

Typhoid and invasive salmonellosis

9th Congress



**Professor Gordon Dougan
Wellcome Trust Sanger Institute
Cambridge University**



Origin of the typhoid and salmonellosis meetings



Carlos Hormaeche

Tikki Pang

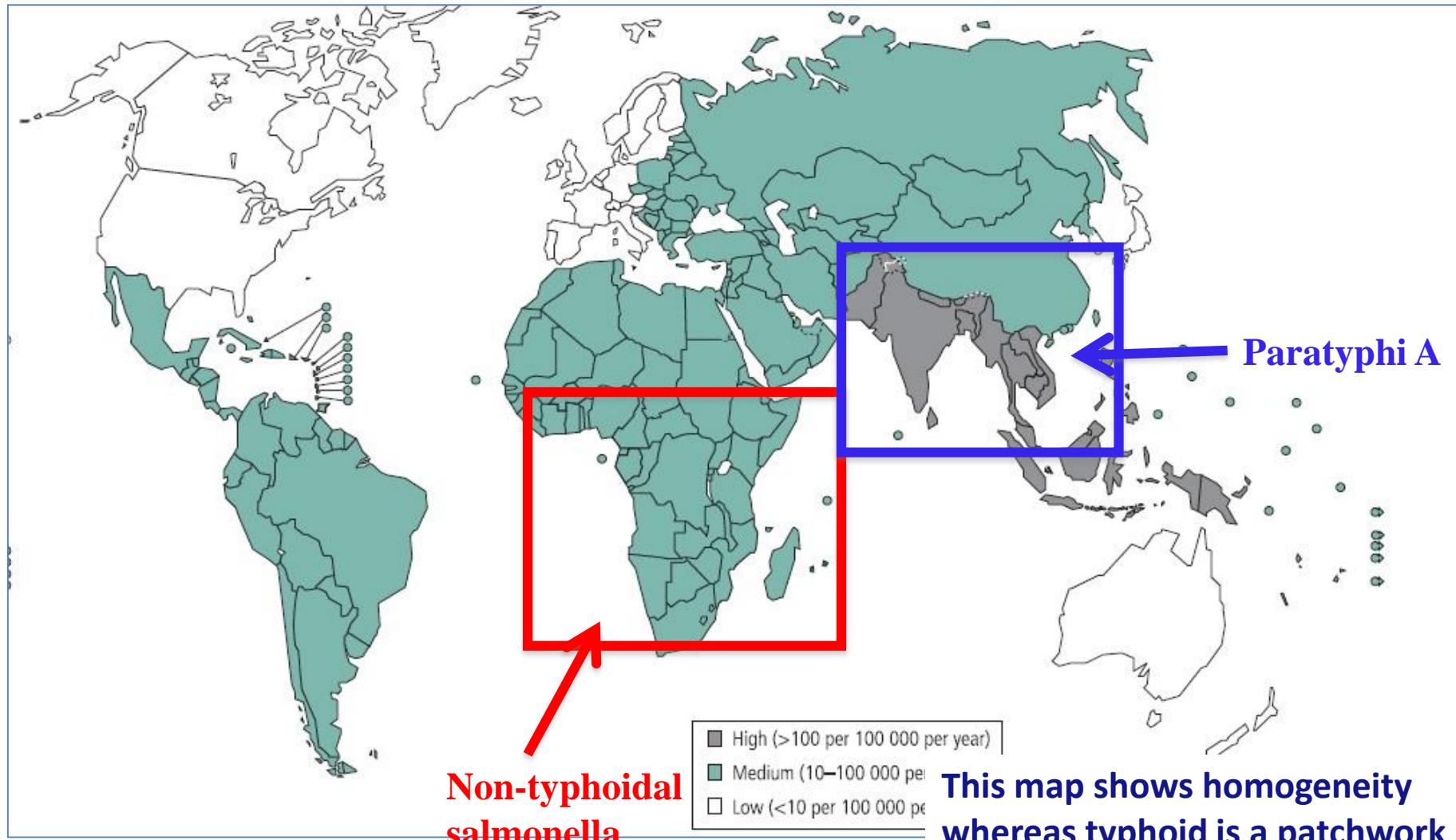
Bernard Ivanoff

Split after first meeting into two, one became ASM Salmonella meetings

26.9 million cases

Typhoid fever by region

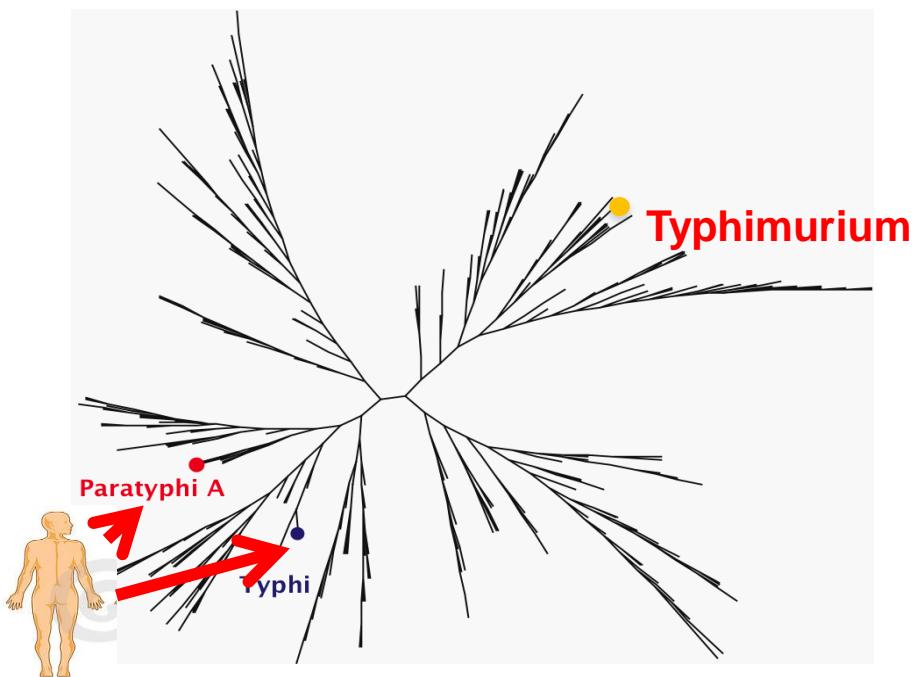
Mortality 1% or
~250,000 per annum



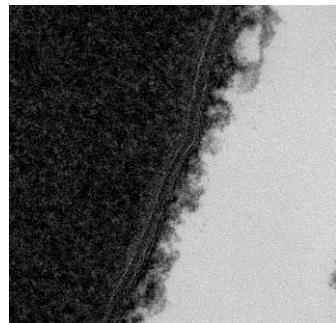
Source: Crump et al, Bulletin WHO, 2004 and Buckle et al 2012

Salmonella typhi, the bacteria

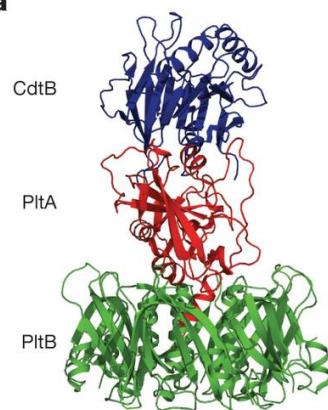
- A monophyletic serovar of *S. enterica* that causes typhoid
 - Only emerged once several thousand years ago!
- Produces Vi capsule and novel toxin
- Human restricted,
 - Non-zoonotic!
- Carrier state



Paratyphi A a distinct monophyletic serovar



a



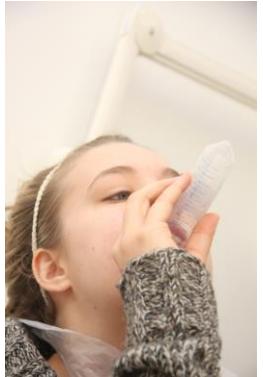
An Outpatient, Ambulant-Design, Controlled Human Infection Model Using Escalating Doses of *Salmonella* Typhi Challenge Delivered in Sodium Bicarbonate Solution

wellcome trust

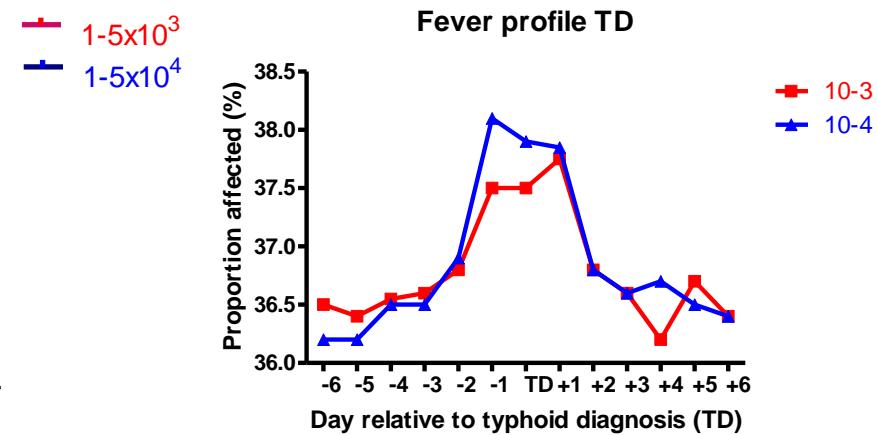
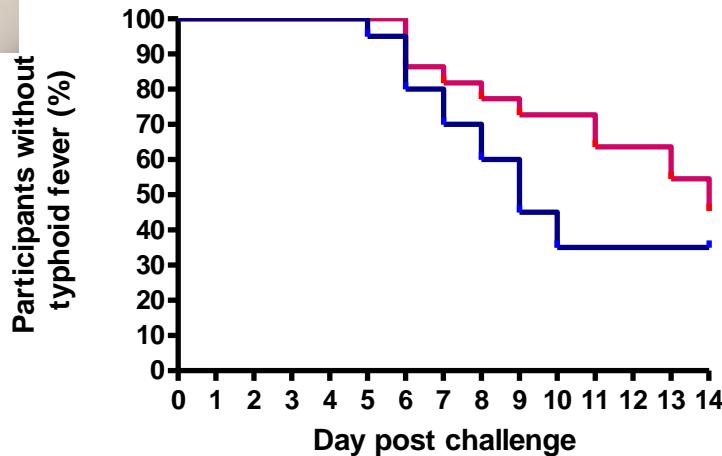
Claire S. Waddington,^{1,2,a,b} Thomas C. Darton,^{1,2,a} Claire Jones,^{1,2} Kathryn Haworth,^{1,2} Anna Peters,^{1,2} Tessa John,^{1,2} Ben A. V. Thompson,^{1,c} Simon A. Kerridge,¹ Robert A. Kingsley,³ Liqing Zhou,^{1,2} Kathryn E. Holt,⁴ Ly-Mee Yu,^{5,c} Stephen Lockhart,^{6,d} Jeremy J. Farrar,^{7,8} Marcelo B. Sztein,⁹ Gordon Dougan,³ Brian Angus,⁸ Myron M. Levine,⁹ and Andrew J. Pollard^{1,2}

- First controlled challenge model for typhoid for ~50 years
- Initially funded by The Wellcome Trust
- Quailes strain of *S. Typhi* recovered from original studies and made at GMP
- Investigating the pathogenesis, immunology, vaccinology of Typhi and Paratyphi A

