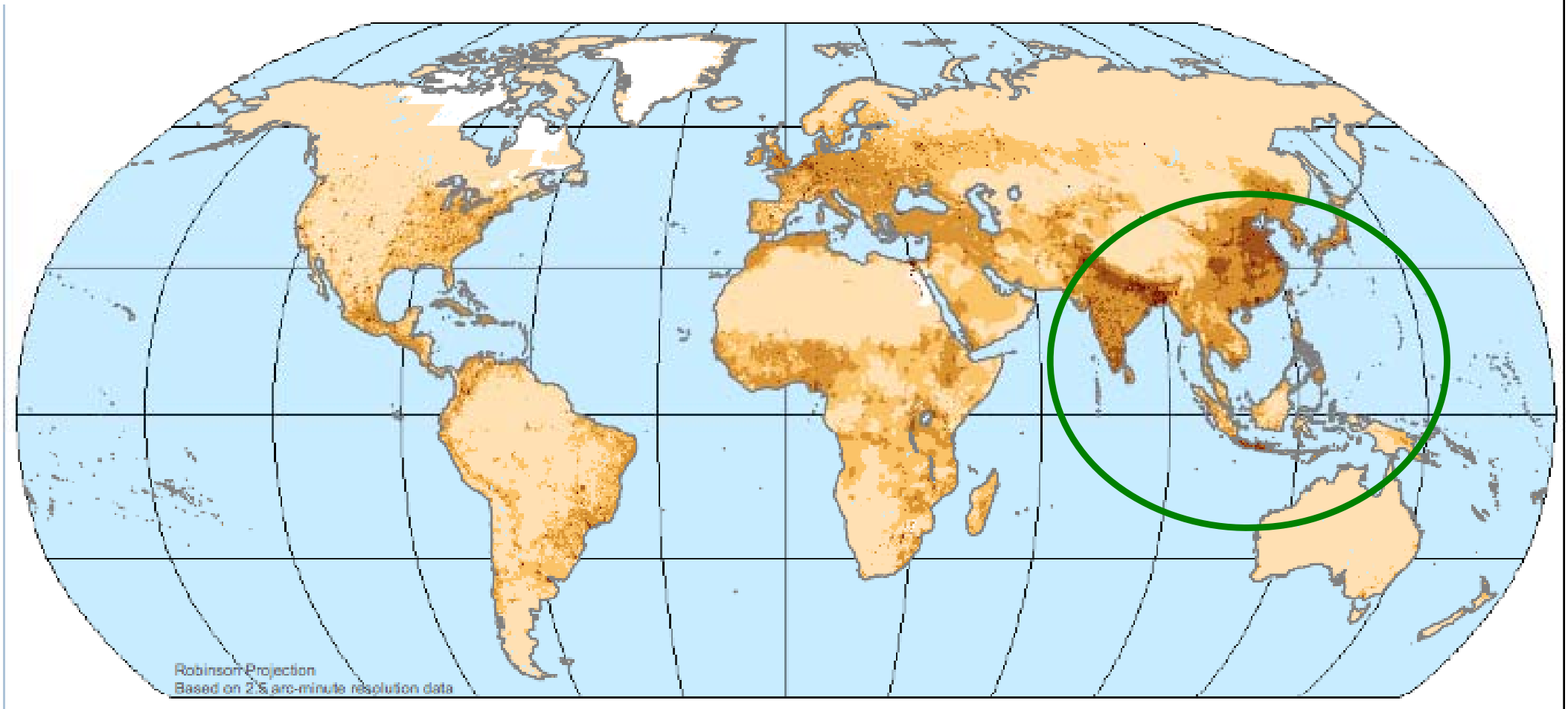
From DAVID H. WALKER, *University of Texas Medical Branch, USA*

The importance of Asia …



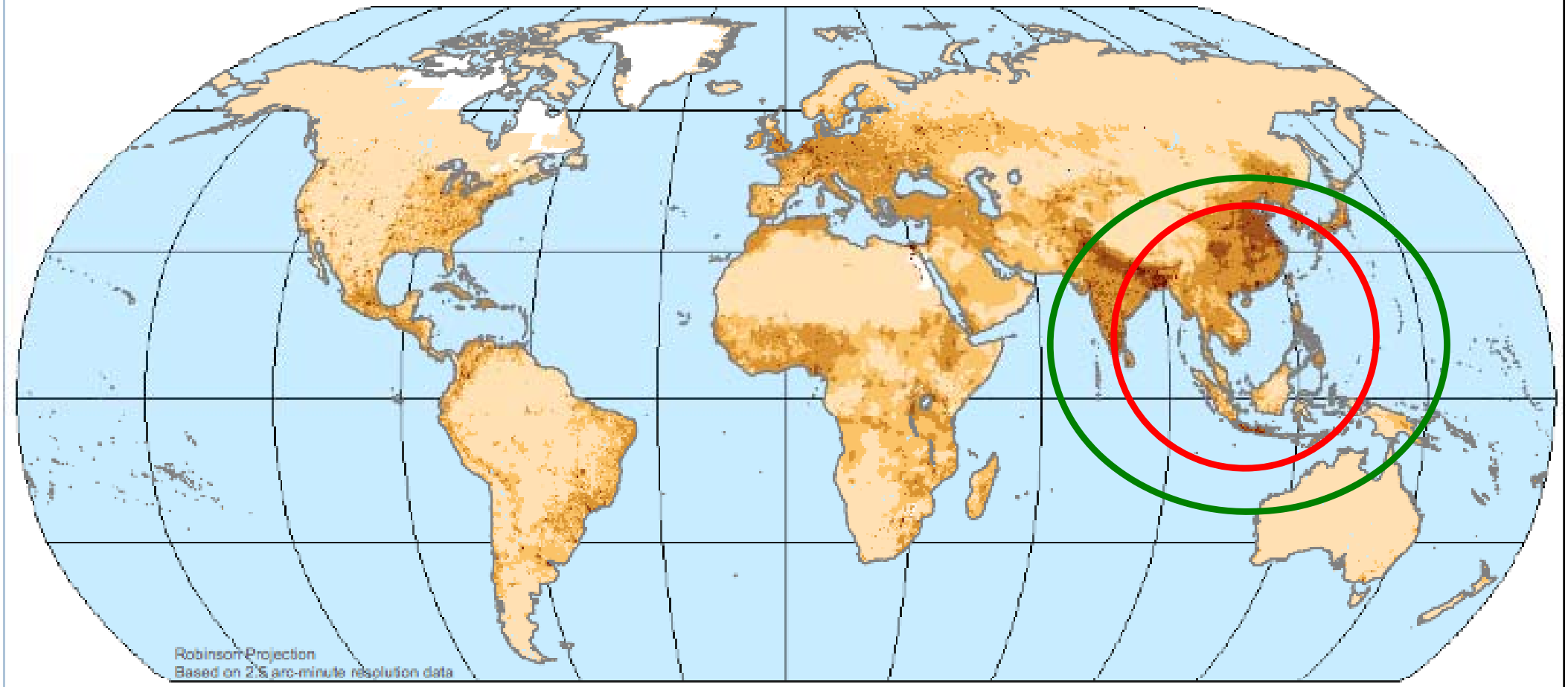
Half of the world's population within 2000 miles of Bangkok !!!

The importance of Asia …



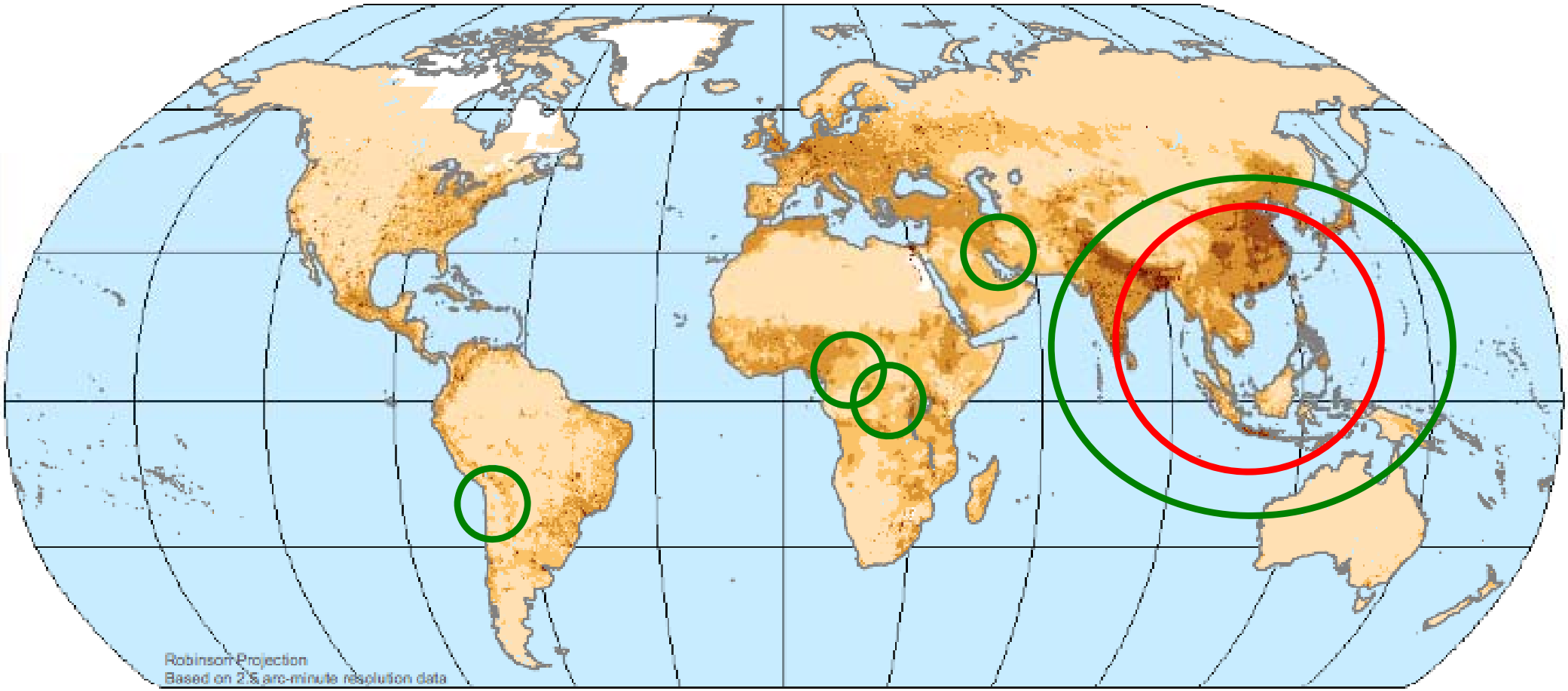
>90 % of reported scrub typhus cases within 3000 miles of Bangkok !

