Fever etiology: bacterial zoonoses and global health

John A. Crump
McKinlay Professor of Global Health
Co-Director, Centre for International Health

Infectious Disease Research Minisymposium
9 September 2014

UNIVERSITY of OTAGO
SAPERE AUDE
Te Whare Wānanga o Otāgo

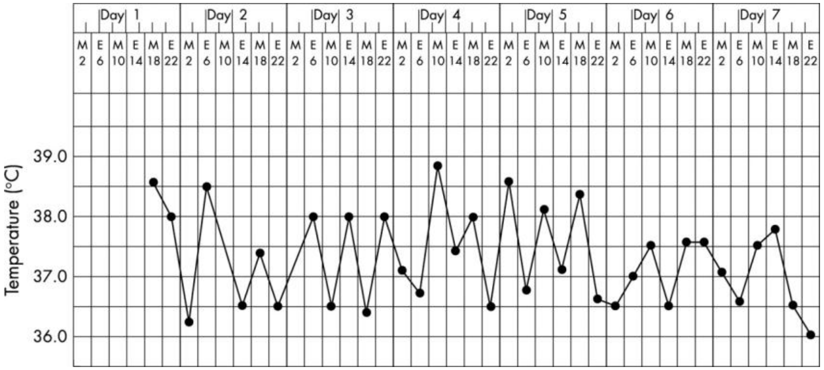
Centre for International Health

Overview

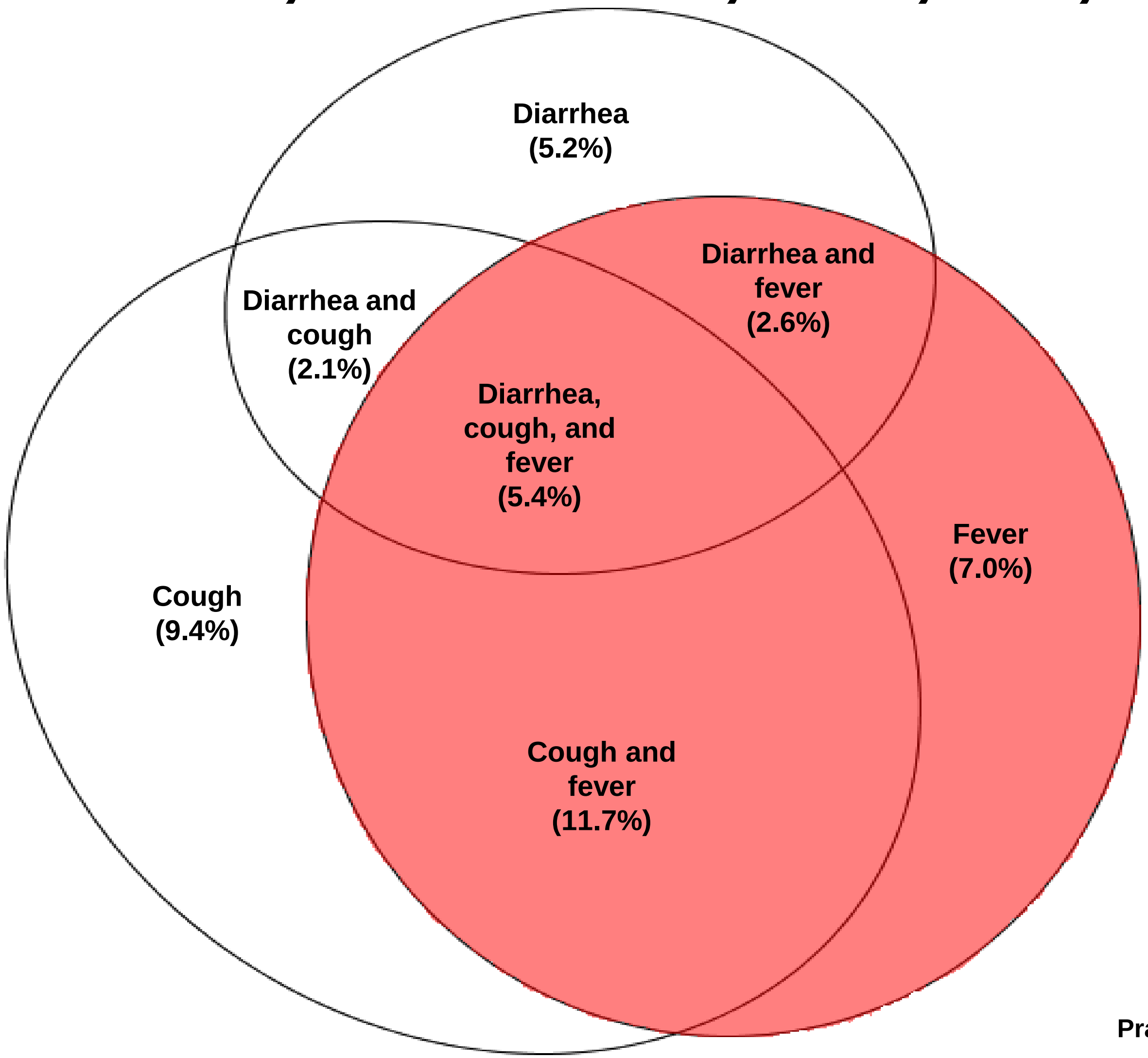
- The challenge of fever in the tropics

- Bacterial zoonoses and febrile illness