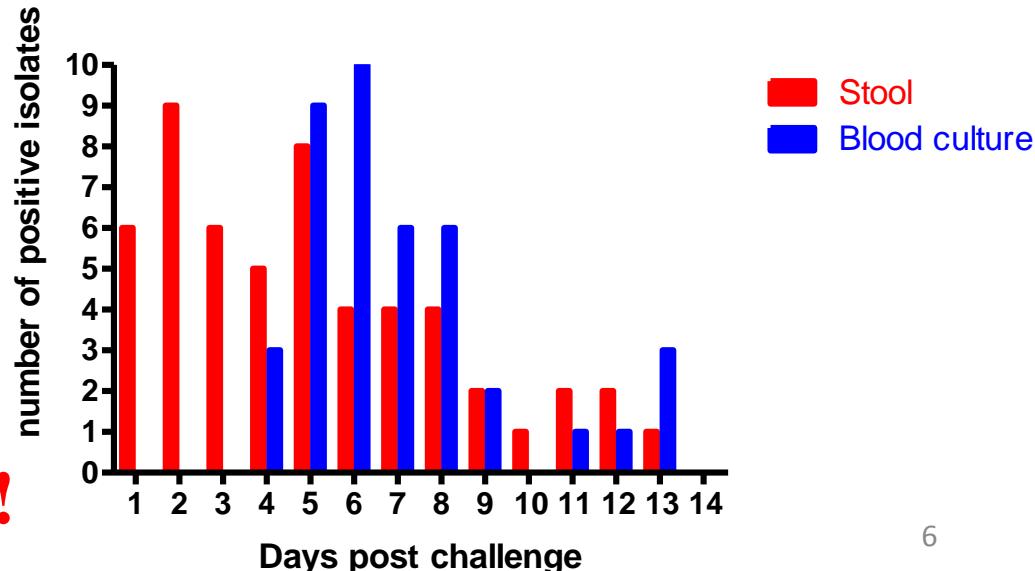




A human challenge model for typhoid/paratyphoid

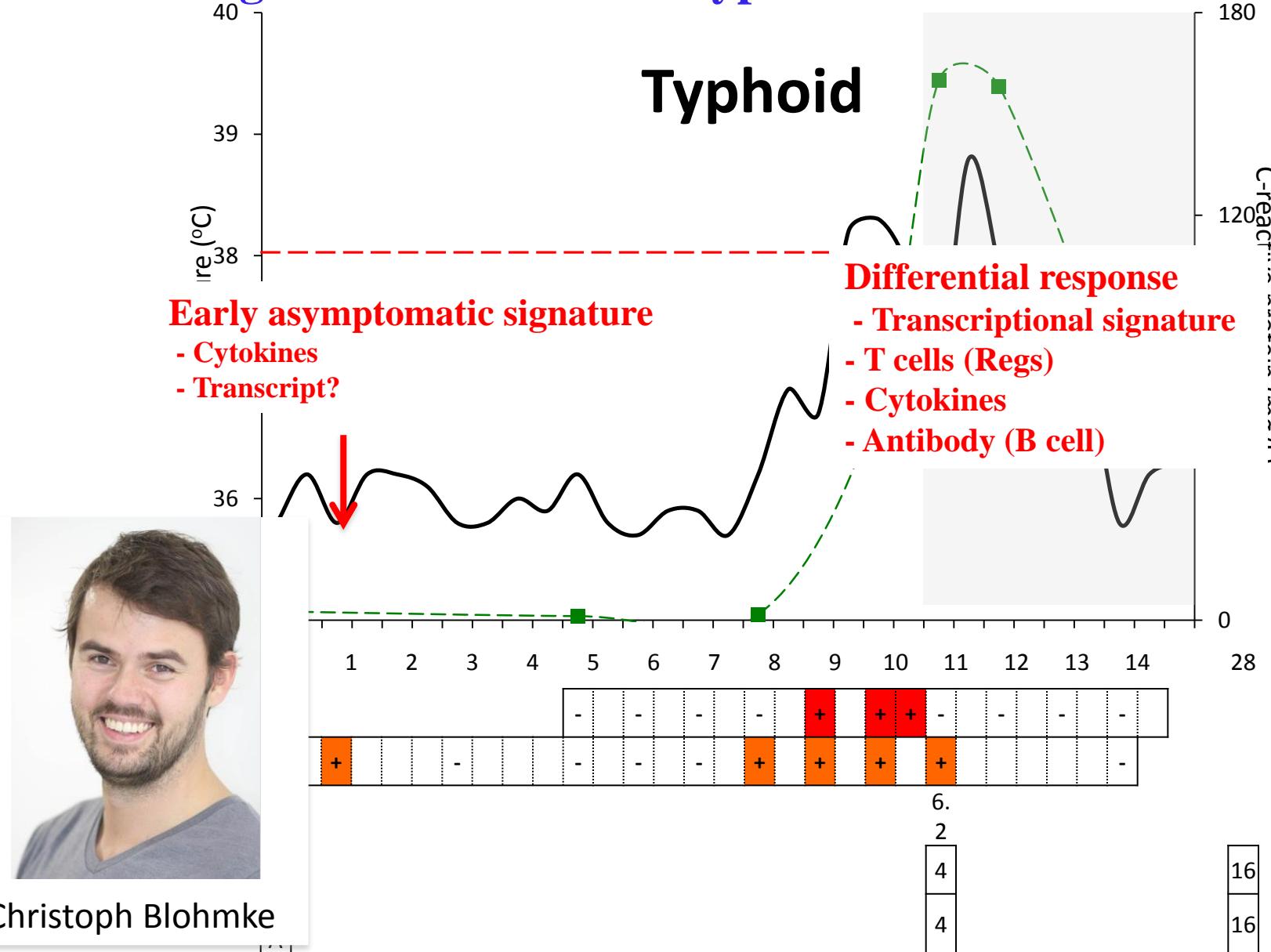


- **1-5x10³ Attack rate
55%**
- **1-5x10⁴ Attack rate
65%**

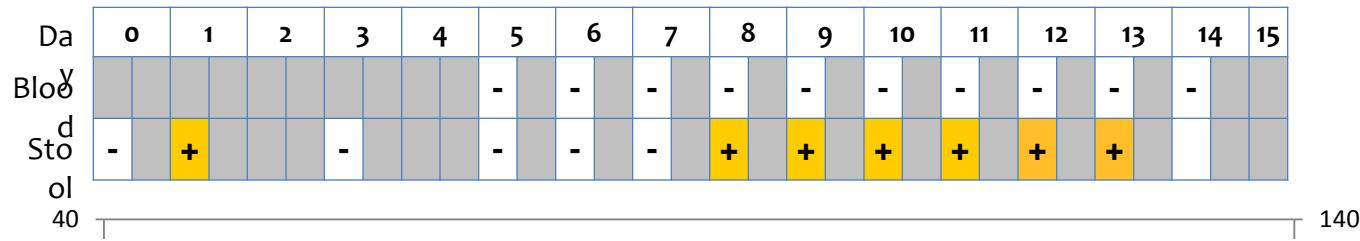


Still looking for volunteers!

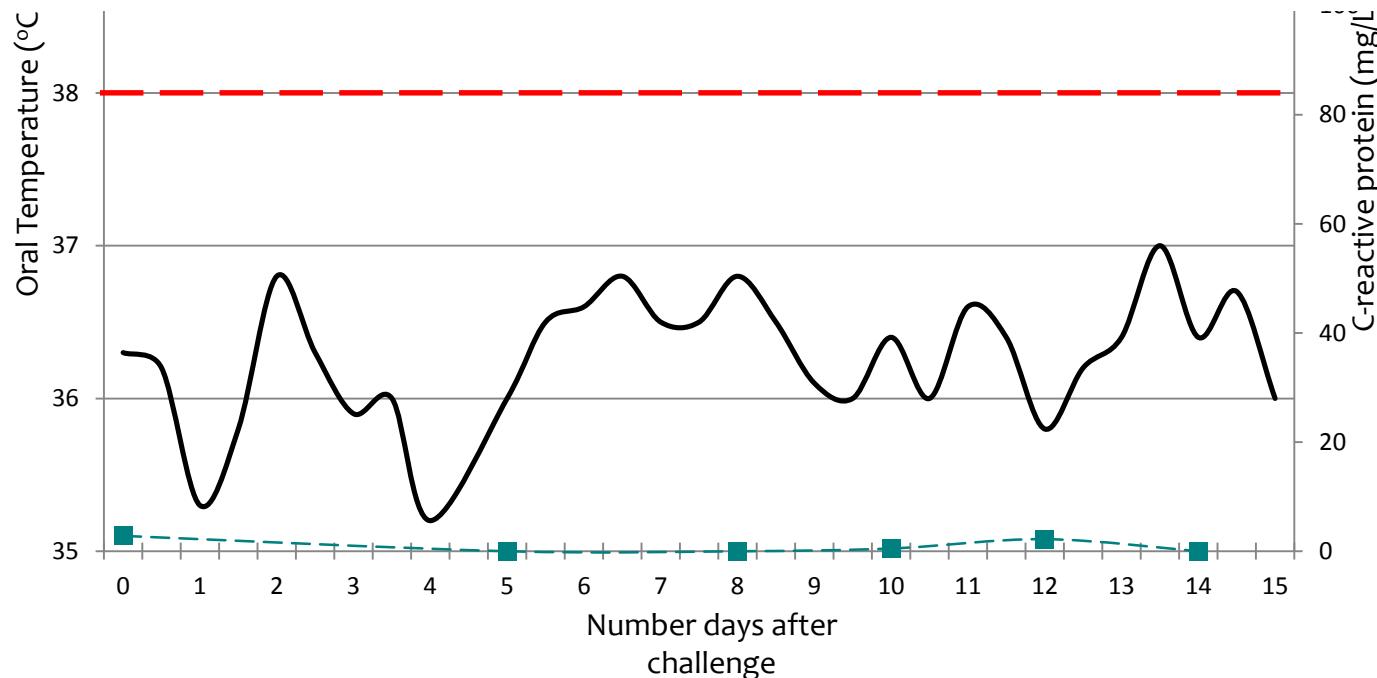
The emergence of typhoid symptoms in an individual challenged with *Salmonella Typhi*



More persistent shedding of S. Typhi without symptoms or obvious blood carriage



- How common is this in the field, is this the real typhoid?
- Typhoid is a stealth infection not always driving disease

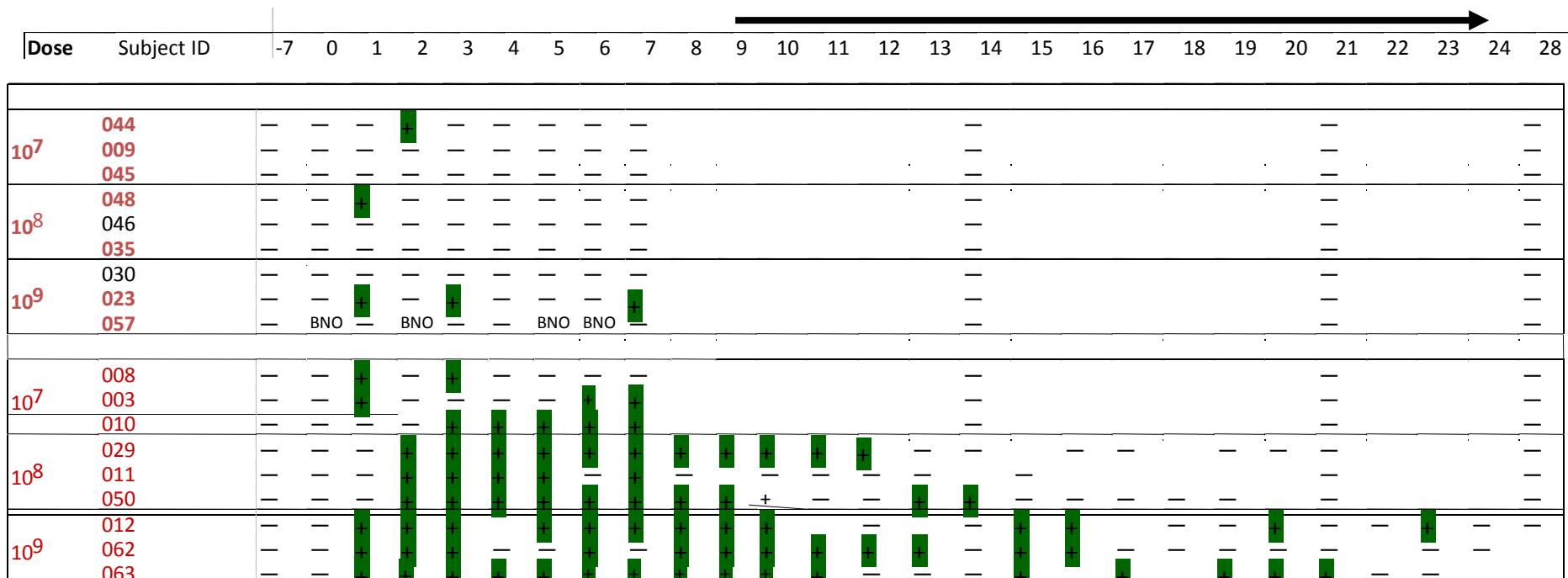


Shedding of S. Typhi Ty2 ssaV (SPI-2) aroC and S. Typhimurium TML ssaV aroC in the stools of volunteers

David Lewis

S. Typhi Ty2 ssaV aroC

Days after oral challenge



S. Typhimurium TML ssaV aroC

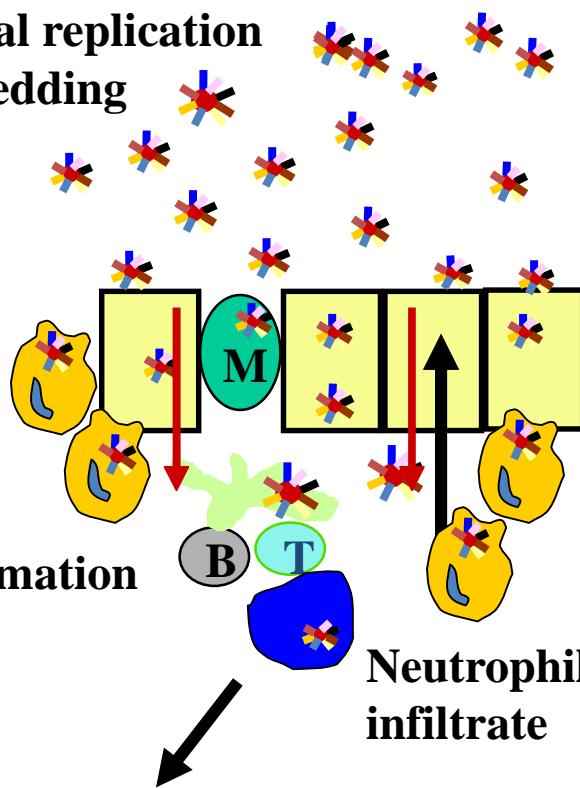
- No Shedding + Shedding

**ACUTE PHASE PROTEINS UP ONLY IN
TYPHIMURIUM VOLUNTEERS**

Theoretical differences in the pathogenicity of zoonotic non-typhoidal salmonellosis and typhoidal disease

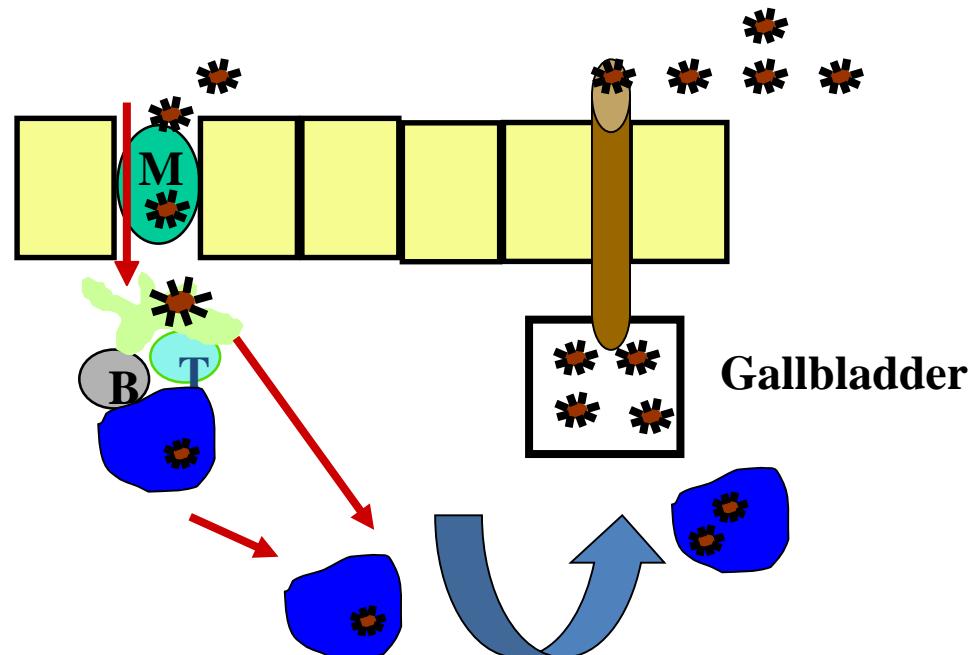
Non-typhoidal Salmonella

Luminal replication
and shedding



Salmonella Typhi

Shedding from
gallbladder



Limited dissemination

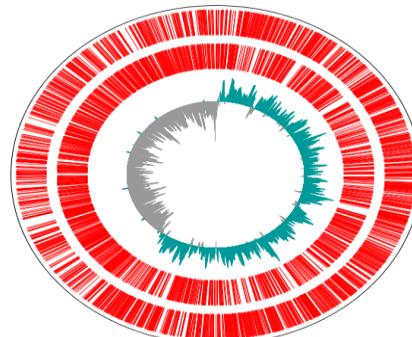
Proposal at the last invasive Salmonellosis meeting in Dhaka, Bangladesh, 2013

- We form an international consortium to map and genotype *S. Typhi*/Paratyphi A across the world
- We create a central web site based on free software to coordinate this
- We design simple SNP-based assays for field testing
- We use this to advocate typhoid control