The importance of Asia …



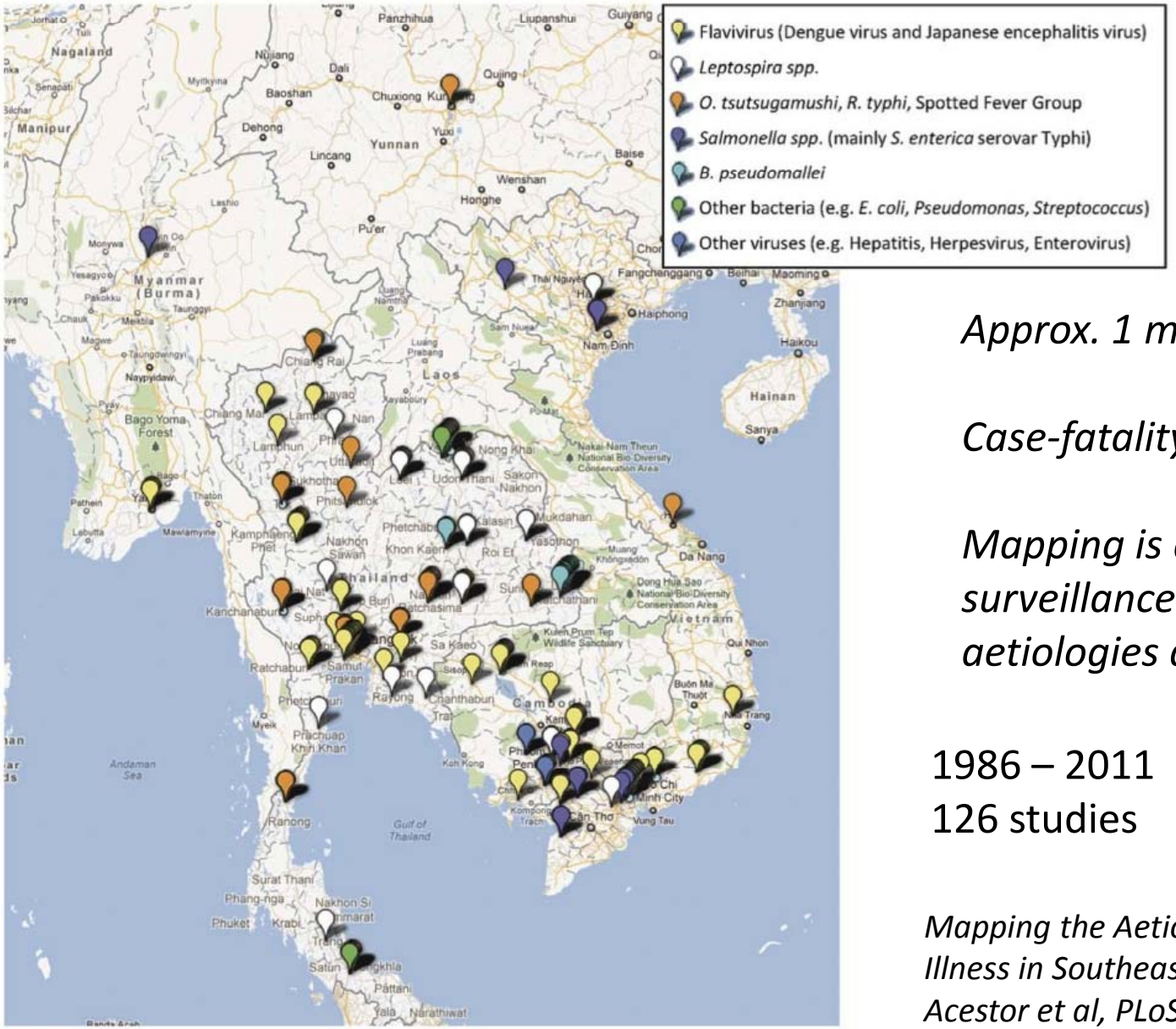
… What about the rest of the world … ??!!

The importance of Asia …



… potential global tropical / subtropical distribution … ?!

How important is scrub typhus?



Approx. 1 mio cases estimated

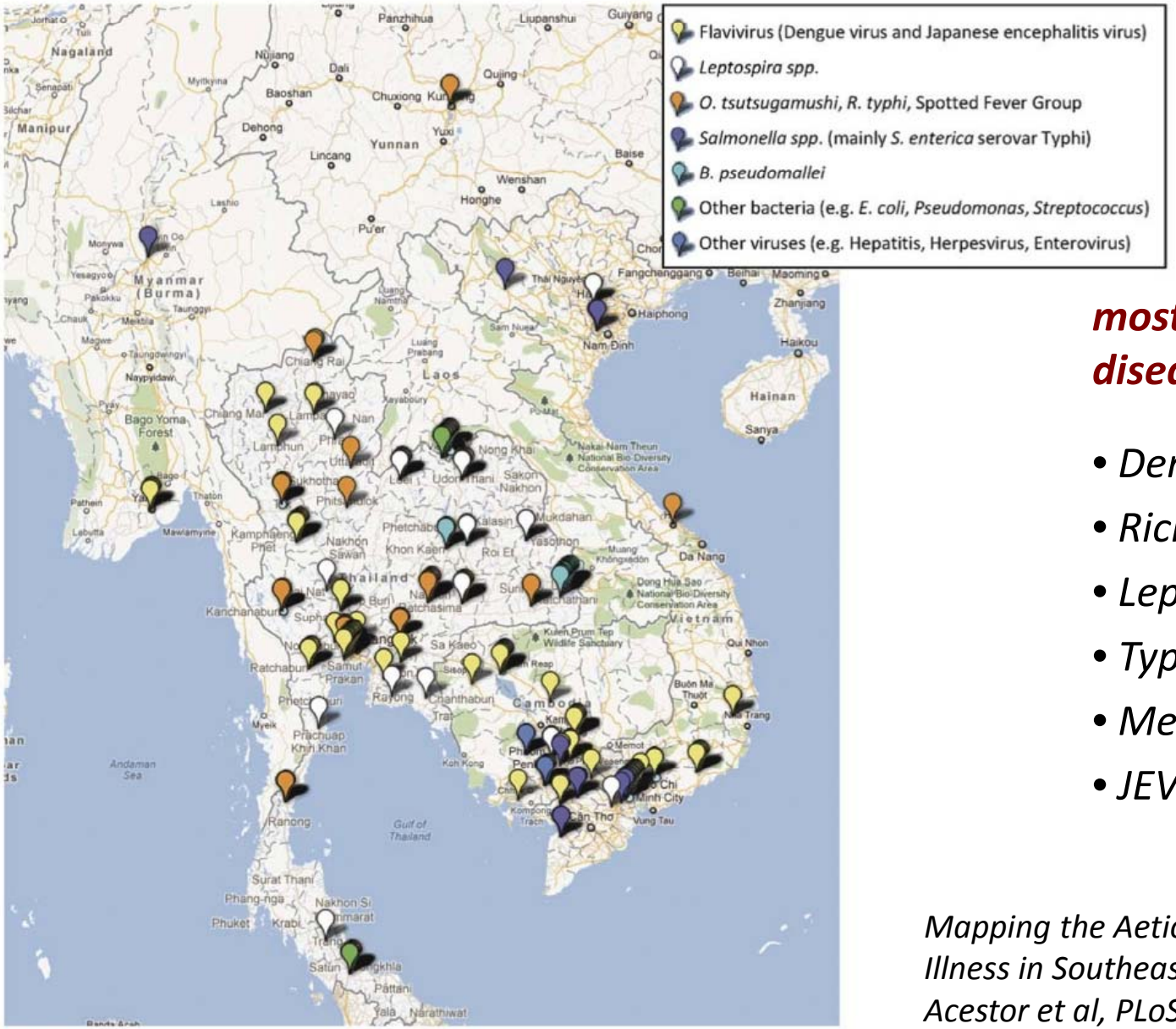
Case-fatality rate 3-7%

Mapping is an excellent surveillance tool to identify aetiologies and localise 'gaps'

1986 – 2011
126 studies

Mapping the Aetiology of Non-Malarial Febrile Illness in Southeast Asia
Acestor et al, PLoS ONE, 2012

How important is scrub typhus?



most frequent reported diseases/pathogens:

- *Dengue*
- *Rickettsial infections*
- *Leptospirosis*
- *Typhoid*
- *Melioidosis*
- *JEV*

Mapping the Aetiology of Non-Malarial Febrile Illness in Southeast Asia
Acestor et al, PLoS ONE, 2012

Typhus in SEA

Thailand
Malaysia
Laos
Vietnam
Cambodia
Myanmar
Bangladesh
India
… ?! etc.