  - Leptospirosis
  - Invasive non-typhoidal *Salmonella*

Diagnostic challenge



Diarrhea, cough, and fever, children <5 years of age, low- and middle-income countries, 1986-2012, n=1,200,986



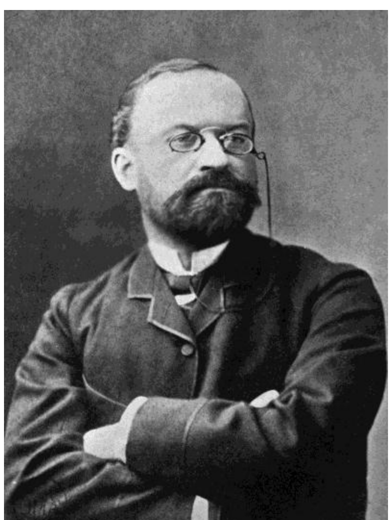
Prasad N. MPH thesis, 2014

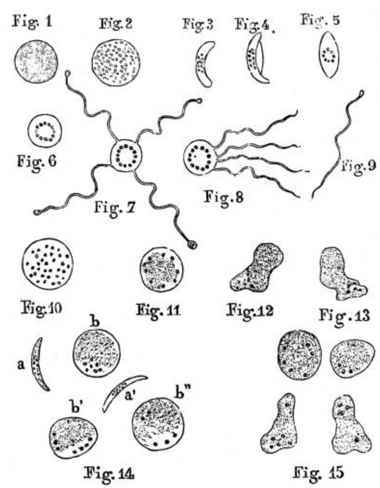
Malaria

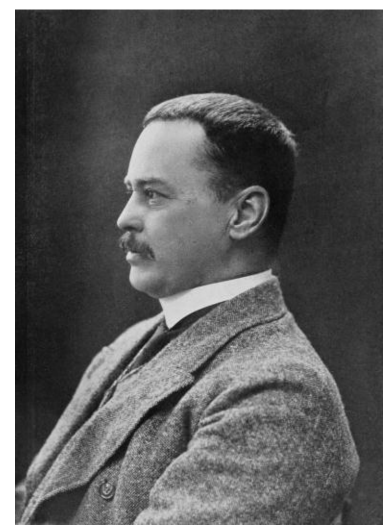
- **1740: Italian *mala* 'bad' + *aria* 'air'**
  - **The fever caused by foul air in marshy districts**