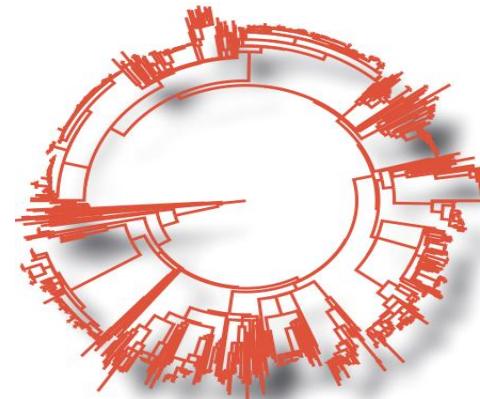
Isolate organisms



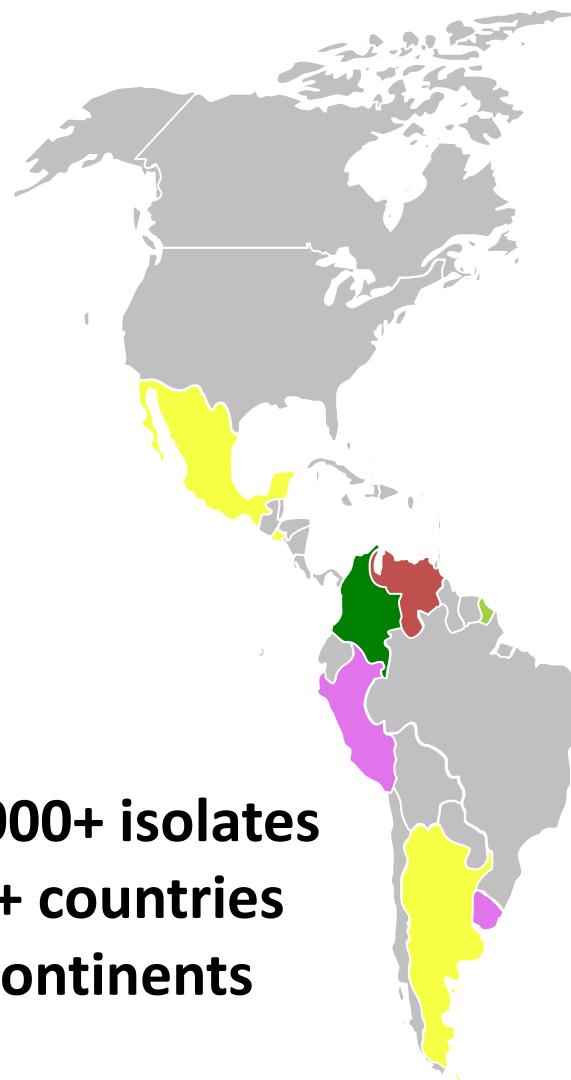
Sequence genomes



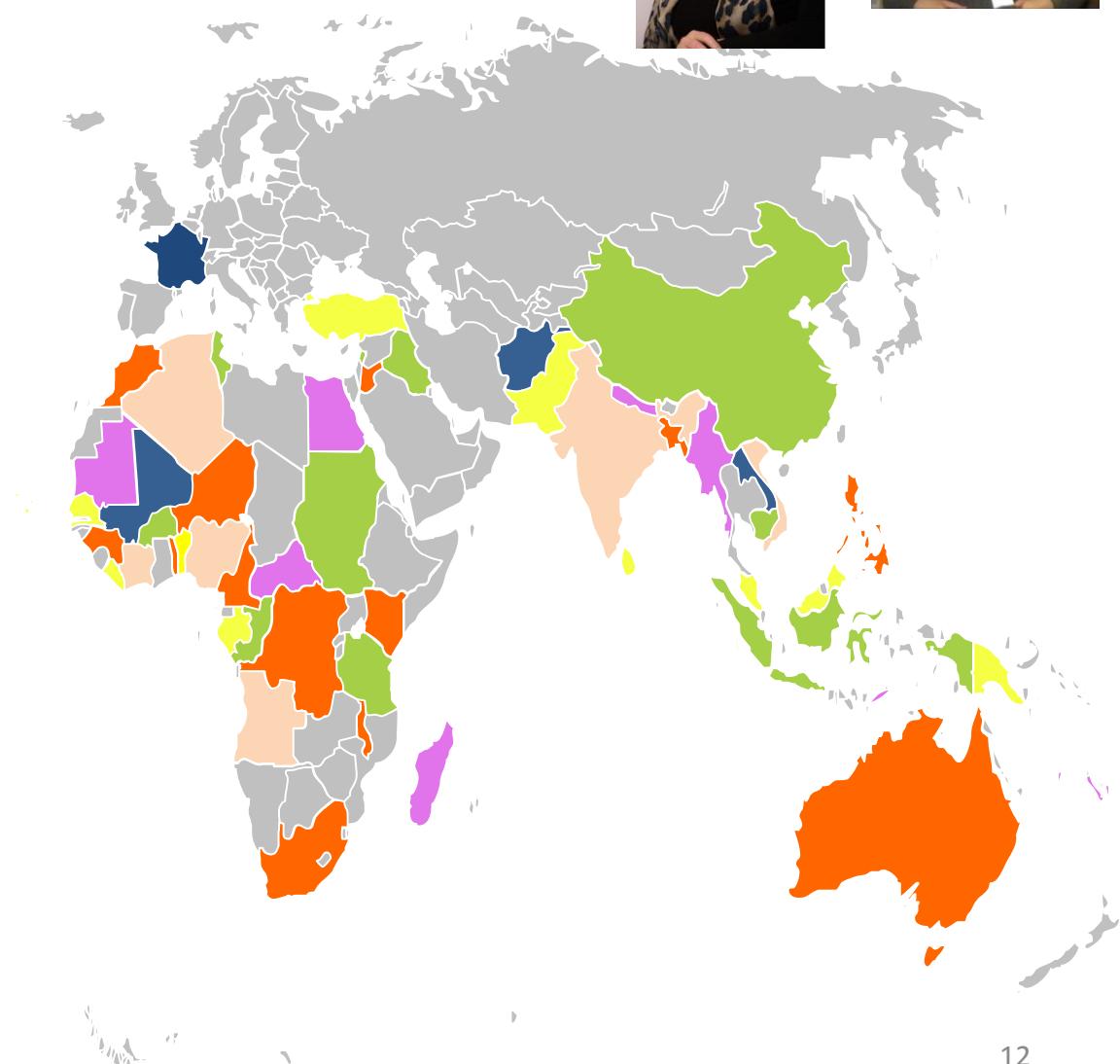
Phylogenetic analysis



Organization of a global collection of *S. Typhi* for genome sequencing



**2,000+ isolates
63+ countries
6 continents**

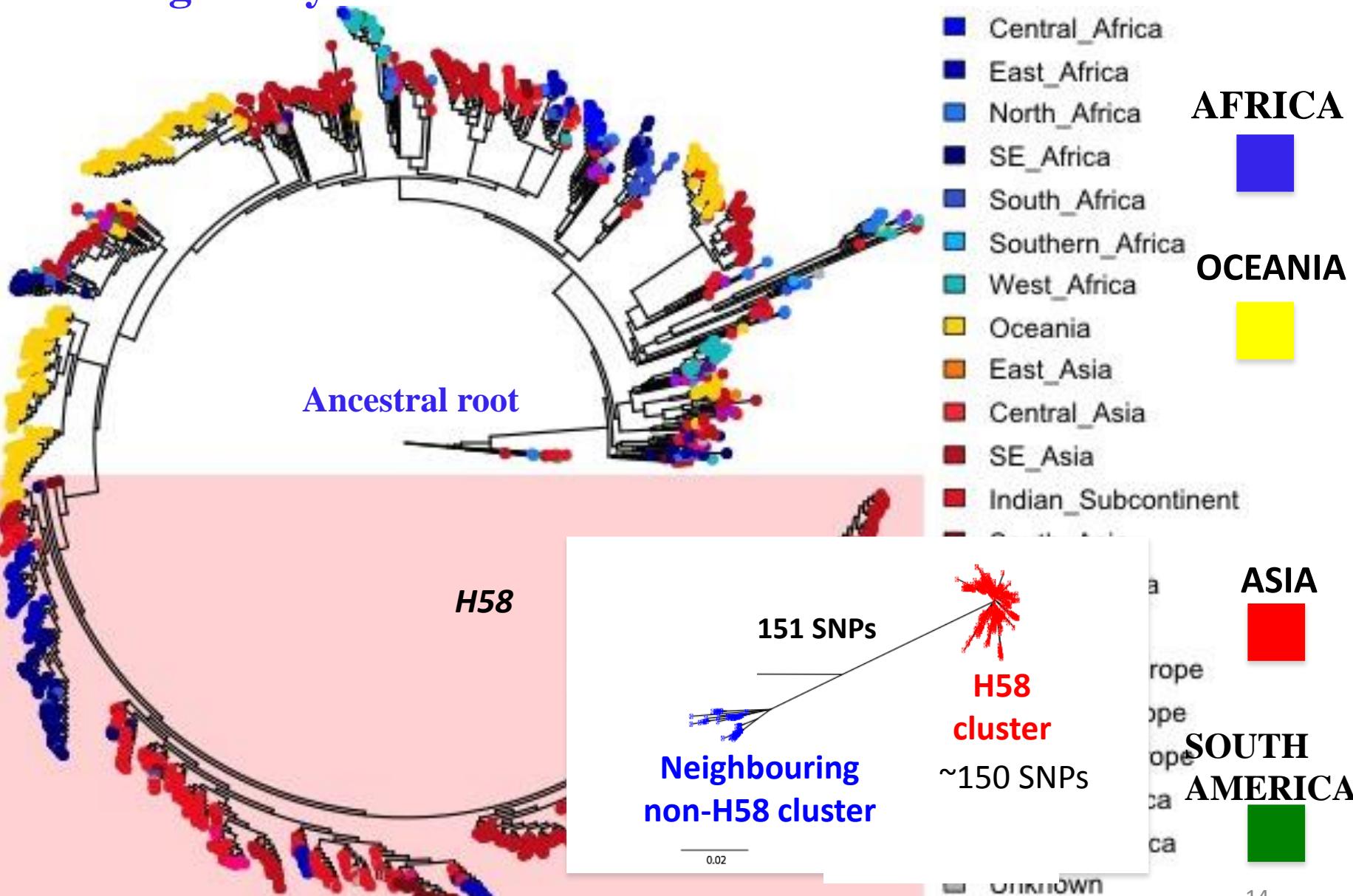


Phylogeographic analysis of the dominant multidrug resistant H58 clade of *Salmonella* Typhi identifies unappreciated inter- and intra-continental transmission events

Vanessa K. Wong, Stephen Baker, Derek Pickard, Julian Parkhill, Andrew J. Page, Nicholas Feasey, Robert A. Kingsley, Nicholas R. Thomson, Jacqueline A. Keane, François-Xavier Weill, David J. Edwards, Jane Hawkey, Simon R. Harris, Alison E. Mather, Amy K. Cain, James Hadfield, Peter J. Hart, Nga Tran Vu Thieu, Elizabeth J. Klemm, Robert F. Breiman, Conall H. Watson, Samuel Kariuki, Melita Gordon, Robert S. Heyderman, Chinyere Okoro, Jan Jacobs, Octavie Lunguya, W. John Edmunds, Chisomo Msefula, Jose Alejandro Chabalgoity, Mike Kama, Kylie Jenkins, Shanta Dutta, Florian Marks, Josefina Campos, Corinne Thompson³, Stephen Obaro, Calman A. MacLennan, Christiane Dolecek, Karen H. Keddy, Anthony M. Smith, Christopher M. Parry, Abhilasha Karkey, E. Kim Mulholland, James I. Campbell, Sabina Dongol, Buddha Basnyat, Muriel Dufour, Don Bandaranayake, Take N. Toleafoa, Shalini Pravin Singh, Mohammad Hatta, Paul Newton, Robert S. Onsare, Lupeoletalalei Isaia, David Dance, Viengmon Davong, Guy Thwaites, Lalith Wijedoru, John A. Crump, Elizabeth De Pinna, Satheesh Nair, Eric J. Nilles, Duy Pham Thanh, Paul Turner, Sona Soeng, Mary Valcanis, Joan Powling, Karolina Dimovski, Geoff Hogg, Jeremy Farrar, Kathryn E. Holt[†] and Gordon Dougan

Nature Genetics in press this week

S. Typhi Haplotype H58 has emerged over the past 20 years to dominate globally



H58 is associated with drug-resistant genotypes

Inner circle: Regions within continents

- South Asia
- South-east Asia
- East Asia
- Western Asia
- North Africa
- Central Africa
- East Africa
- West Africa
- Southern Africa
- South Africa
- Africa
- Oceania
- Australia
- Western Europe
- Eastern Europe
- Southern Europe
- North America
- South America
- Unknown

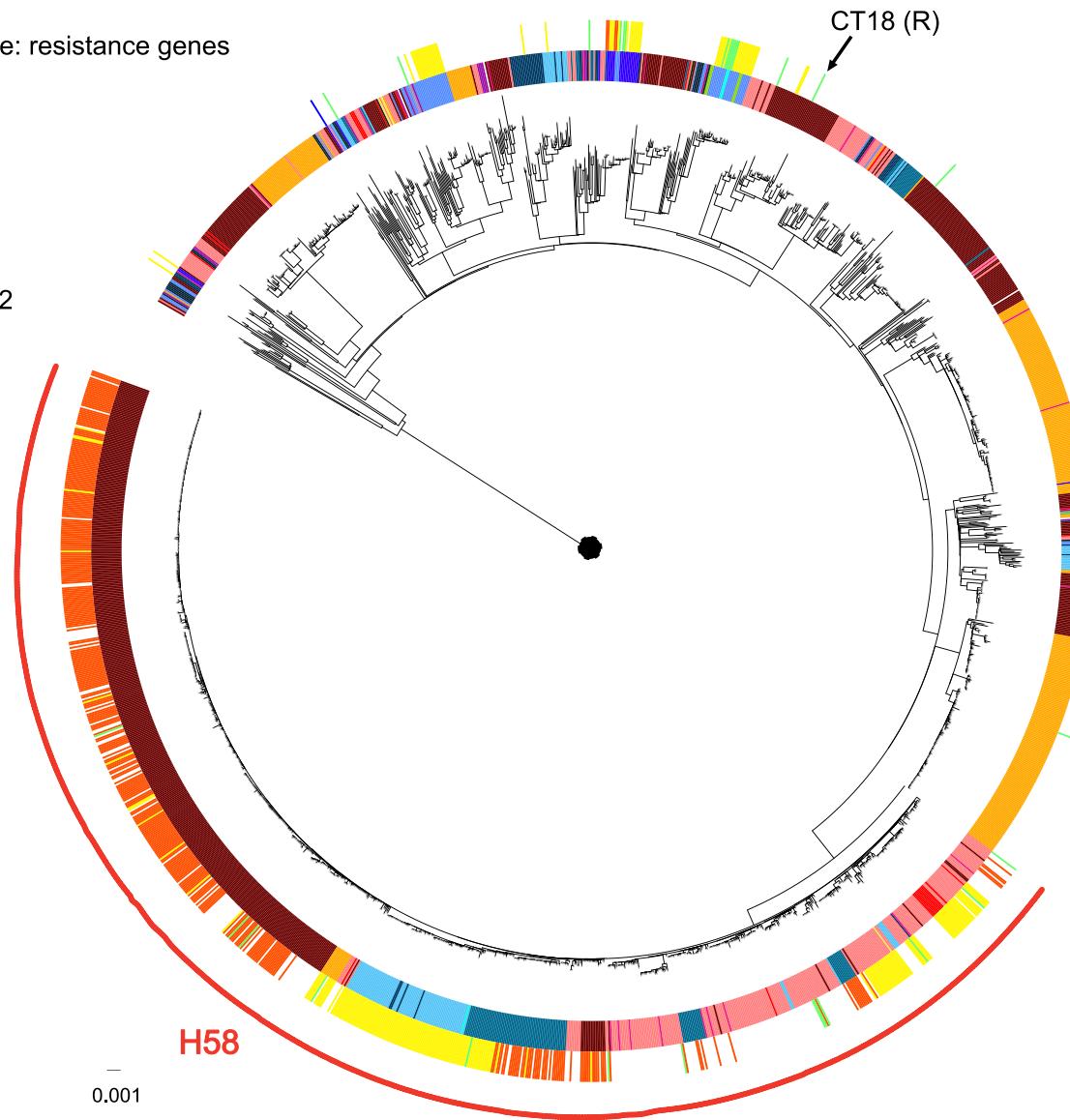
Outer circle: resistance genes

- 0
- 1-3
- 4-6
- 7-9
- 10-12

H58

0.001

CT18 (R)