"Dengue-like illness"

"Typhus-like illness"

- *burden 15-25% ST, 10-15% MT*
- *estimated 1 mio cases per year*
- *seroprevalence 30-50%*

Fringe habitats - rural

Markets - urban

***ST and MT** (and SFG) combined = leading cause of treatable undifferentiated febrile illnesses*

"Scrub typhus is probably one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization in the region "

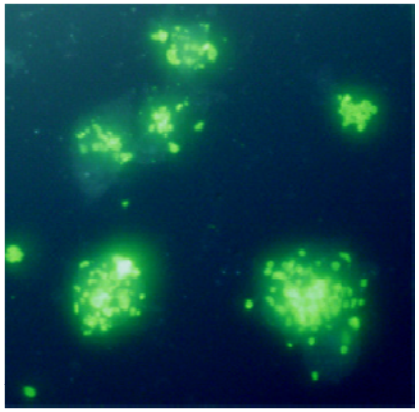
World Health Organization, 1999

Rickettsial Disease

- Eschar (scrub typhus, spotted fevers)
- Skin rash
  "spotted fever", "exanthematic typhus"
- *"Typhus"* - *typhos* [smoky, hazy]
  - neurological symptoms

Complications

- Meningo-encephalitis
- Pneumonitis – ARDS
- Acute renal failure
- Severe hepatitis
- Myocarditis
- Coagulopathies – DIC



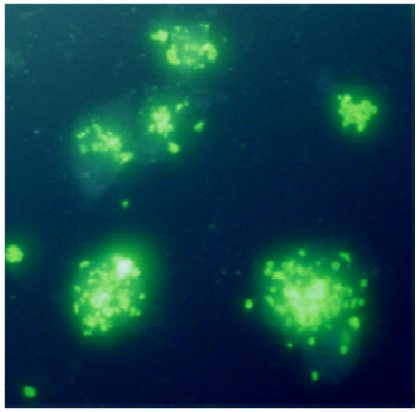
O.tsutsugamushi in cell culture

Coma – delir - confusion

Differential Diagnosis 'Typhus'

Typhus-like illness

- Scrub typhus
- Murine Typhus
- Leptospirosis
- Spotted Fever Rickettsiosis
- Dengue
- Japanese Encephalitis
- Typhoid (*Salm.* Typhi)
- Bacterial meningitis
- (Malaria)



O.tsutsugamushi in cell culture

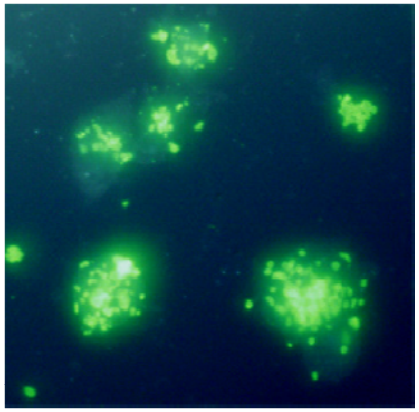
Coma – delir - confusion

… the problems …

- Clinical presentation is similar
- Diagnostic targets time-dependent
- Endemic diseases = ‘background’ immunity
- Conventional microbiology not useful
- Difficult-to-culture pathogens
- Serological cross-reactivity – antigen usage
- Ill-defined positivity cut-off titers
- *etc*.

A real challenge!

Need of diagnosis to guide treatment !



O.tsutsugamushi in cell culture

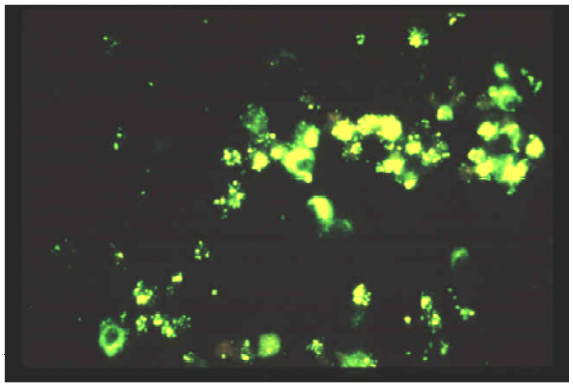
Coma – delir - confusion

DD: Inoculation lesion vs. Eschar

- *manipulated insect bite!*
- *Staphylococcal lymphadenitis*
- *Brown recluse spider bite*
- *Typhus (Scrub Typhus, Tick Typhus)*
- *Rickettsialpox*
- *Tularemia*
- *Cat scratch disease*
- *Rat-bite fever*
- *Ecthyma gangrenosum*
- *Pyogenic granuloma*
- *Cutaneous anthrax*
- *Plague (flea bite site)*
- *Cutaneous tuberculosis*
- *Cutaneous leishmaniasis*
- *Lymphogranuloma venereum*
- *Aspergillosis*
- *Mucomycosis*

Rickettsial Diagnostics

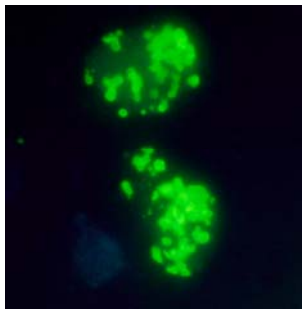
- ***In vitro* isolation**
  - High specificity, low sensitivity
  - Slow (weeks)
  - Requires biocontainment lab
- **Serology**
  - IFA is the traditional “Gold Standard”
  - ELISAs
  - Rapid tests
- **Molecular**
  - Many targets, nested PCR, realtime PCR, LAMP
  - Not ideal in resource poor settings
  - Limited sensitivity (?)
- **Antigen-capture**
  - None available



Scrub typhus diagnostics

Current gold standard Immuno-fluorescence assay (IFA)

- Microscopic slide with pooled antigens
- Anti-human IgM/IgG FITC conjugate
- Dilution series

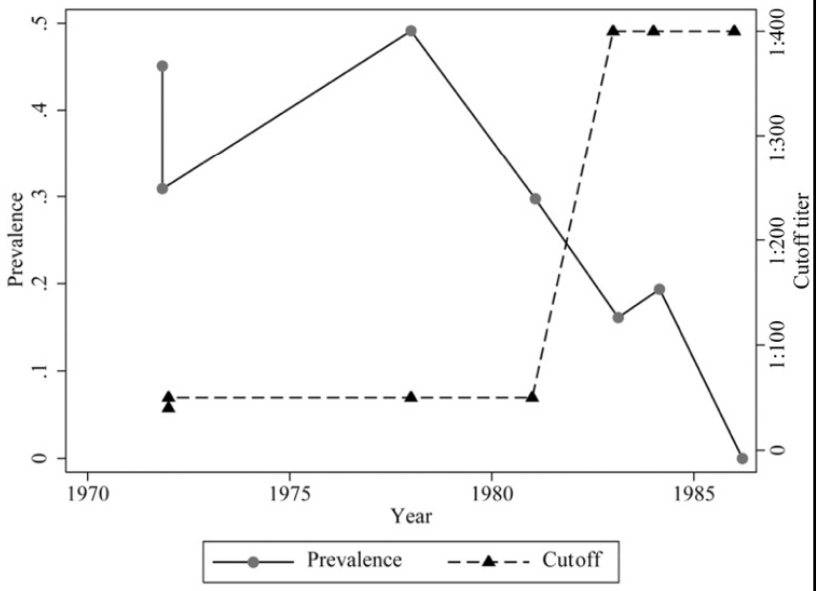


Lack of consensus

- *No standardisation of IFA slides around the world*
- *No consensus on cut-off titers*
- *Little / no evidence for titers or antigen used*
- Single serum cut off IgM ≥1:400
- Paired serum: ≥ four-fold titer rise