- **1880: malaria parasites discovered by Laveran**

- **1897: mosquitoes identified as vector by Ross**





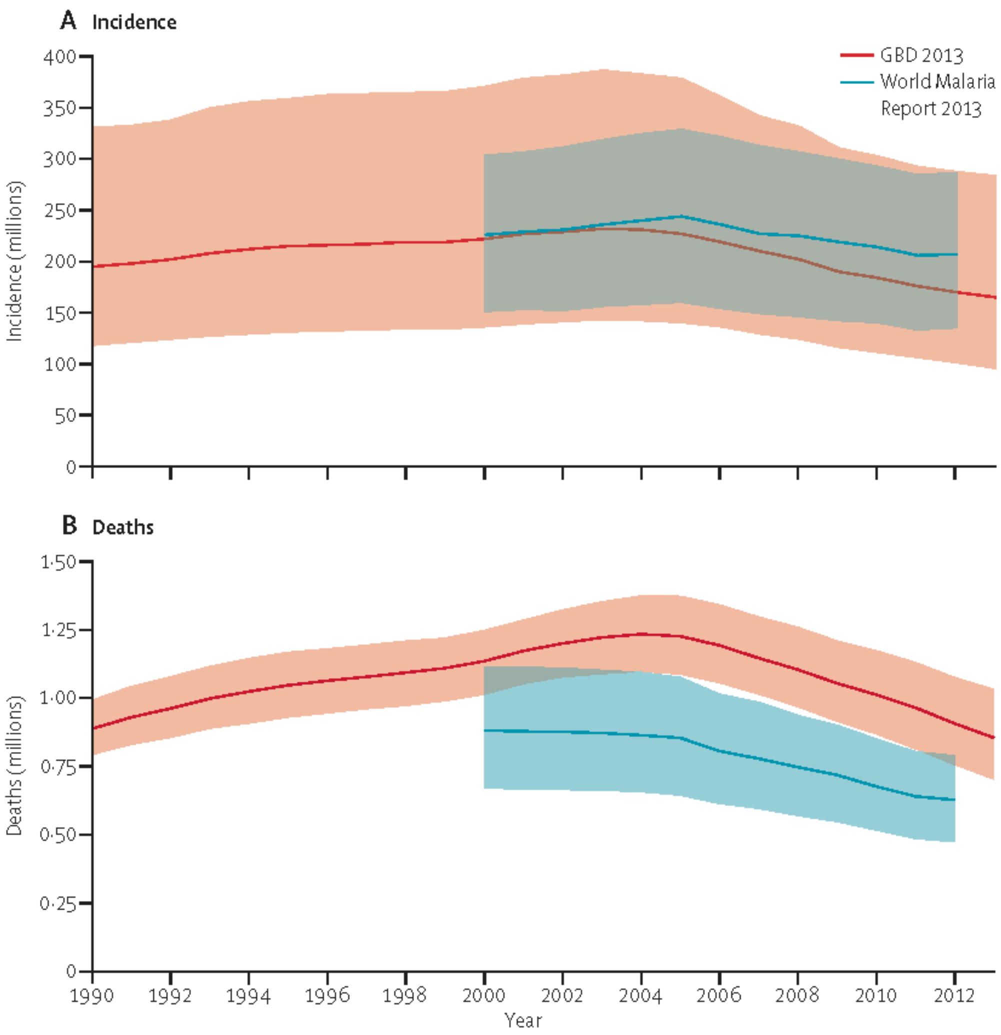


Progress with malaria control and treatment

- **1955-78: Global malaria eradication**
  - Residual insecticides
  - Antimalarial drug treatment
  - Surveillance
  - Succeeded in temperate zones and areas with seasonal malaria
  - Was not attempted in most of sub-Saharan Africa

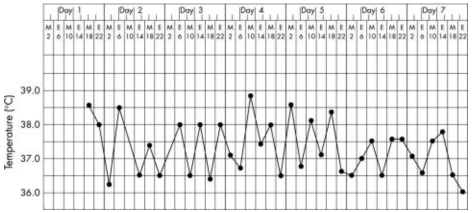
- **1998-present: Scale-up of malaria interventions**
  - Indoor residual spraying
  - Insecticide-treated nets
  - Expansion of malaria rapid diagnostic tests and artemisinin-based combination therapies
  - Considerable focus on sub-Saharan Africa

Trends in global malaria incidence and deaths, 1990 to 2012



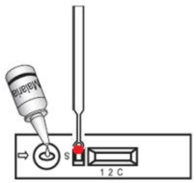
Murray CJ, et al. Lancet 2014; in pres

What to do with the patient with fever and a negative test for malaria?