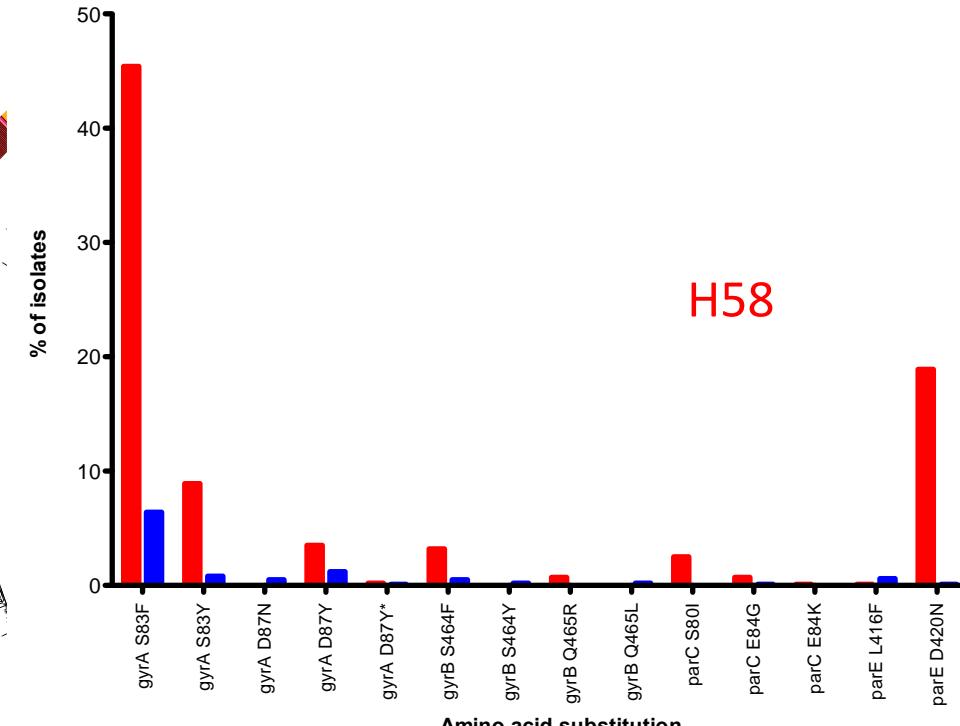
H58 is a hot spot for quinolone related mutations

Inner circle: Regions within continents

- South Asia
- South-east Asia
- East Asia
- Western Asia
- North Africa
- Central Africa
- East Africa
- West Africa
- Southern Africa
- South Africa
- Africa
- Oceania
- Australia
- Western Europe
- Eastern Europe
- Southern Europe
- North America
- South America
- Unknown

Outer circle: *gyr* and *par* mutations

- One *gyrA*
- One *gyrB*
- Two *gyrB*
- One *gyrA* & *parC*
- One *gyrA* & *parE*
- Two *gyrA* & one *parC*
- Two *gyrA* & one *parE*
- One *gyrA*, *gyrB* and *parE*



H58

0.001

H58

Like all Typhi, H58 is still undergoing genome streamlining, through transposition

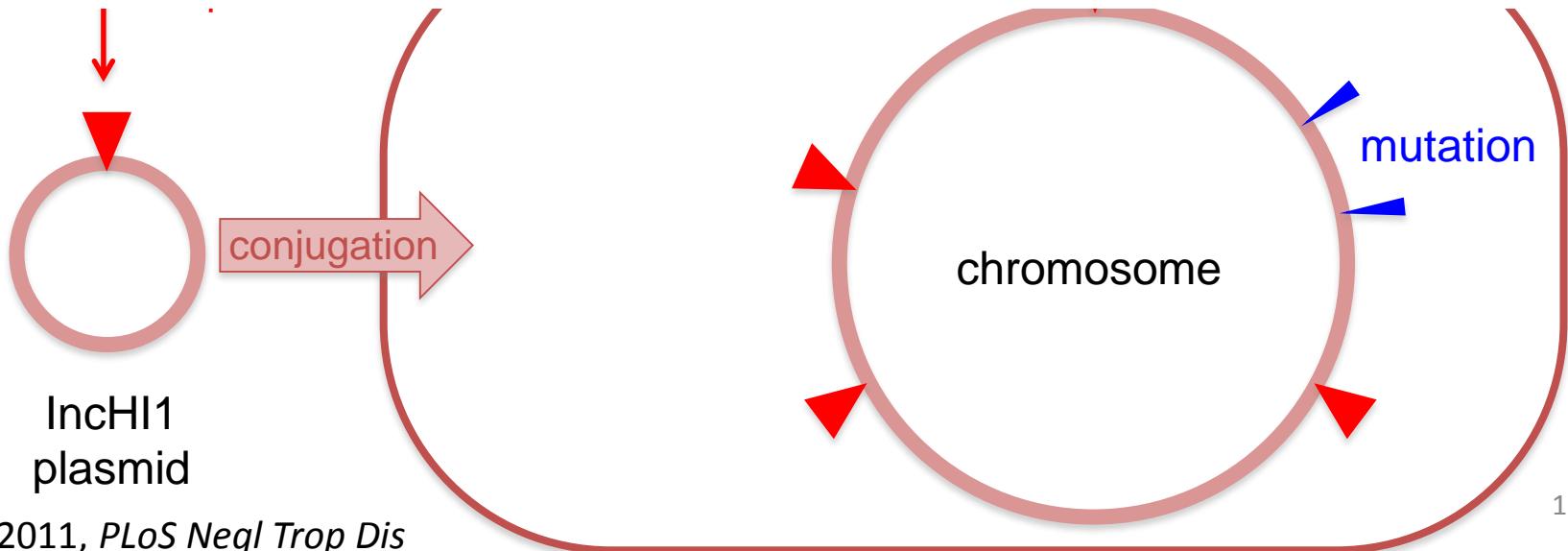
Multidrug resistance transposon

trimethoprim-sulfamethoxazole

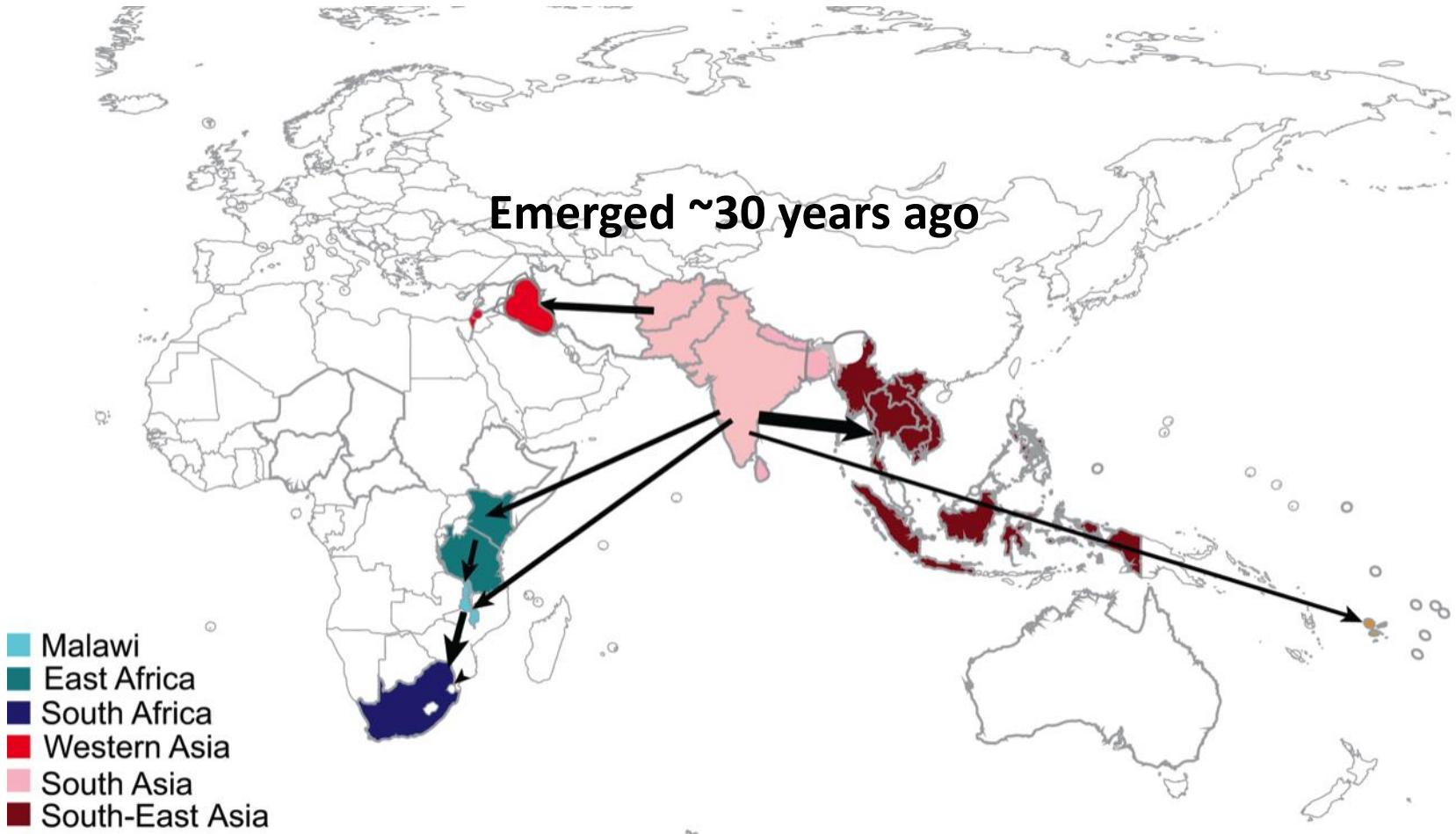


Genomic signature of multidrug-resistant *Salmonella enterica* serovar typhi isolates related to a massive outbreak in Zambia between 2010 and 2012.

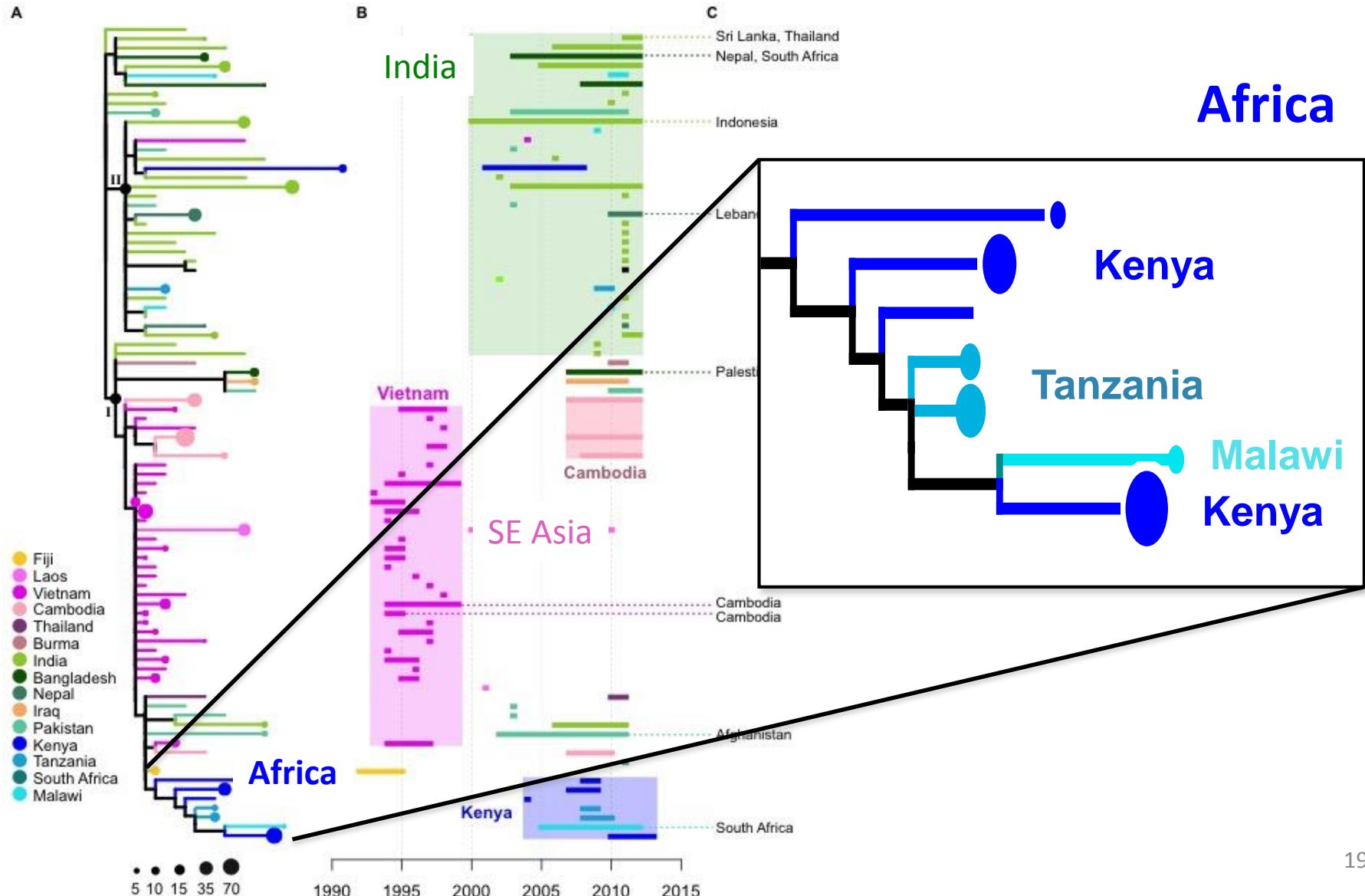
Hendriksen RS, Leekitcharoenphon P, Lukjancenko O, Lukwesa-Musyani C, Tambatamba B, Mwaba J, Kalonda A, Nakazwe R, Kwenda G, Jensen JD, Svendsen CA, Dittmann KK, Kaas RS, Cavaco LM, Aarestrup FM, Hasman H, Mwansa JC. *J Clin Microbiol*. 2015 Jan;53(1):262-72