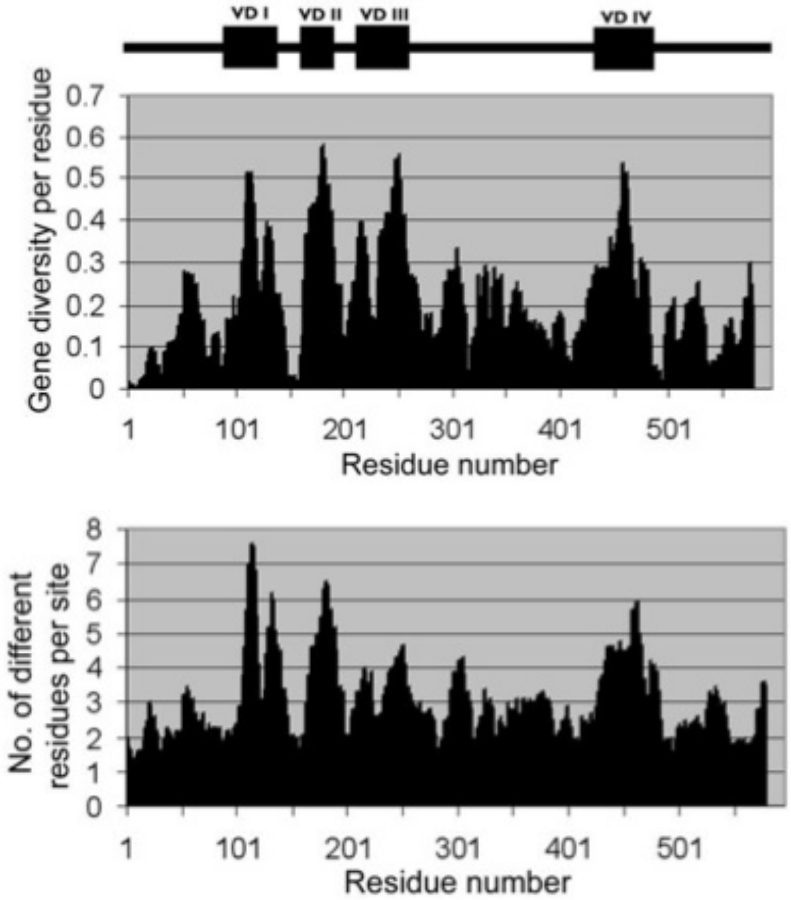
Problems

- Antigen selection – WCA purity (cross-reactivity)
- Subjective endpoint (reader variability)
- Standardization
- UV-fluorescent microscope



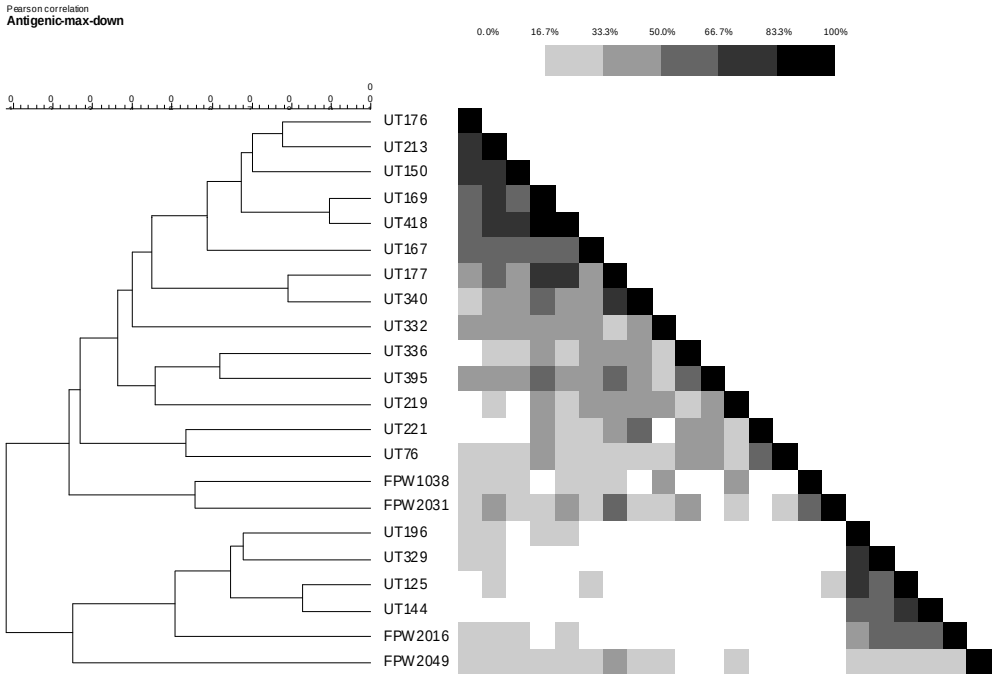
Blacksell et al., Clin Inf Dis, 2006

Antigenic heterogeneity



Antigenic heterogeneity of the 56kDa gene of *O. tsutsugamushi*

Kelly et al., CID, 2009

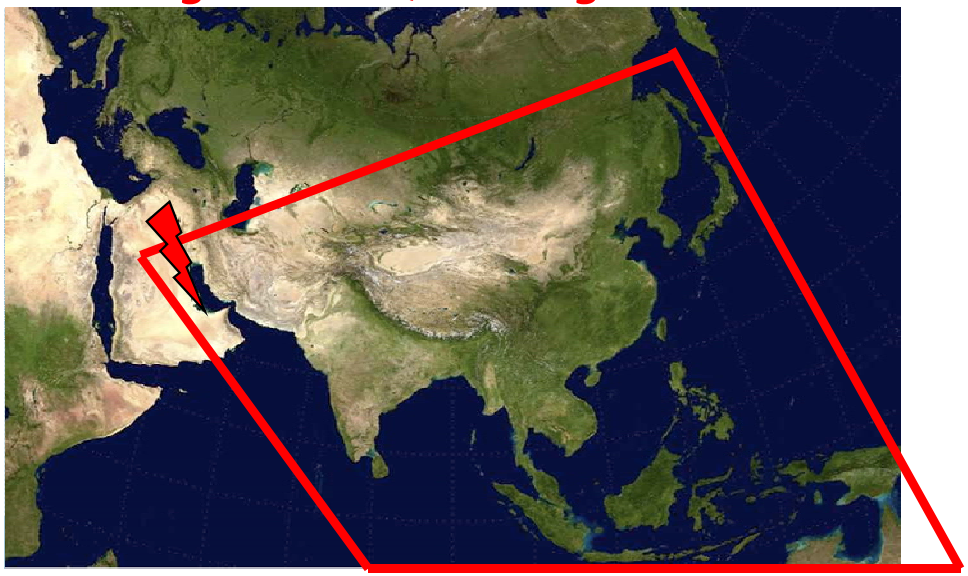


IFAs made with patient isolates, tested patient sera

Problems for both serological diagnosis and vaccine development

Orientia chuto sp. nov.

'Tsutsugamushi Quadrangle'

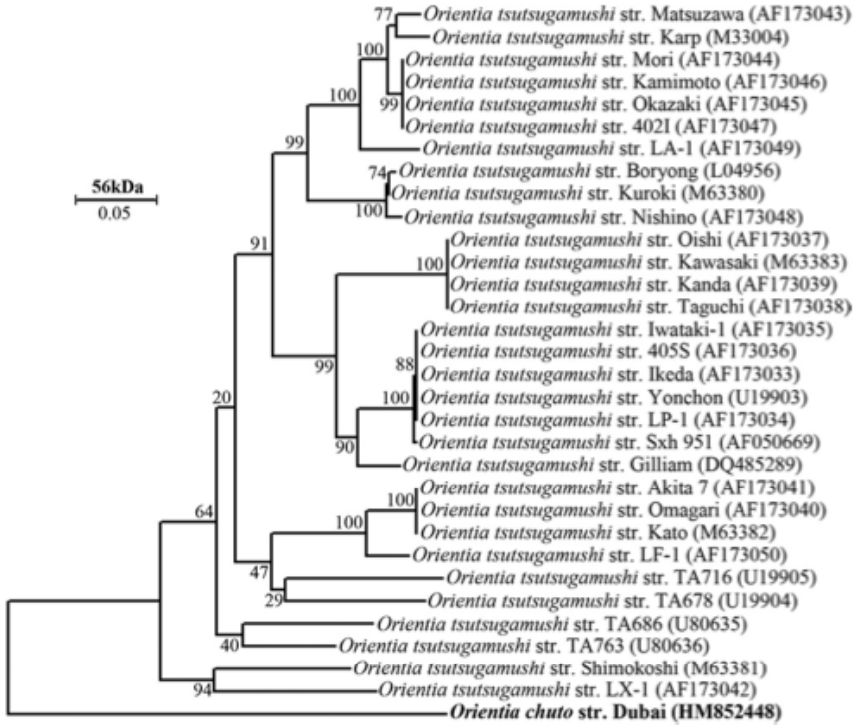


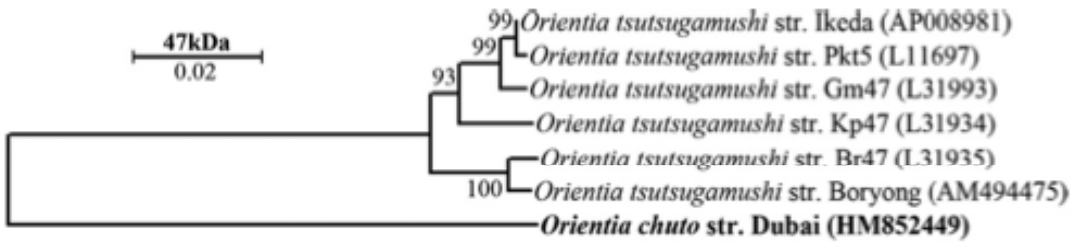
Percent similarities per gene (difference)