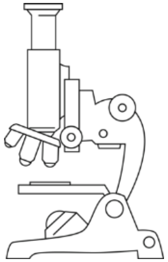


Fever

↓



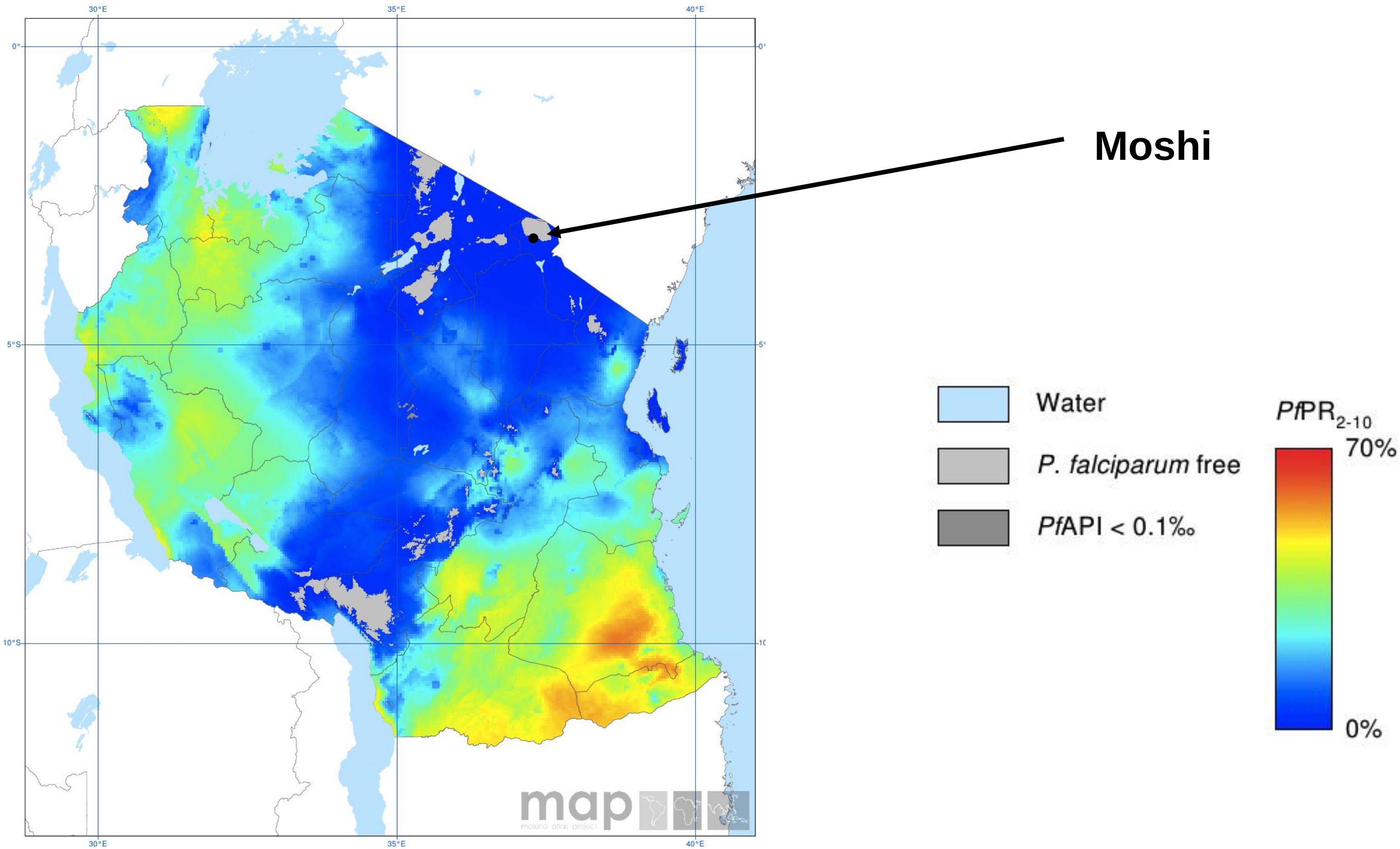
Test for malaria



Positive	Negative
↓	↓
Prescribe antimalarial	?

Kilimanjaro Christian Medical Centre, Moshi, Tanzania

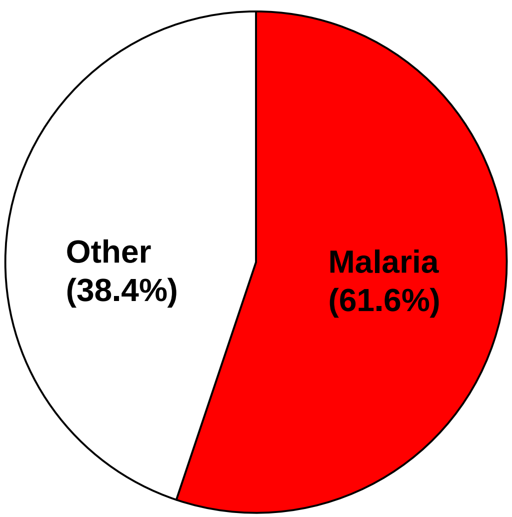
Proportion of people with malaria, Tanzania, 2010