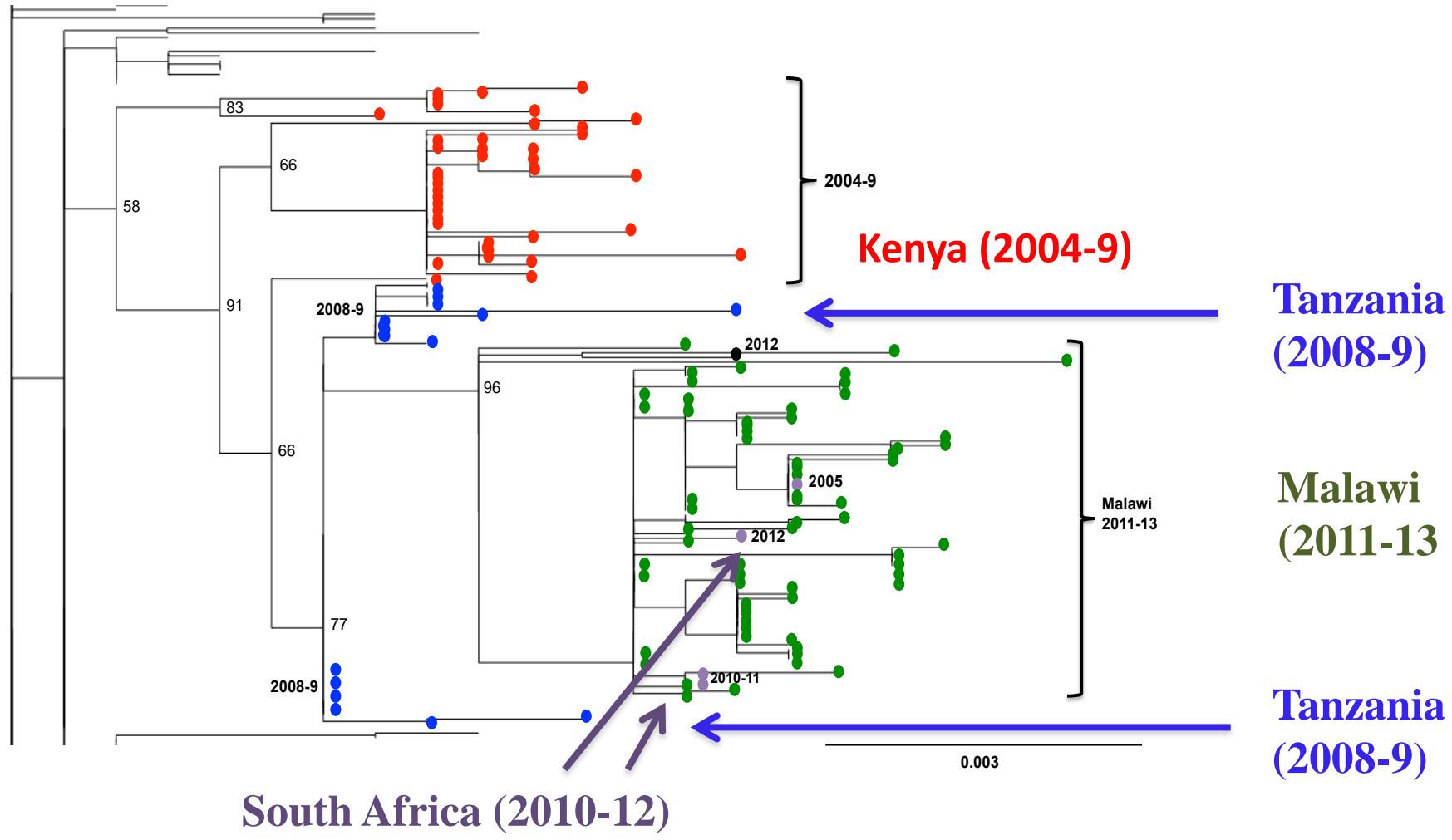
The dissemination of *S. Typhi* H58



Global dissemination of S. Typhi H58 as a timeline

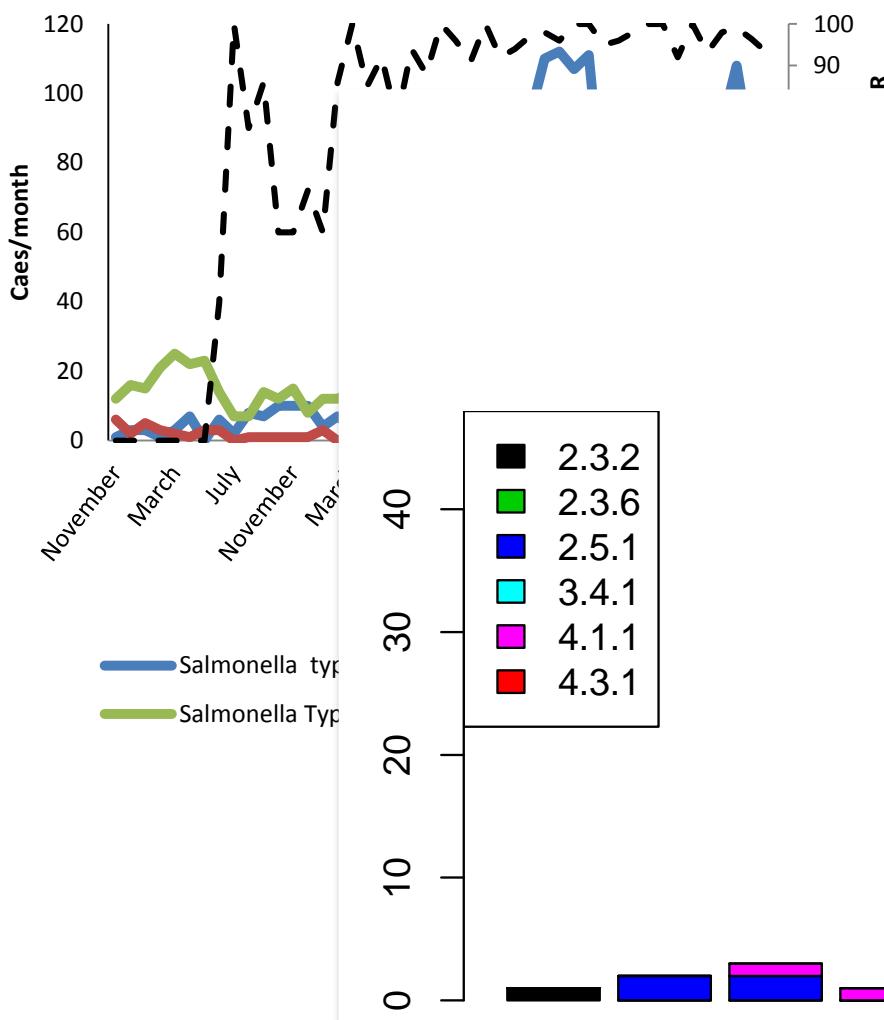


Phylogenetic relationship of African isolates within the H58 tree provides evidence of a recent expansion/epidemic



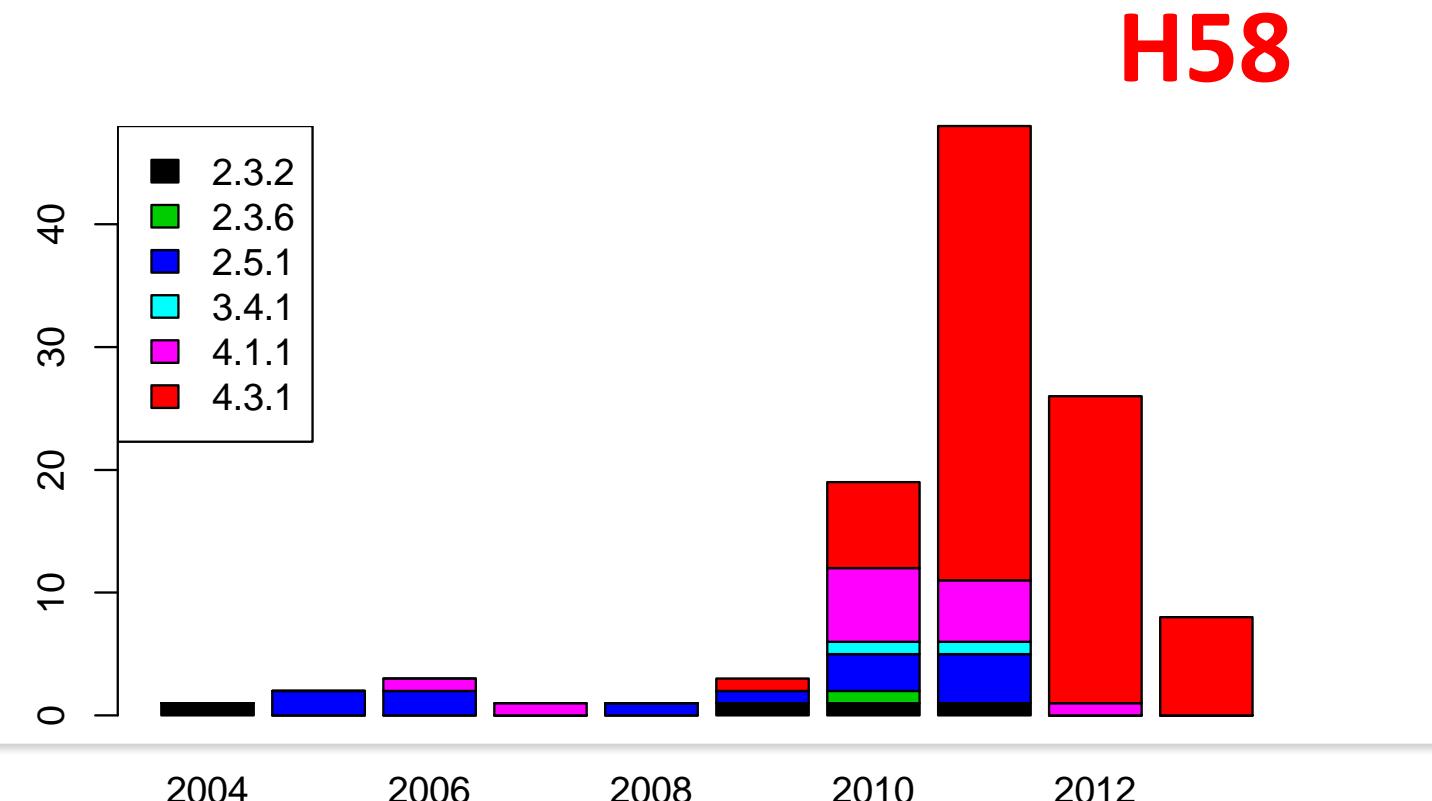
Need to fill in the gaps

Salmonella BSI at QECH, November 2010-August 2014



Genotypes in Malawi

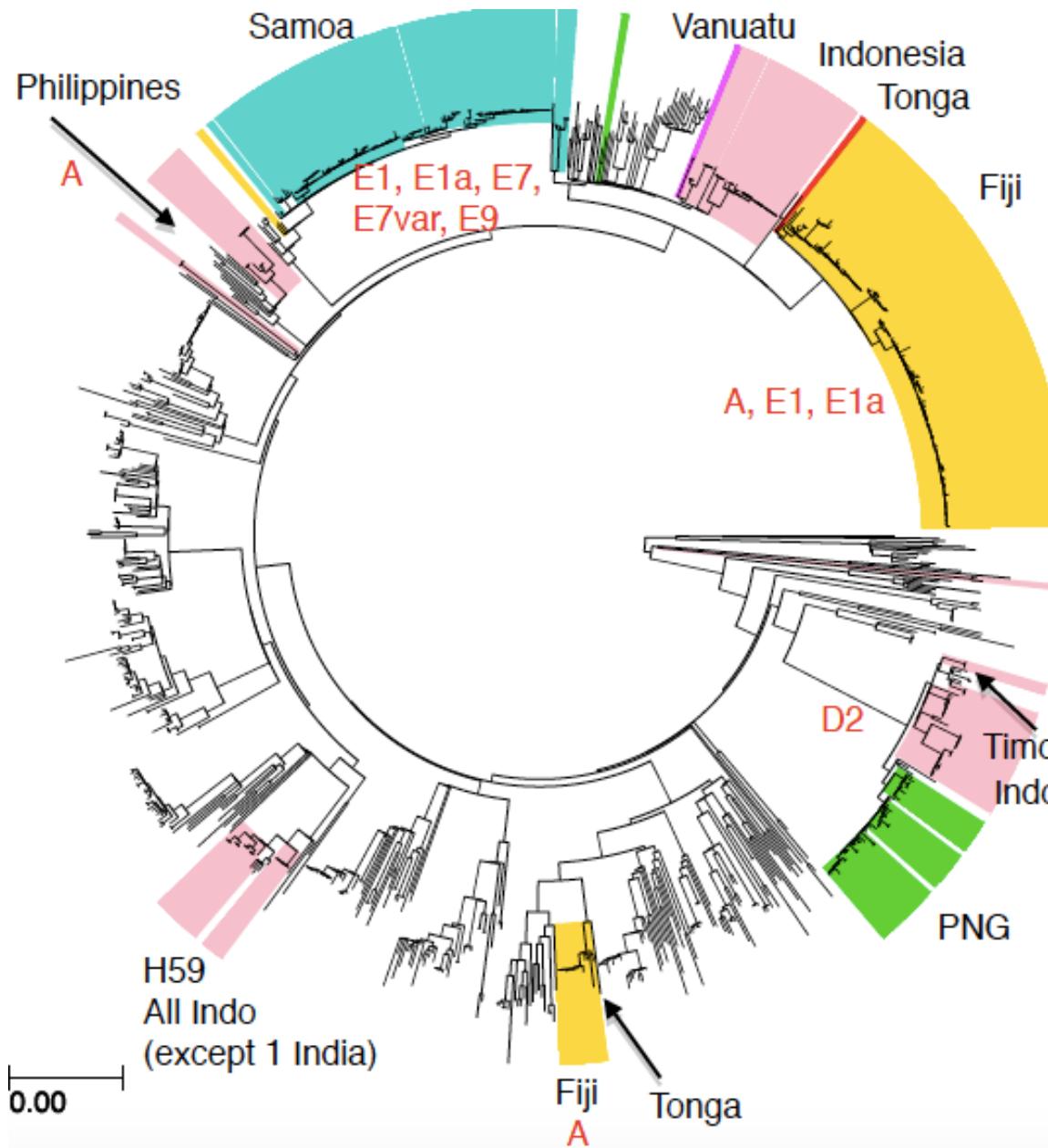
H58



Why is the H58 lineage so successful?