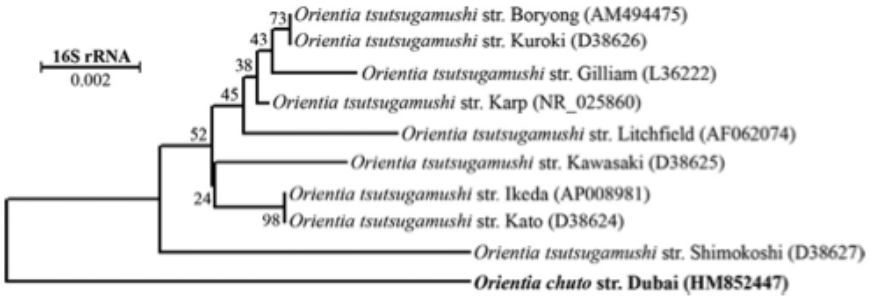
16S – 2%

47kDa – 18%

56kDa – 46%



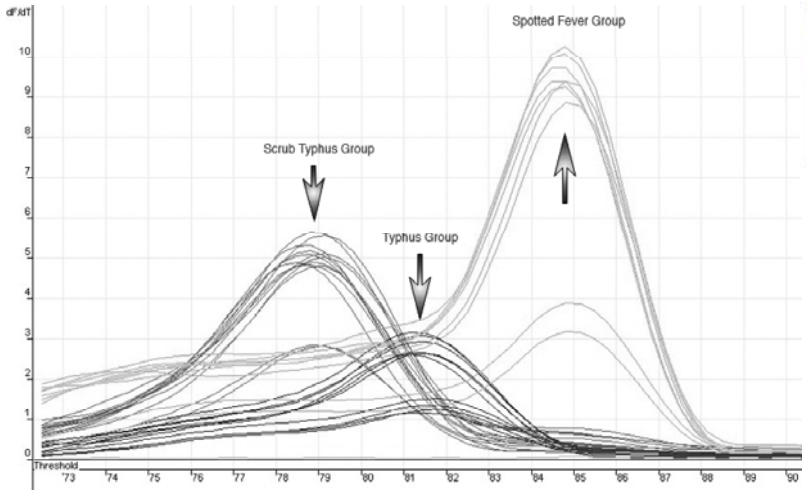


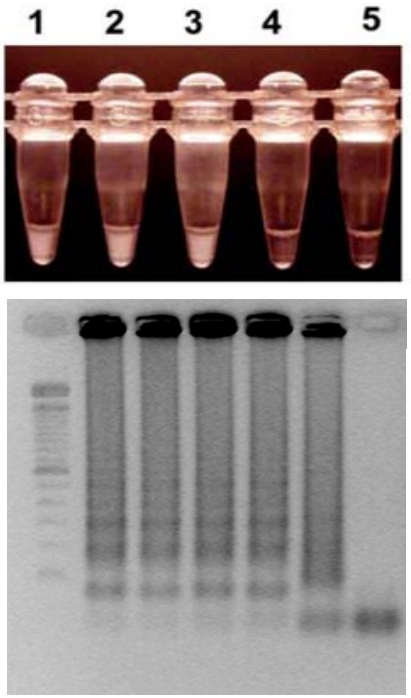


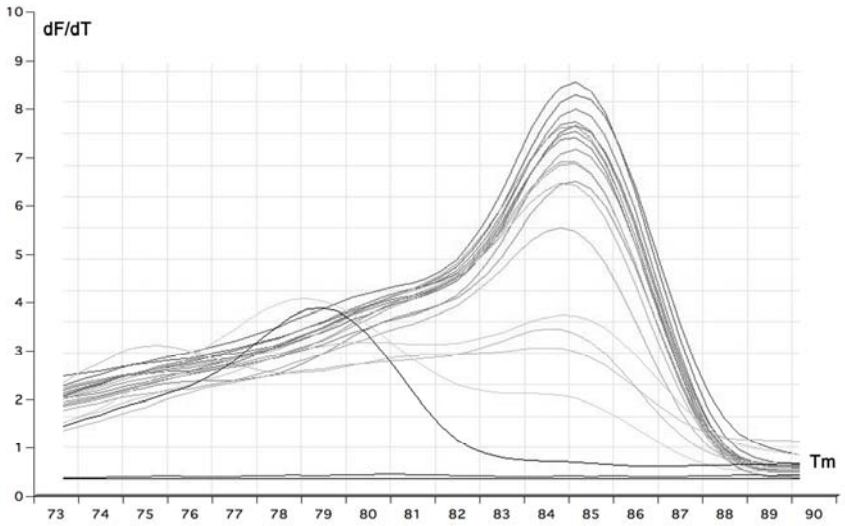
Izzard et al., *Jour Clin Micro*, 2010

Identifying rickettsaemic cases

- Multiplex Realtime PCR (broad range)
- Loop-mediated isothermal PCR (low cost)
- Highly sensitive quantitative Realtime PCR (quantitative)

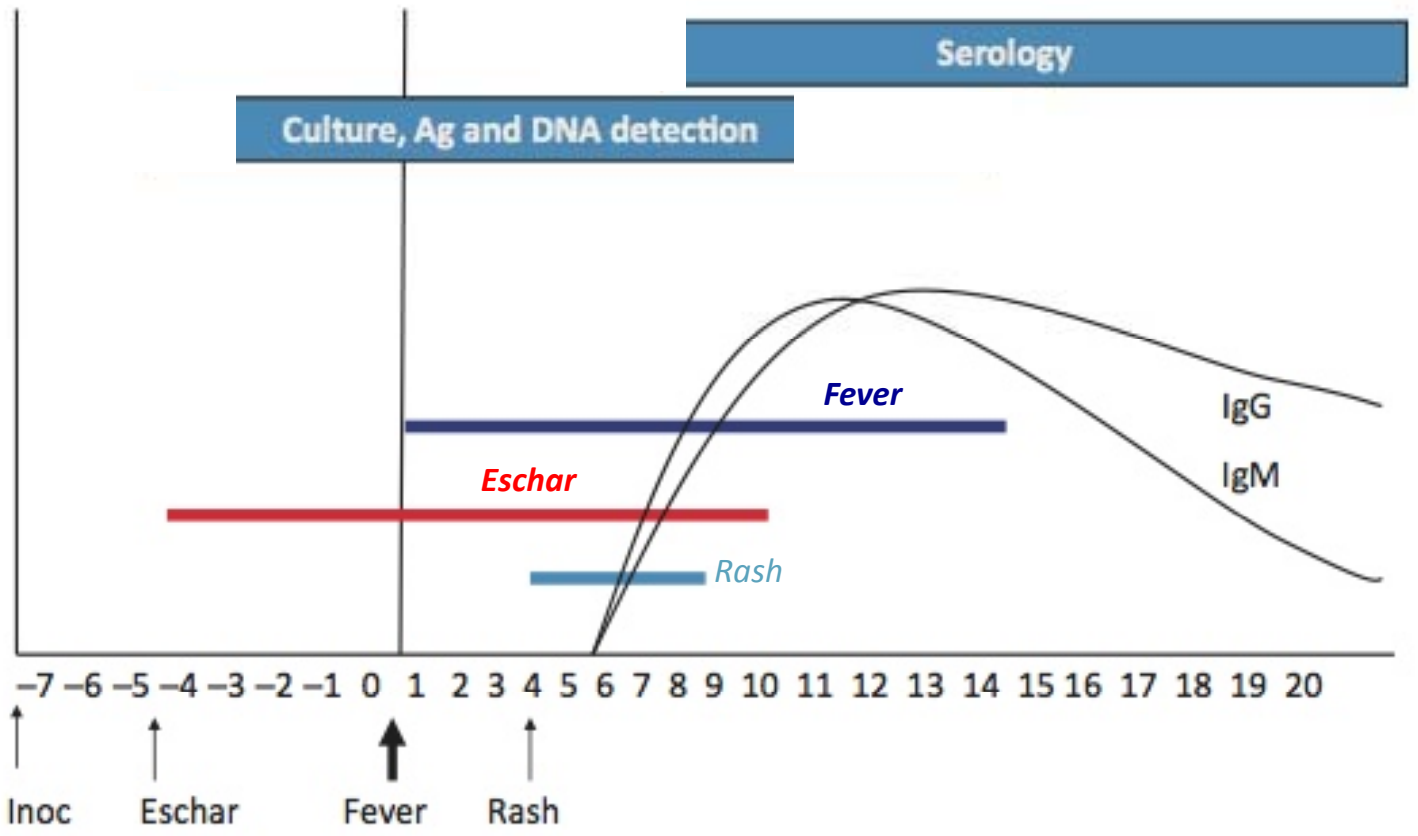






Jiang J et al., 2004, 2008
Sonthayanon P et al., 2009
Paris DH et al., 2008, 2009

Diagnostic Dynamics



PCR has limitations (approx. 60% positivity) – only useful in rickettsaemic phase