- Is it just antimicrobial resistance ? (what about other AMR lineages which are less successful)
- H58 has a unique SNP repertoire with some interesting mutations e.g. effectors
- How do you assess fitness in a host restricted pathogen?
- Human challenge?
- More in depth molecular clinical analysis

S. Typhi in the Pacific region are generally island-specific

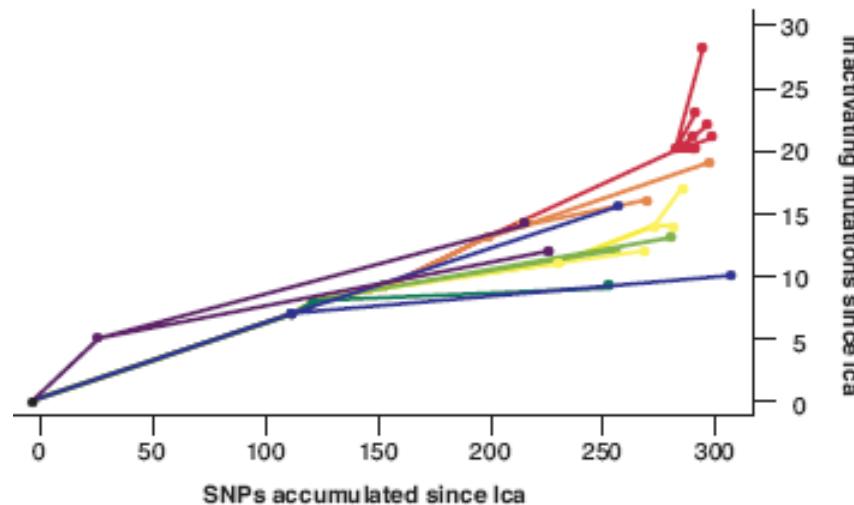


Is this an opportunity
for an eradication
programme?

H58 not dominant yet

How did typhoid evolve?

- Single point of origin followed by global dissemination
- Human restriction
- Gene acquisition (e.g. Vi and typhoid toxin)
- Genome degradation (~300 pseudogenes)
- Change of niche from gut to systemic system & carriers

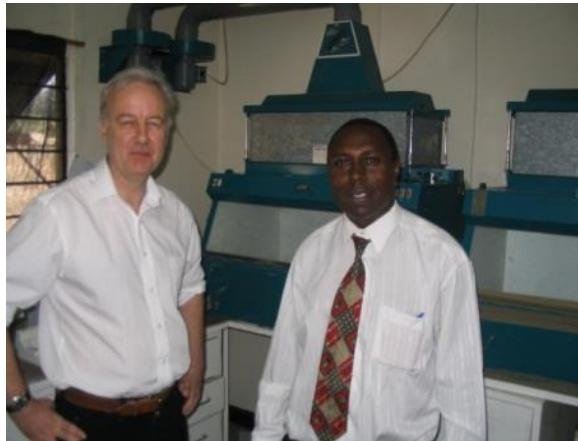
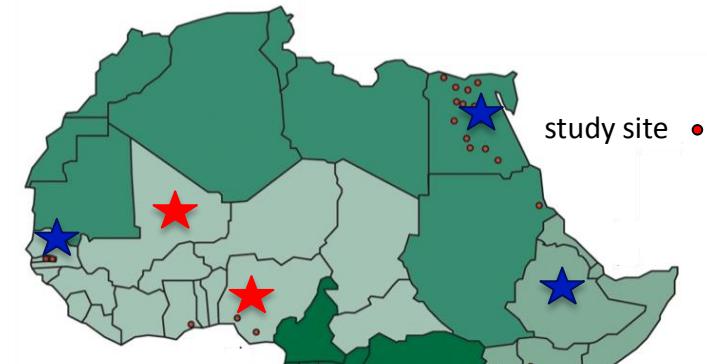


Invasive non-typhoidal Salmonellosis in Sub-Saharan Africa – does this involve human to human transmission?

Non-typhoidal Salmonella (NTS) common in blood stream infections in sub-Saharan Africa

Dominant serotypes

S. Typhimurium	50-80%
S. Enteritidis	10-40%



Sam Kariuki



Rob Kingsley



Chinyere Okoro²⁵

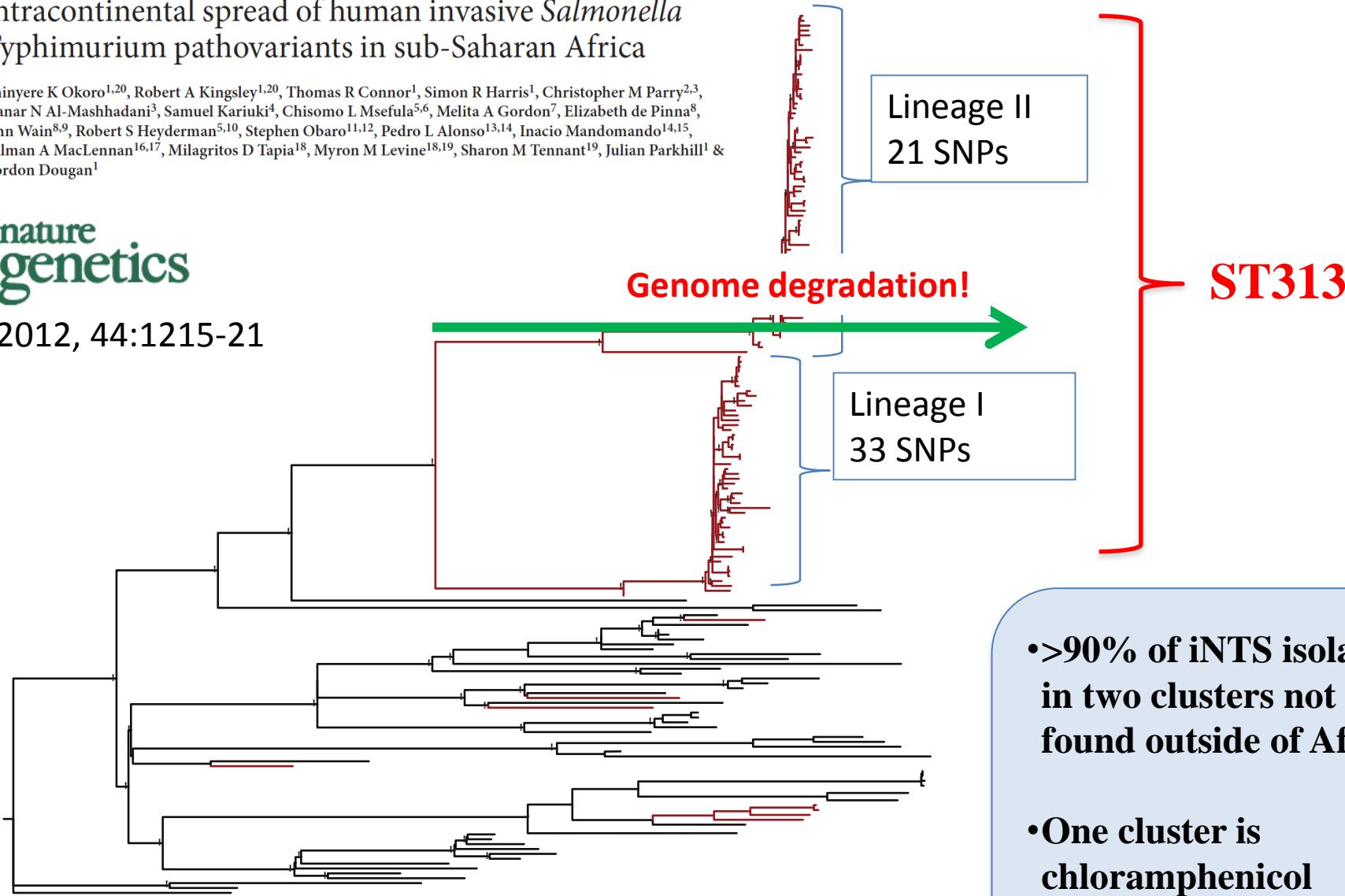
S. Typhimurium iNTS isolates are from two related clades ST313

Intracontinental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa

Chinyere K Okoro^{1,20}, Robert A Kingsley^{1,20}, Thomas R Connor¹, Simon R Harris¹, Christopher M Parry^{2,3}, Manar N Al-Mashhadani³, Samuel Kariuki⁴, Chisomo L Msefula^{5,6}, Melita A Gordon⁷, Elizabeth de Pinna⁸, John Wain^{8,9}, Robert S Heyderman^{5,10}, Stephen Obaro^{11,12}, Pedro L Alonso^{13,14}, Inacio Mandomando^{14,15}, Calman A MacLennan^{16,17}, Milagritos D Tapia¹⁸, Myron M Levine^{18,19}, Sharon M Tennant¹⁹, Julian Parkhill¹ & Gordon Dougan¹

nature
genetics

2012, 44:1215-21

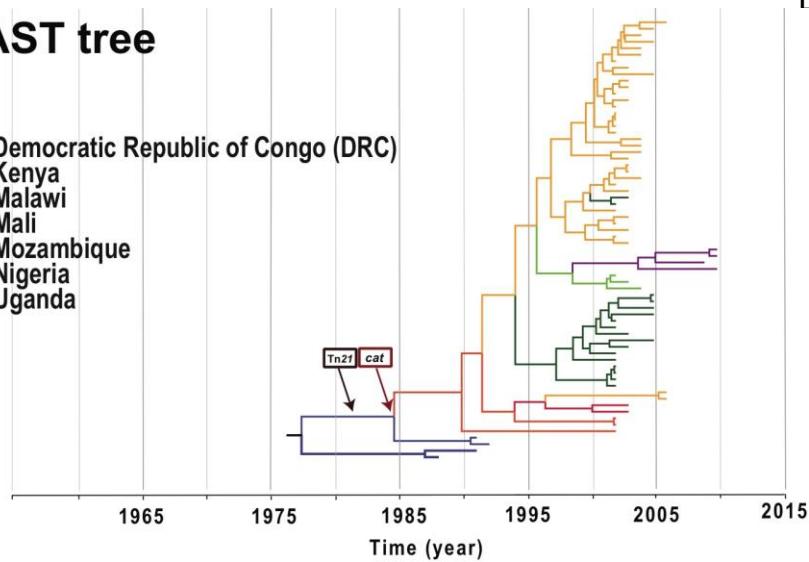


- >90% of iNTS isolates in two clusters not found outside of Africa
- One cluster is chloramphenicol resistant

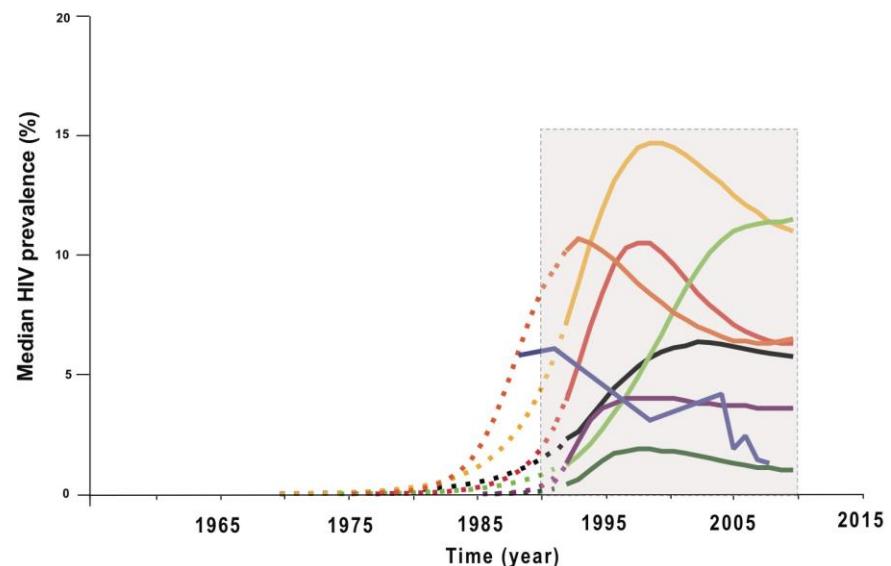
Time-dependent phylogeographic analysis using BEAST

BEAST tree

- Democratic Republic of Congo (DRC)
- Kenya
- Malawi
- Mali
- Mozambique
- Nigeria
- Uganda

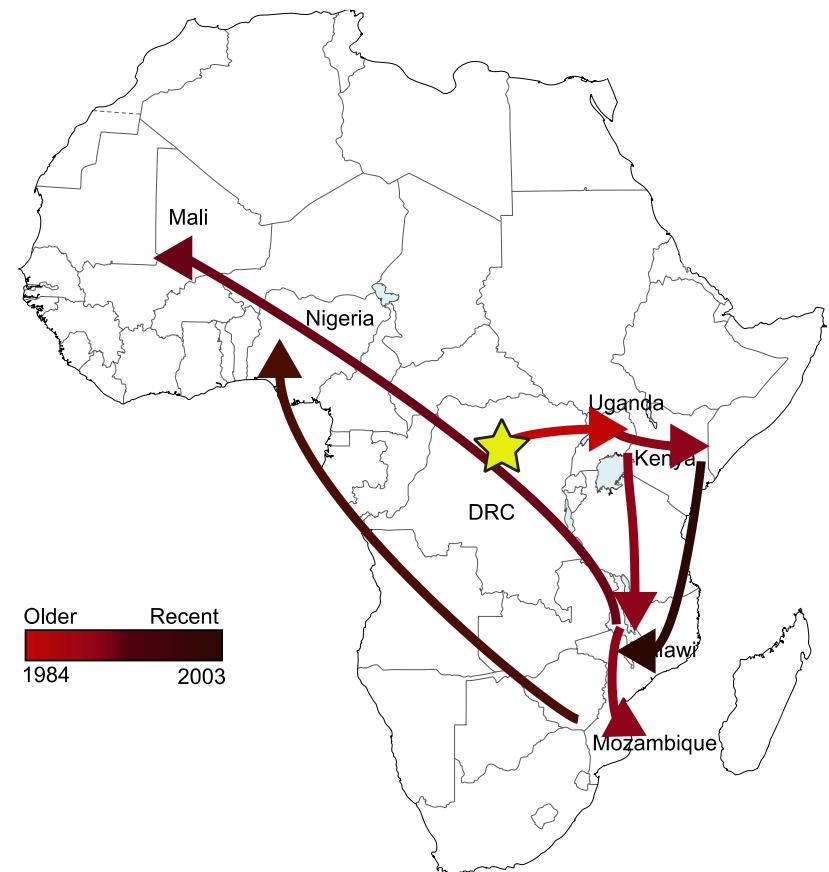


HIV Prevalence



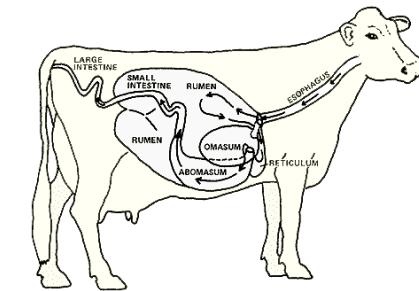
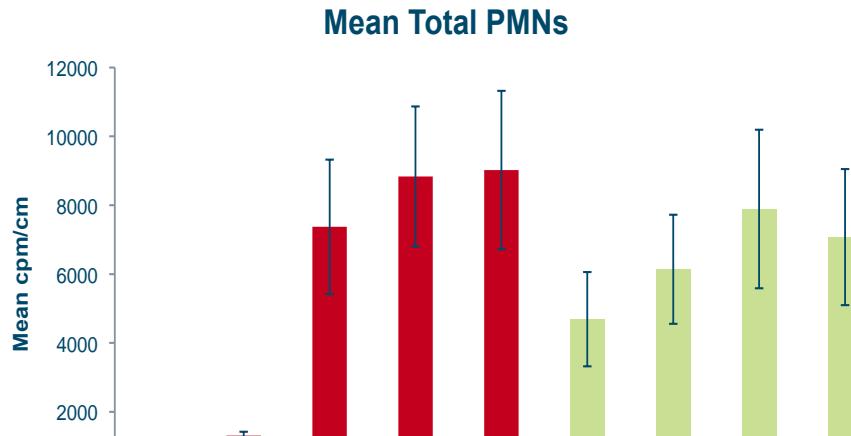
Bayesian evolutionary analysis by sampling trees