Direct bacterial detection – importance of antigen-characterisation

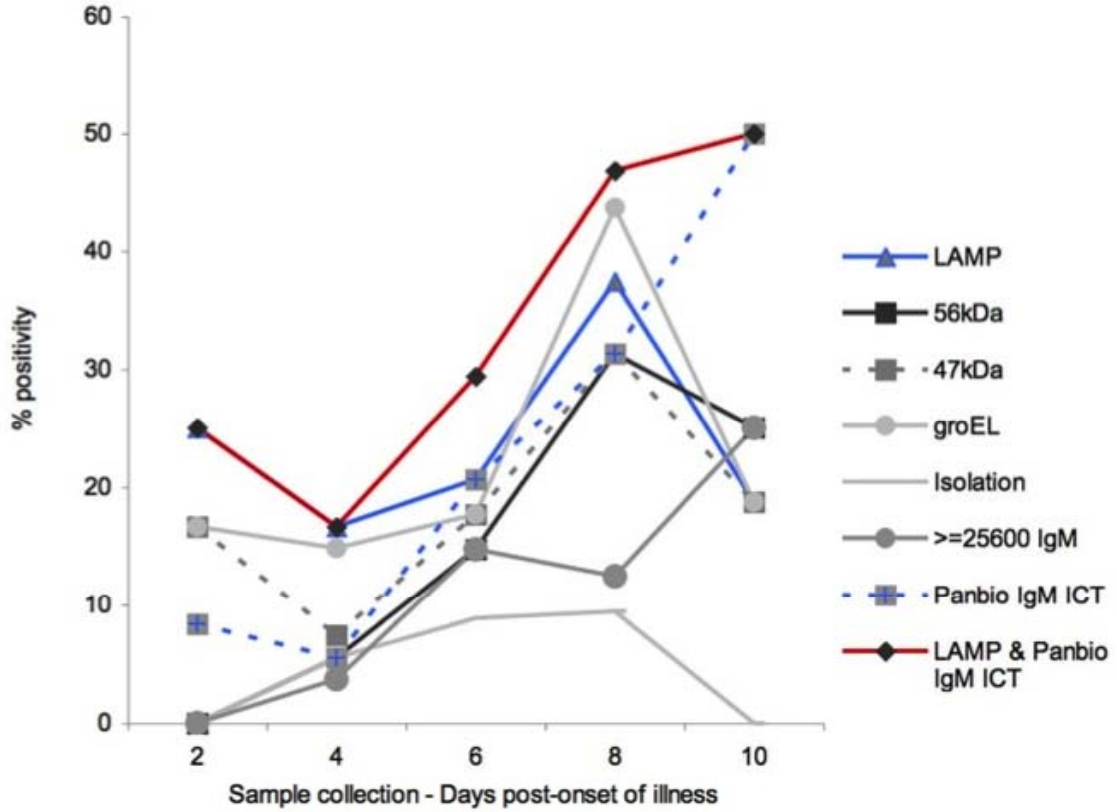
Paris, Richards, Day, Ch124, Med Mol Micro

Scrub Typhus Infection Criteria 'STIC'

Validation criteria for Dg assays?!

- **STIC (rigorous criteria)**
  - Isolation
  - Dynamic serology
  - 2/3 PCR pos.
  - Single IgM >1:12'800

- **Clinical validation of LAMP**
  - Fever study (N-Thailand)
  - Se ≈ 50-56% / Sp ≈ 96-99%
  - comparable to nPCR and realtime PCR



PCR / LAMP were superior to serology at <10 days of fever

Combination with ICT improved overall positivity (Se) and expanded the spectrum of OT detection over time

Paris DH et al., 2011

Imperfect gold standard model (Bayesian Latent Class model)

Profile	Number
11111	.
11110	.
11101	.
11011	.
10111	.
⋮	
00000	.
TOTAL	N

- **No 'gold standard'**
- Assume that Se and/or Sp of gold standard is imperfect
- Considers **the true prevalence** instead
- True prevalence is determined by true status (infected or not infected) of each individual

- Need at least **3 tests in a single population**
- *Not assuming any test is perfect gold standard*

  (All five tests are imperfect)

LCM – approach for scrub typhus

Parameters	STIC as gold standard*	Bayesian LCM**
Prevalence	34.2% (26.9-42.0)	23.3 (16.0-32.3)
Blood culture for *Orientia tsutsugamushi*		
Sensitivity	16.4% (7.8-28.8)	24.1 (12.1-41.8)
Specificity	100%	100
PCR assays†		
Sensitivity	49.1% (35.4-62.9)	65.2 (46.2-82.5)
Specificity	100%	98.0 (92.7-100.0)
IFA IgM††		
Sensitivity	83.6 (71.2-92.2)	69.4 (53.9-83.9)
Specificity	100	84.0 (76.3-90.2)

†Positive is defined as 2 out of 3 PCR assays (groel, p47 and p56 PCR assays) showed positive result
††Positive is defined as either admission IgM titer of IFA ≥1:12,800 or paired IFA IgM ≥4-fold increase
**Value shown are mean estimates with 95% confidence interval*
***Values shown are median estimates with 95% credible interval*

LCM – approach for scrub typhus

Parameters	STIC as gold standard*	Bayesian LCM**
Nested 56kDa-based PCR assay		
Sensitivity	40.0 (27.0-54.1)	56.4 (47.8-65.6)
Specificity	99.1 (94.9-100.0)	98.4 (96.2-100.0)
47kDa-based real-time PCR assay		
Sensitivity	47.3 (33.7-61.2)	62.9 (53.7-71.4)
Specificity	98.1 (93.4-99.8)	96.6 (93.2-98.4)
GroEL-based real-time PCR assay		
Sensitivity	56.4 (42.3-69.7)	71.4 (61.9-79.4)
Specificity	96.2 (90.6-99.0)	93.4 (90.1-95.8)

*Value shown are mean estimates with 95% confidence interval
**Values shown are median estimates with 95% credible interval

LCM – approach for scrub typhus

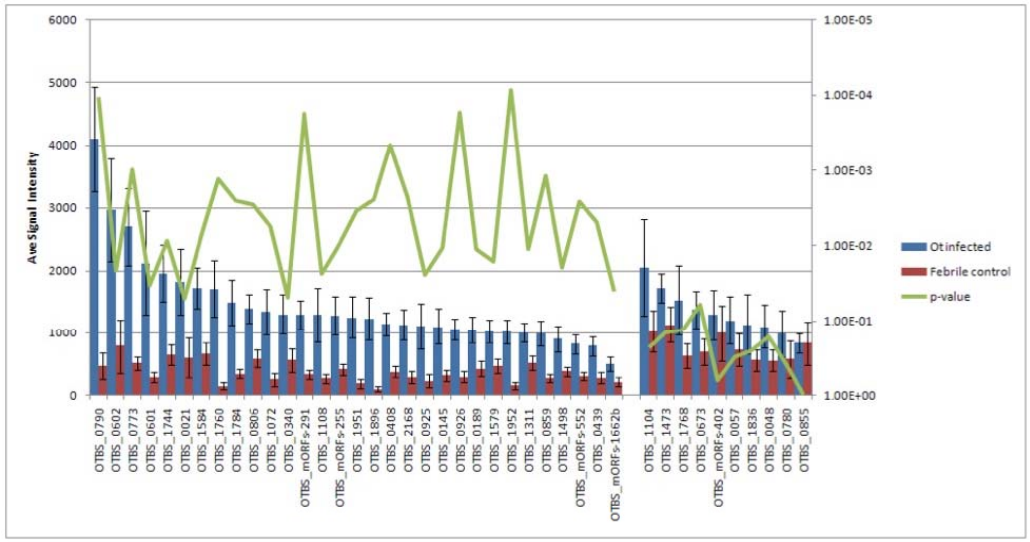
Parameters	STIC as gold standard*	Bayesian LCM**
Admission IFA IgM (cut-off ≥1:12,800)		
Sensitivity	36.4 (23.8-50.4)	50.0 (43.2-58.6)
Specificity	100	99.2 (96.9-100.0)
Paired IFA IgM (≥4 fold increase)		
Sensitivity	47.3 (33.7-61.2)	19.4 (15.6-25.0)
Specificity	100	84.8 (83.6-86.6)
PanBio IgM + Eschar		
Sensitivity	47.3 (33.7-61.2)	75.0 (65.0-86.1)
Specificity	93.4 (86.9-97.3)	96.0 (93.0-99.2)

*Value shown are mean estimates with 95% confidence interval
**Values shown are median estimates with 95% credible interval

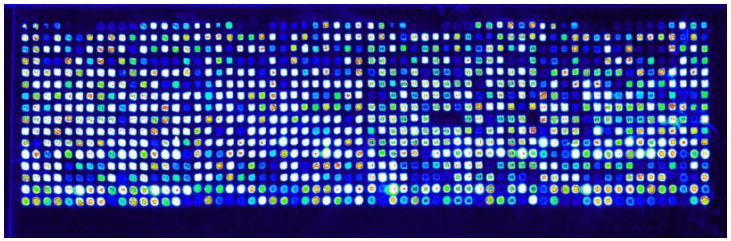
… the future …

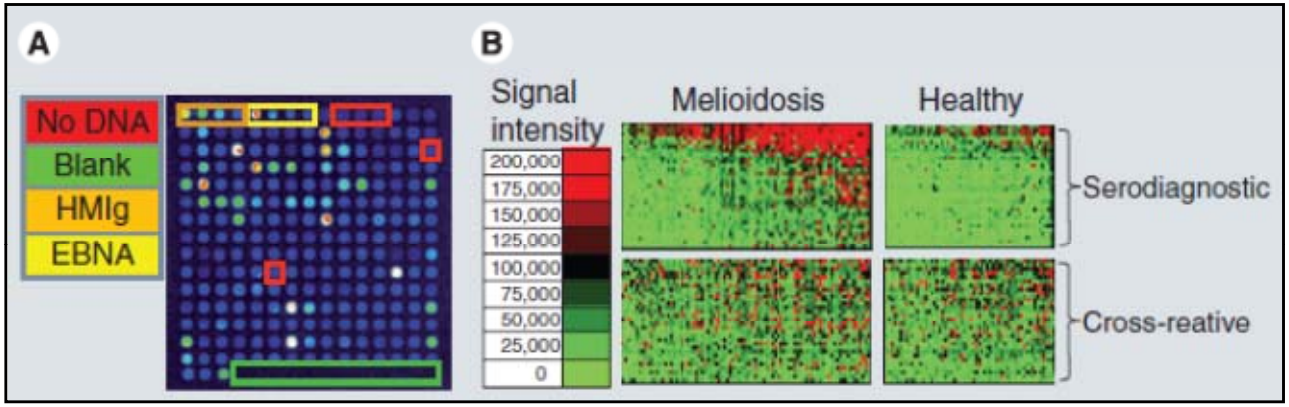
Aims:

- Simple, affordable point-of-care diagnostics
- Specific antigen selection
- Standardized assay, non-subjective endpoint



O. tsutsugamushi protein array





Felgner P et al., PNAS 2009
Paris DH et al., AJTMH 2013

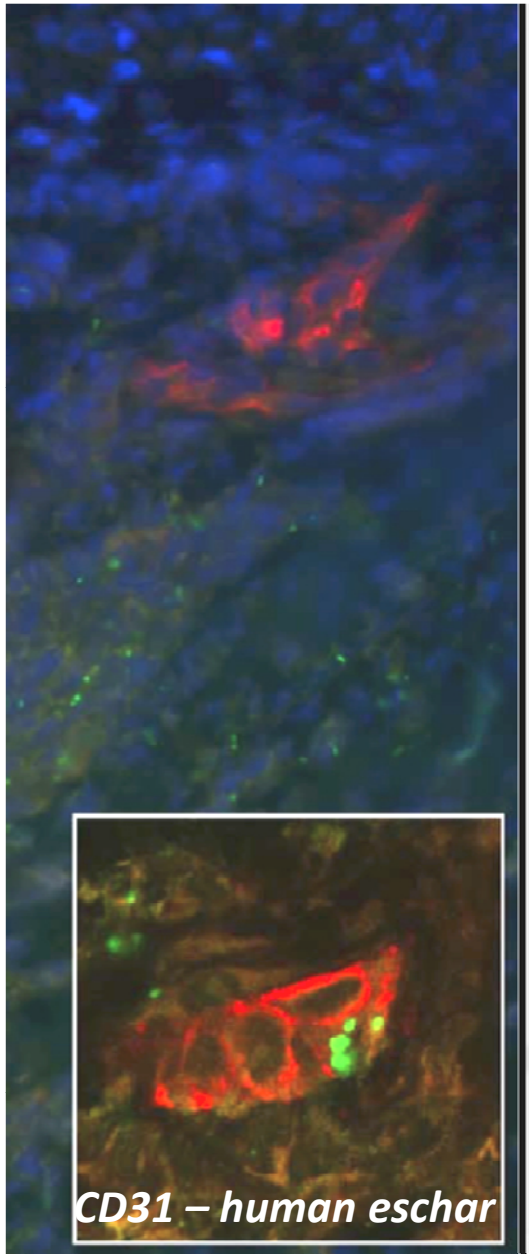
Pathogenesis - Pathophysiology

Human pathophysiology

- Immuno-pathogenic response mechanisms
  - Coagulation disorders
  - Oxidative stress, role of reactive radicals
  - Cytokines, and cytotoxic immune cells
  - Vascular permeability ?
- Cell biology
  - Attachment, invasion, mechanisms of cell injury
  - *Orientia*-mediated intracellular events

Animal models

- Replicate human disease
- Natural disease dynamics / time course data
- Standardized challenge model for diagnostic and vaccine evaluation studies



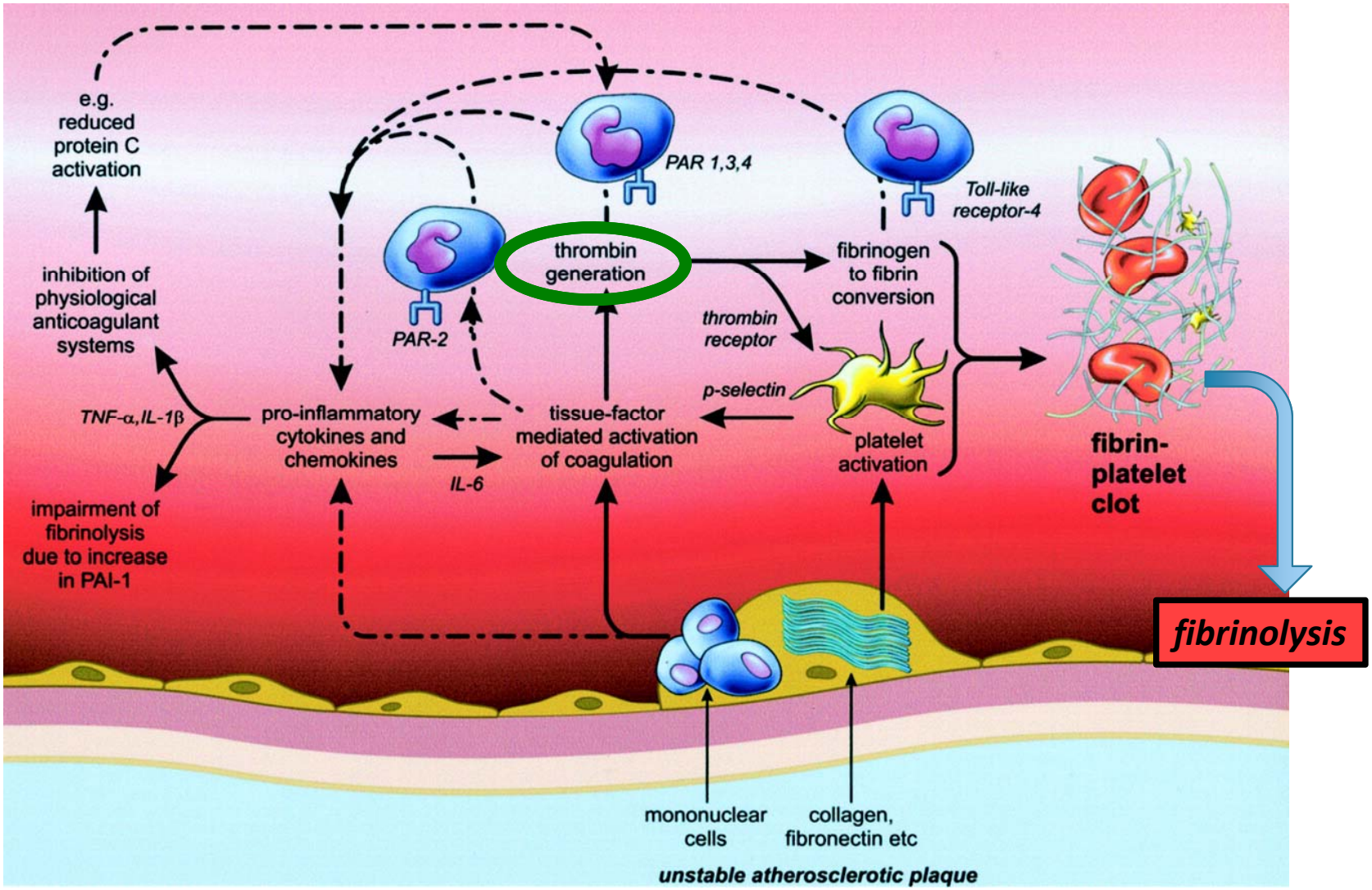
Coagulation

Procoagulant
sol TF
TAT complexes

Anticoagulant
sTM
AT
Prot C

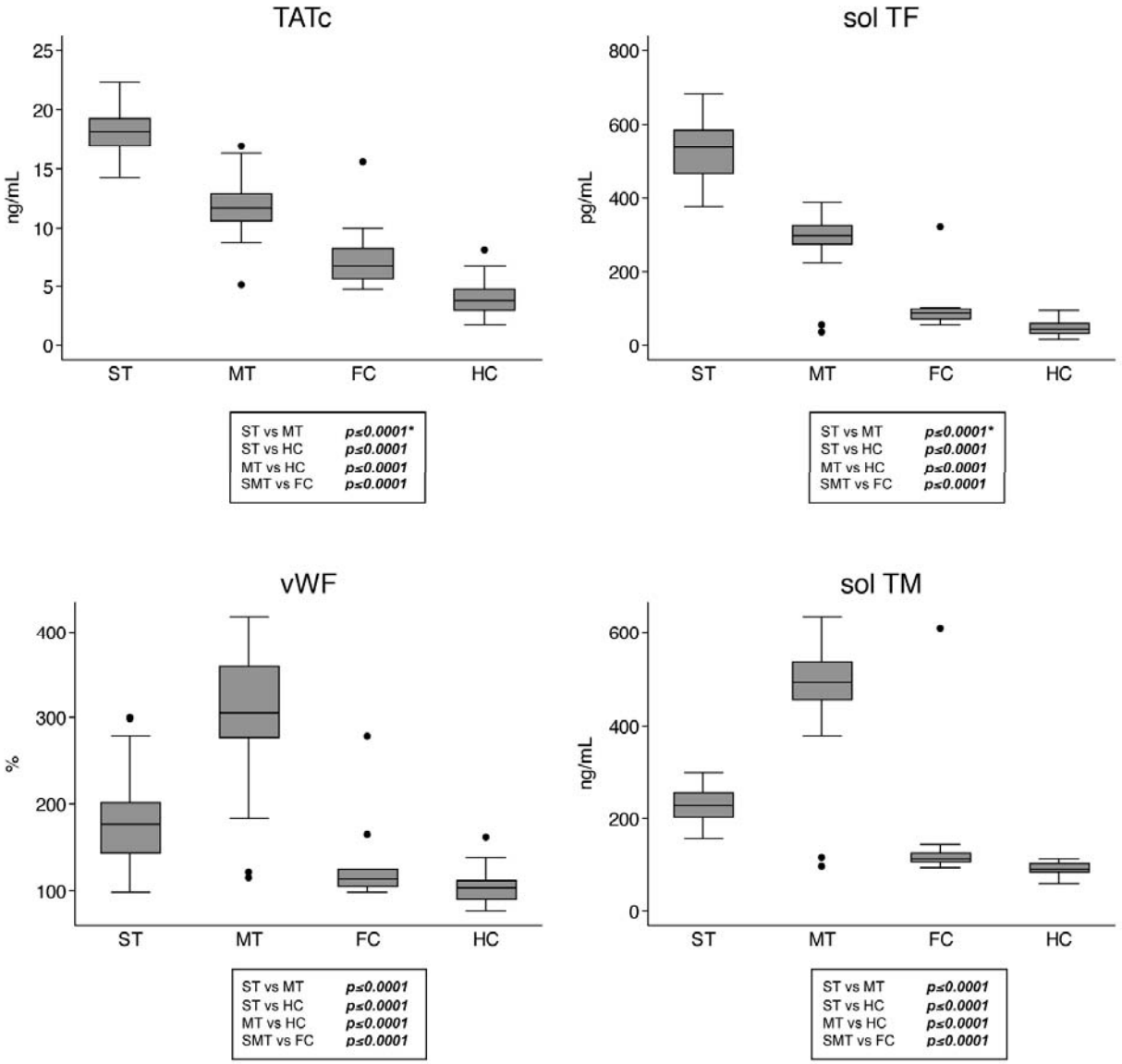
Fibrinolysis
PAI-1
PAA
tPA

Endothelial
vWF
sol TM



Levi M et al. 2004, *Circulation*

Coagulation profiles of ST and MT



Scrub Typhus

"pro-coagulant and inflammatory profile"

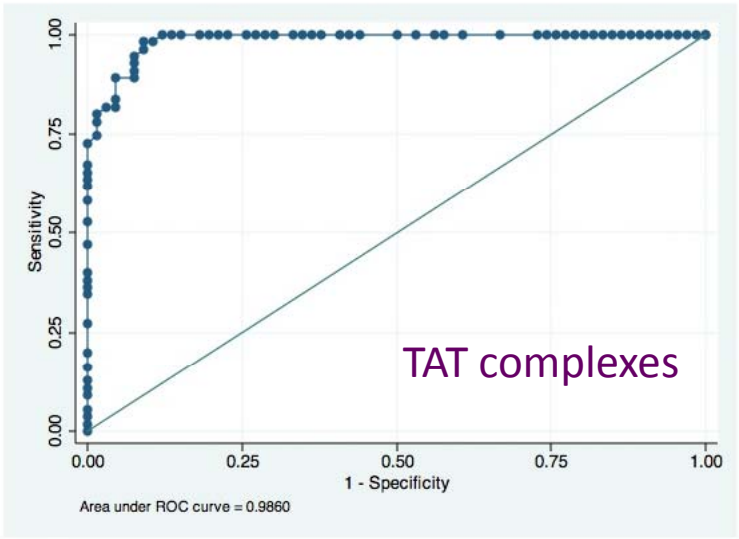
Murine Typhus

"endothelial damage profile"

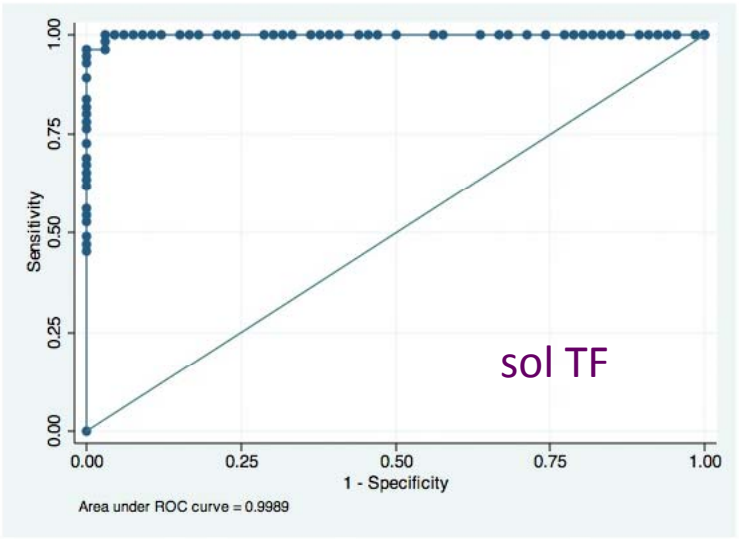
Paris DH et al., 2012

ROC curves

ST
(n=60)

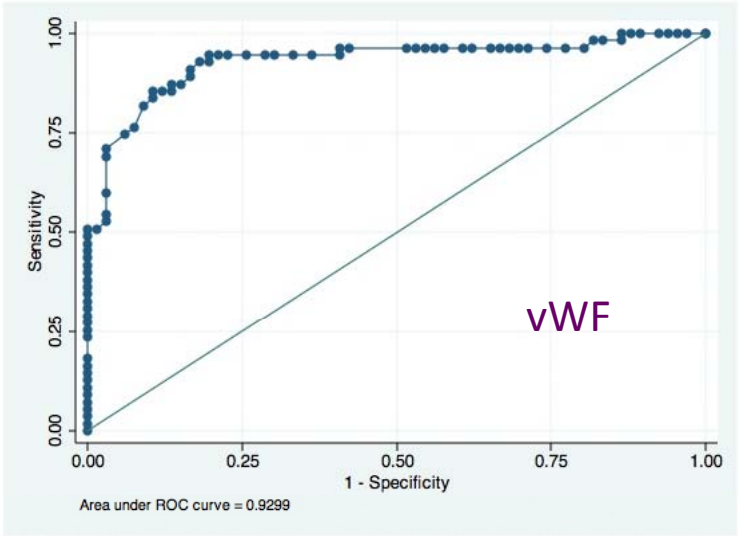


A

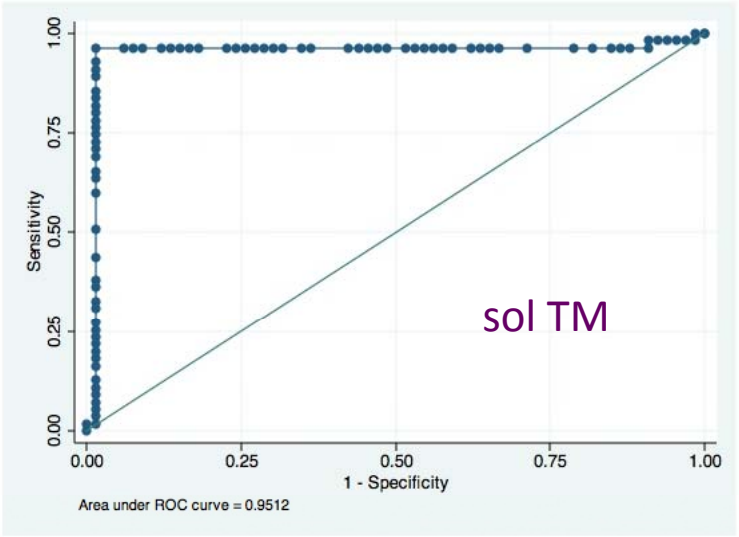


B

MT
(n=60)



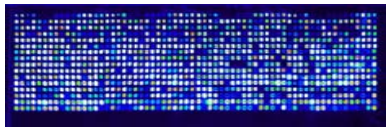
C



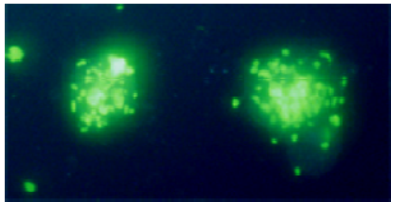
D

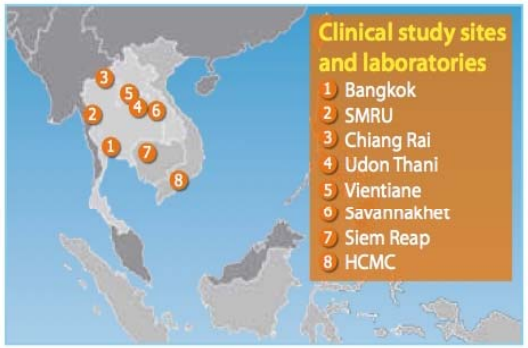
What do we want to achieve … ?!

- ***Improve Diagnostics***
  - New platform, non-subjective, broad, sensitive
  - Appropriate sample type and target selection (DNA/ag - serology combination?)
  - Progress into ag-detection based assays
  - Recombinant proteins - immune response assays *(ELISAs, ELISpot assays, FACS)*



- ***Host pathogen interactions***
  - Characterize immune response / antigens / strains
  - Immune mechanisms – why is natural immunity not protective?
  - APCs - Immune evasion / modulation mechanisms
  - Cell biology - pathogen markers, virulence factors etc.
  - Genome sequencing of strains
  - Clinical network – prospective validation





Thank you !

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