BEAST – integrates temporal, geographic and phylogenetic information



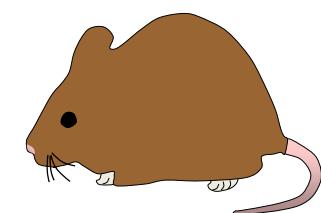
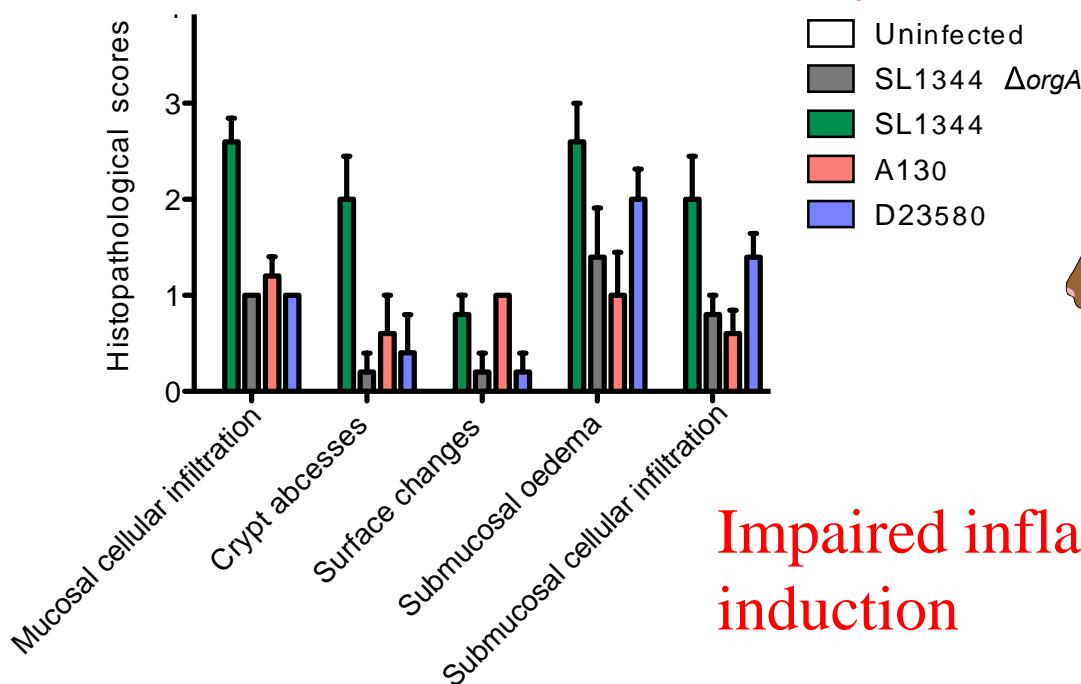
Some *S. Typhimurium* ST313 have altered enteropathogenicity

Bovine models -
Calf ileal loop
experiments



Aspect of this may be true for *S. Enteritidis*
in Sub-Saharan Africa (Nick Feasy)

Streptomycin-
pretreatment in
murine models



Impaired inflammasome
induction

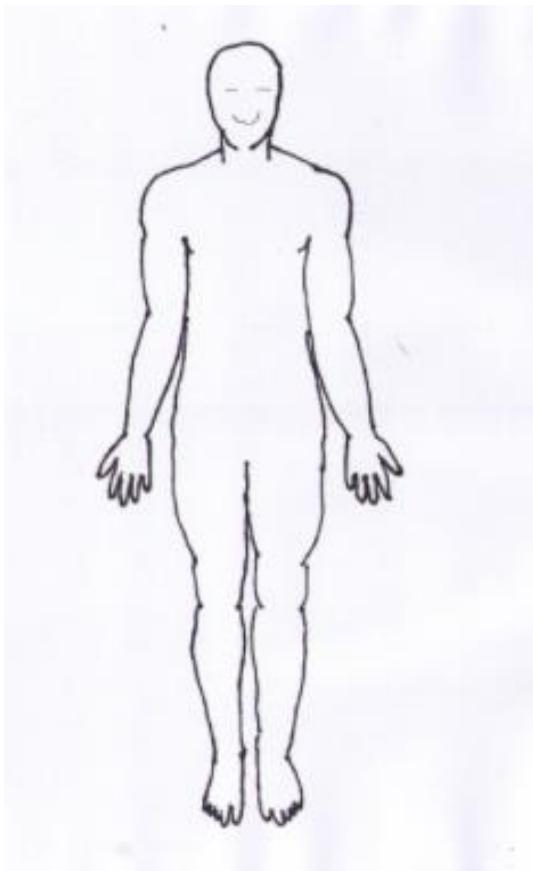
S. Typhimurium ST313 summary

- Two clades emerged to spread across Sub-Saharan Africa associated with the HIV epidemic
- Is the ST313 wave over?
- ST313 have signatures of host adaptation
- ST313 was in Africa before HIV (in primates or children locally?)
- Is there human to human transmission of some sort?

Sam Kariuki was right, journal editors and referees were wrong!

Does remarkable pathogen evolution in an IL12/23 β 1 receptor deficient individual throw light on how typhoid evolved?

IL12/23 β 1 receptor deficient and chronically infected with recurrent S. Enteritidis infection



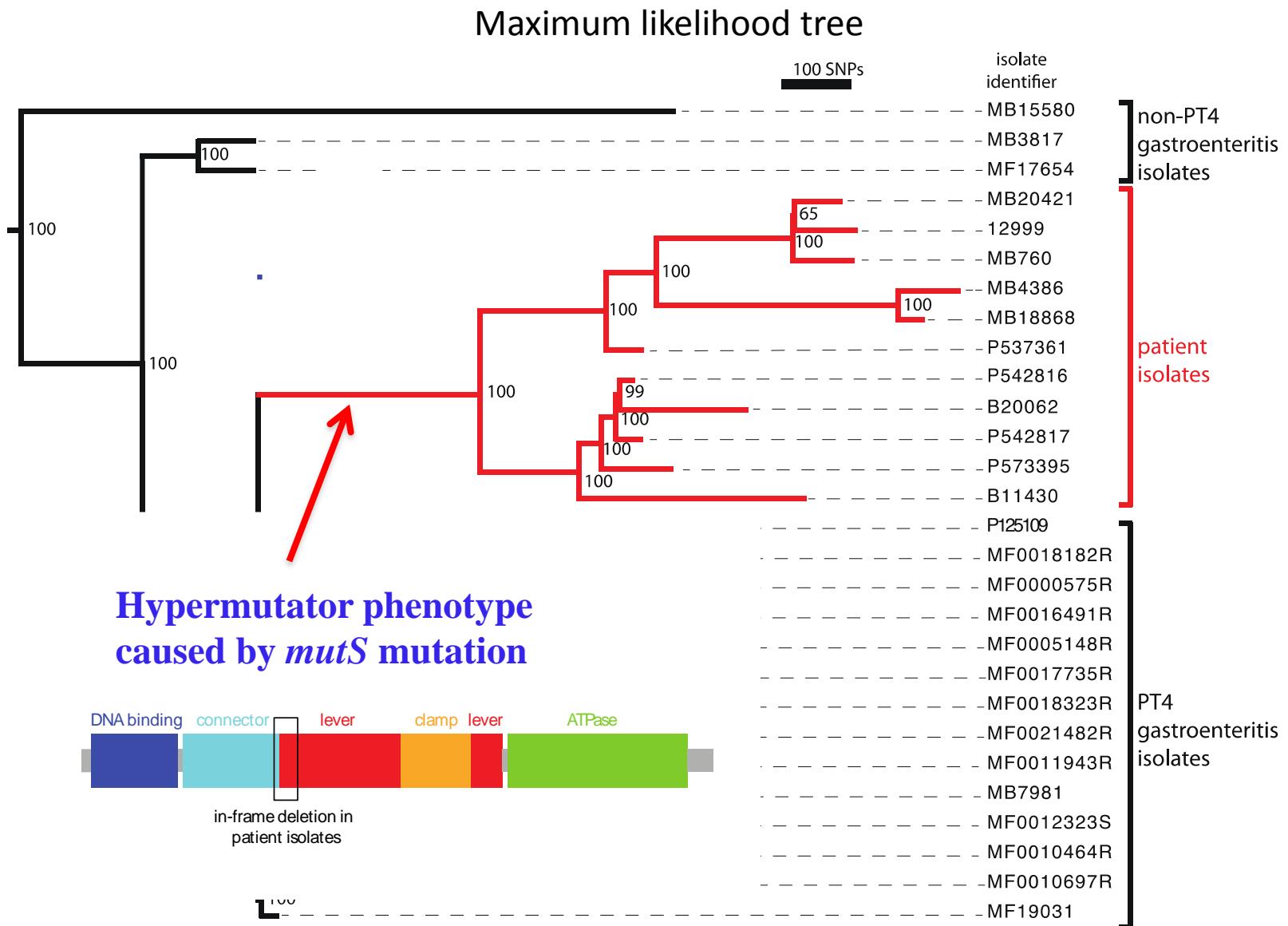
- Age 12 presents with clinically severe bacteremia with *Salmonella* Enteritidis
- Treated with **antibiotics** and **interferon γ**

Next decade

- >50 episodes of bacteremia becoming less clinically severe over time
- No stool carriage just systemic site
- Eventually no signs of chronic infection CRP, albumin, IgG

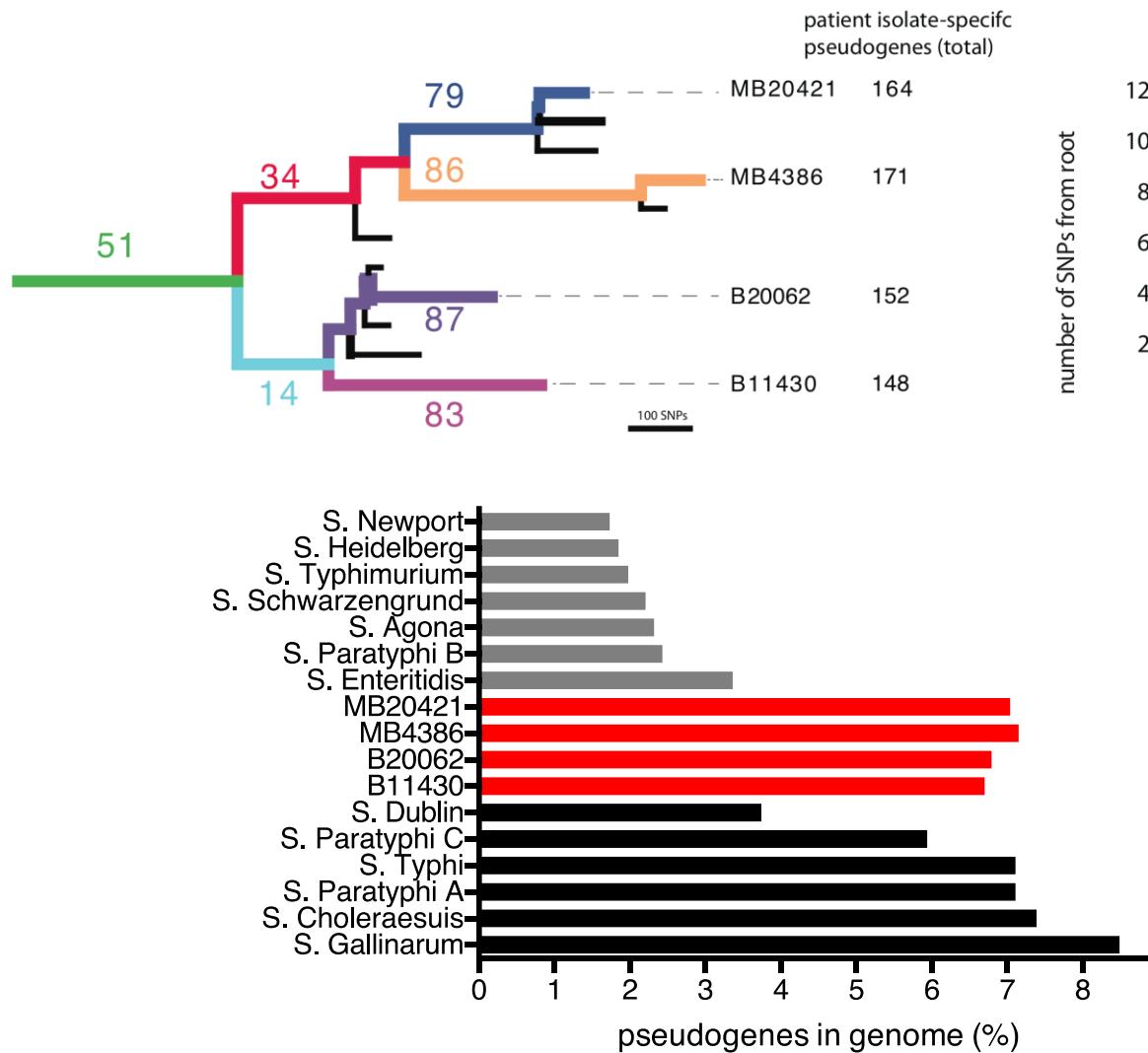
Bacteria originally typed as *Salmonella enteritidis* but became increasingly difficult

Patient isolates show extensive mutation accumulation and genome degradation

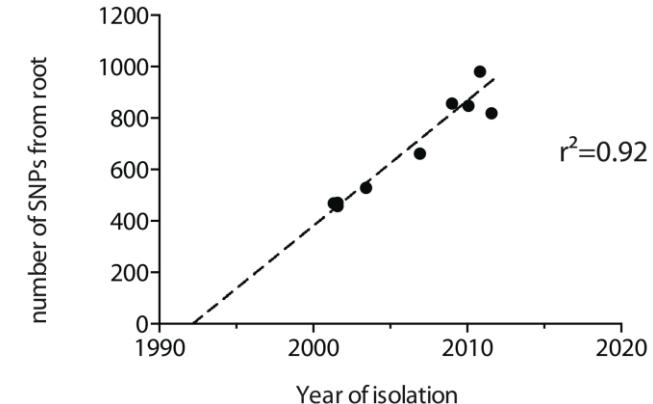


Hallmarks of host-adaptation: genome degradation

Complete pseudogene analysis of four patient isolates sequenced by PacBio



Molecular clock rate



Gastrointestinal serovars

Patient isolates

Extra-intestinal serovars

level comparable to host-adapted/restricted serovars

Convergent evolution with *S. Typhi* and *S. Paratyphi A*

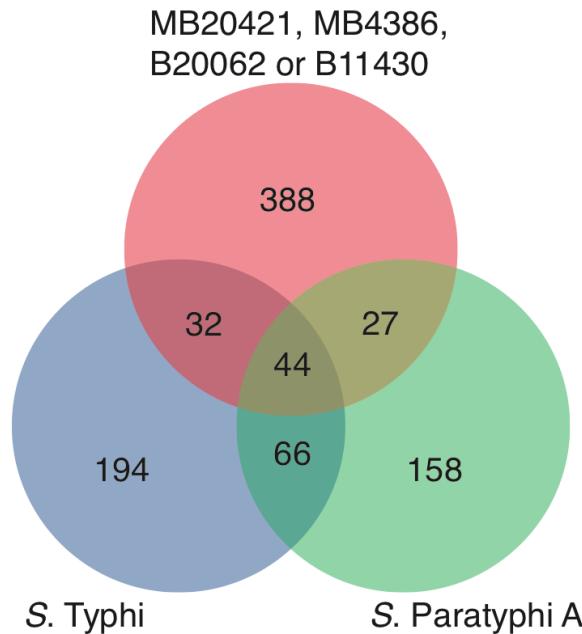
Intestinal metabolome disrupted

Adhesins and intestinal shedding genes gone

SPI-1 dead

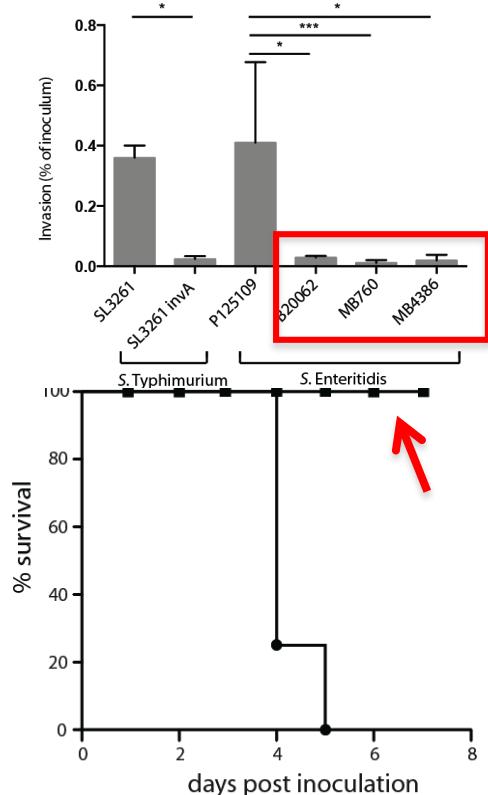
SPI-2 stream lined

O antigen ligase gone



Pseudogenes by pathway/ locus

	Patient isolates				S. Typhi			S. Paratyphi A	
Central anaerobic metabolism									
Nitrogen utilization	narK	narX	narG	narV	narV	narW	narZ	narW	
Tetrathionate utilization	ttrB	ttrR			ttrS			ttrB	
Vitamin B12 synthesis	btuR	cbiJ	cbiK	cbiL	cbiN	cbiC	cbiJ	cbiK	cbiM cbiA
Adhesion									
non-fimbrial adhesins	shdA	misL	bigA		shdA	misL	bigA	shdA	
fimbrial sef locus	sefC	sefR			sefA	sefC	sefD	sefR	sefD sefR
fimbrial sth locus	sthB	sthC			sthC	sthE			
Type III secretion									
SPI-1 effectors	sipA	sopA	sopD		slrP	sopE2	sopA	slrP	sopA
SPI-2 effectors	ssel	sseJ	sspH2	steC	sseK1	steB	sopD2 sseJ	sseK2	steB sopD2 sseJ sseK2 sifB steC



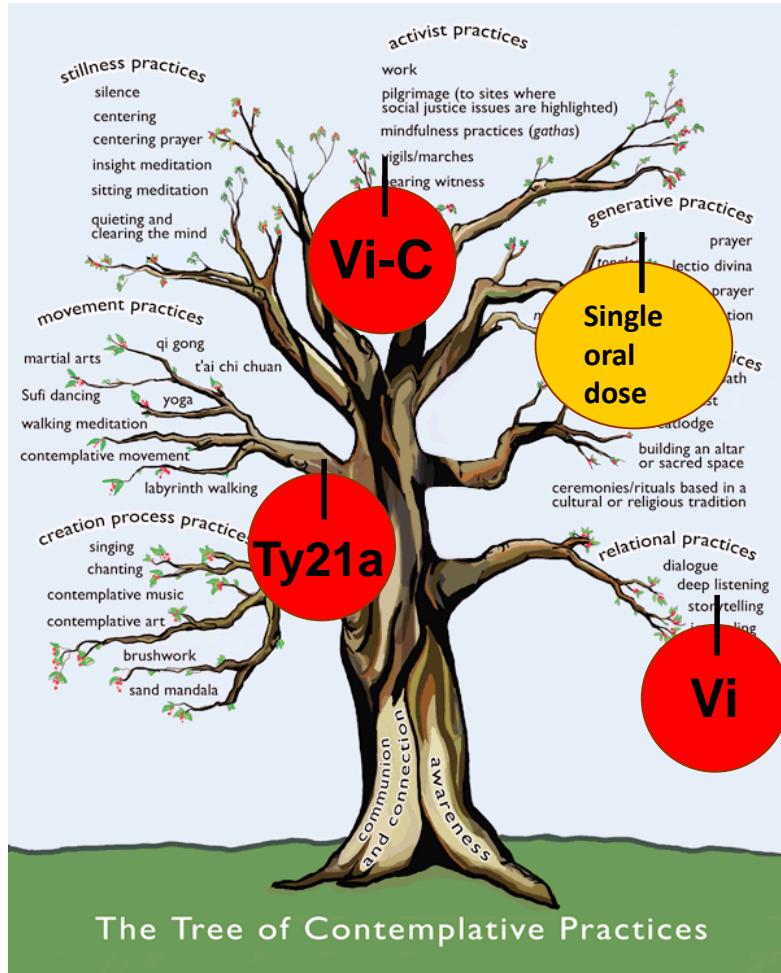
What does this study tell us, if anything?

- **Microbes can evolve (mutate) at dramatically different rates in different settings**
- **Compromised individuals or cohorts may be sources of adapted microbes. Can we spot adaptation to humans early?**
- **Factors such as antimicrobial treatment are exerting unprecedented selective pressures (we see this in most pathogens)**



Elizabeth Klemm

We have typhoid vaccines that work.....but....



Vi capsule, Paratyphi A O side chain

- Length of polysaccharide ?
- Typhi/Paratyphi carrier proteins?
- What is a correlate of protection (antibody, bactericidal ?

Clinical Project	Clinical Trial Phase
Ty21a	Licensed
M01ZH09	Phase 2, adults and children
Ty800	Phase 2, adults (2008)
CVD909	Phase I, adults (2010)



What are the immediate challenges?

Can we.....

- ...get conjugate vaccines used effectively?**
- ...get any typhoid vaccine used broadly?**
- ...get diagnosis working at the bed-side?**
- ...track and define carriers more effectively**
- ...eradicate typhoid!**

**Advocacy and driving vaccines to WHO
prequalification and licensing**

The urgent need for new typhoid diagnostics

***Salmonella Typhi* and *Salmonella Paratyphi A* elaborate distinct systemic metabolite signatures during enteric fever**

Elin Näsström¹, Nga Tran Vu Thieu², Sabina Dongol³, Abhilasha Karkey³, Phat Voong Vinh², Tuyen Ha Thanh², Anders Johansson⁴, Amit Arjyal², Guy Thwaites^{2,5}, Christiane Dolecek^{2,5}, Buddha Basnyat³, Stephen Baker^{2,5,6*}, Henrik Antti^{1*}

Metabolomics



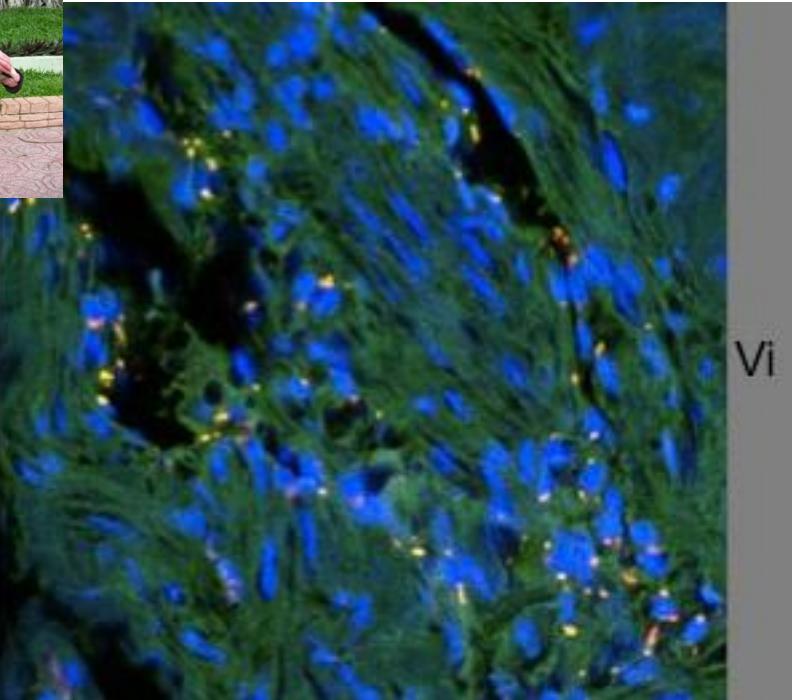
PCR

Immune profiling with a *Salmonella Typhi* antigen microarray identifies new diagnostic biomarkers of human typhoid

Li Liang¹, Silvia Juarez¹, Tran Vu Thieu Nga², Sarah Dunstan², Rie Nakajima-Sasaki¹, D. Huw Davies¹, Stephen McSorley³, Stephen Baker² & Philip L. Felgner¹

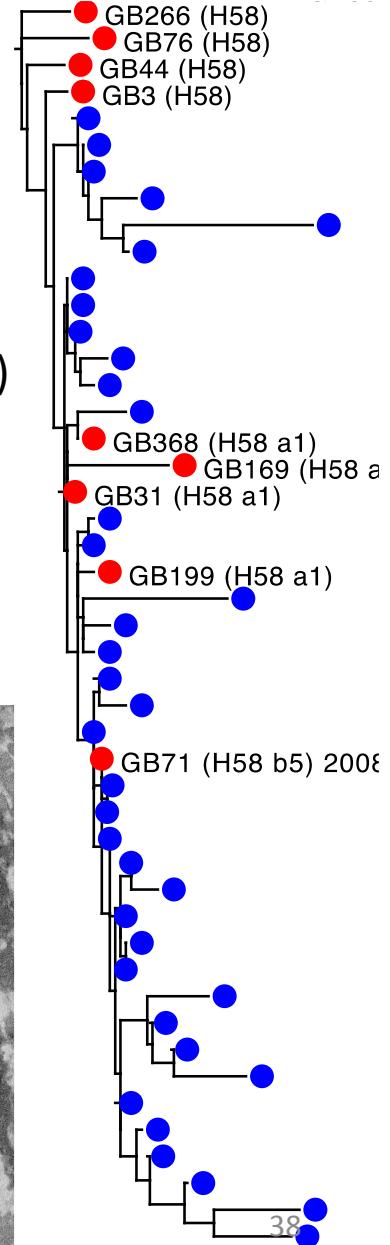
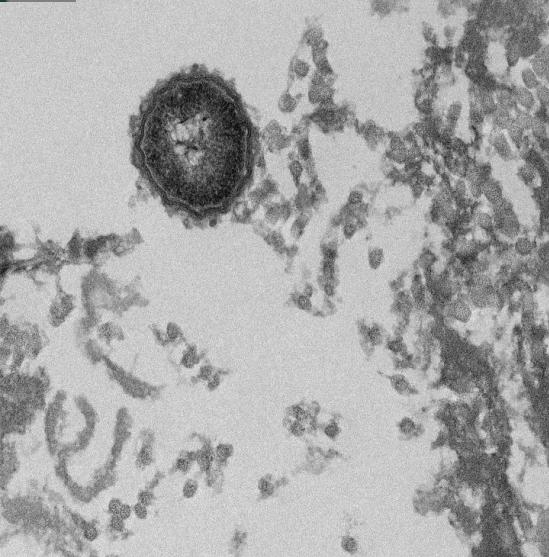
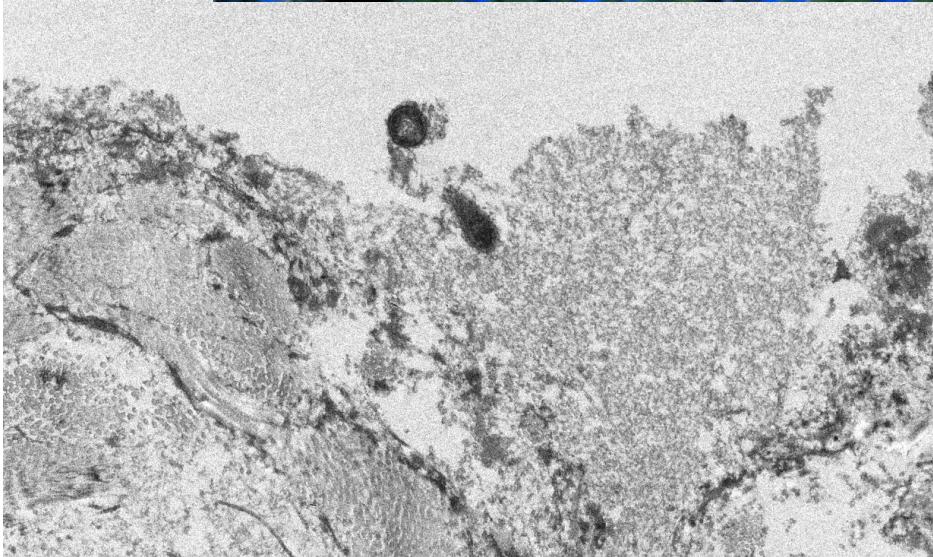
Serology, new antigens

What is a typhoid carrier in 2015 and where are they!



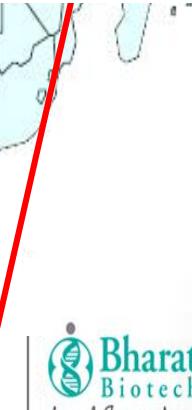
Vi

- Gall bladder (2010)
- Acute typhoid (2004-2008)





Wellcome Trust Strategic Award on Typhoid



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