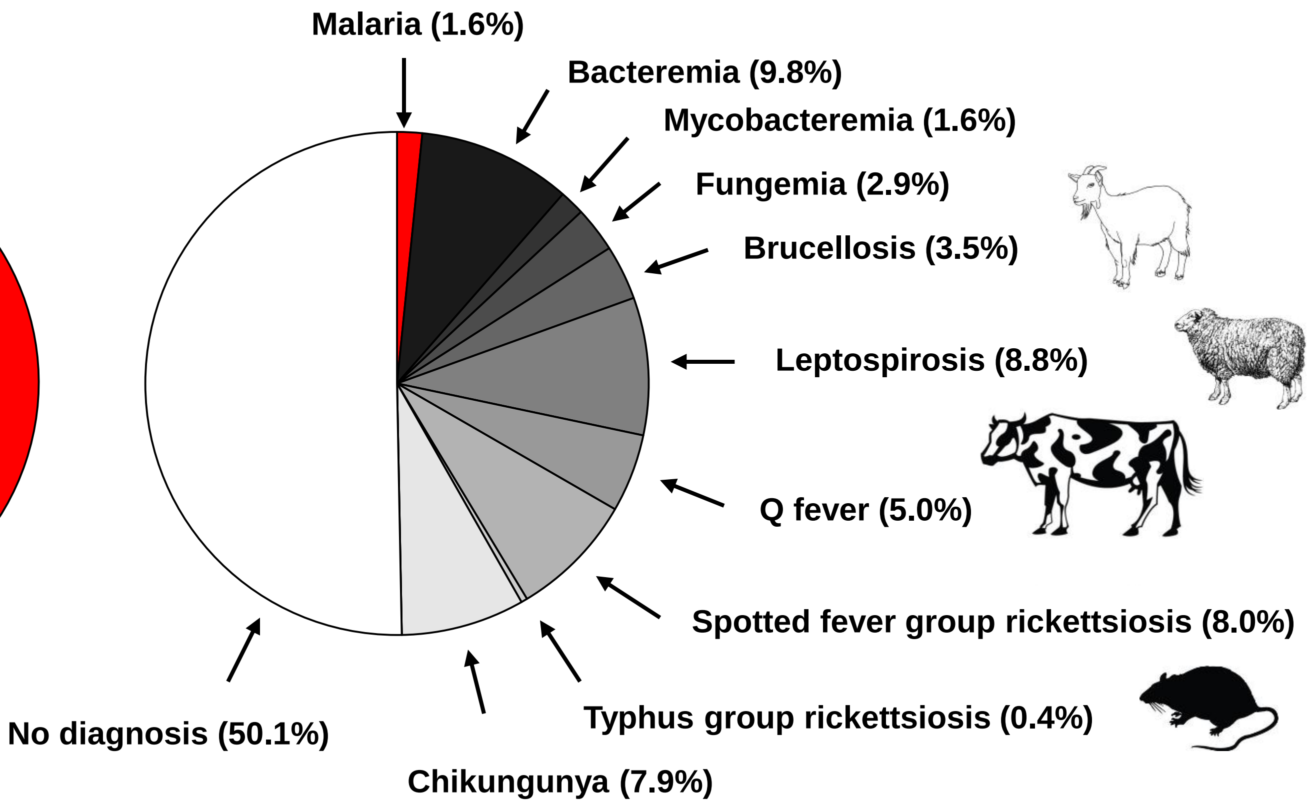
Hay, S.I. et al. PLoS Medicine 2009; 6: e1000048
www.map.ox.ac.uk

Clinical diagnosis versus laboratory diagnosis of febrile inpatients, northern Tanzania, 2007-8 (n=870)

Clinical diagnosis



Laboratory diagnosis



Crump JA, et al. PLoS Neglect Trop Dis 2013; 7: e2324

Leptospirosis

- *Leptospira* spp
  - Pathogenic (n=13) and non-pathogenic species
  - ~25 serogroups
  - >250 serovars
  - Classification system complex with poor concordance between serogroups and genetic groups

- Disease
  - Febrile illness in humans, sometimes severe
  - Reproductive disease, milk drop, growth failure, death in livestock

Leptospira spp epidemiology in humans

- Reservoir species
  - Rodents
  - Livestock

- Exposure
  - Direct contact: urine, tissues
  - Environmental exposure: water, soil

- Tropics
  - Rodents in urban slums (e.g., Brazil)
  - Rice fields and sugar cane plantations (e.g., Asia)
  - Few data from Africa

Predominant reactive serogroup among patients with confirmed leptospirosis, northern Tanzania, 2007-8

Serogroup	n positive	Median reciprocal titer	Potential local reservoir
Mini	13	200	Cattle, hedgehogs, dogs
Australis	13	400	Rats, cattle, hedgehogs, dogs, donkeys
Icterohaemorrhagiae	4	200	Rats, cattle, dogs, goats, pigs, donkeys
Celledoni	3	800	Rodents
Djasiman	1	400	Dogs
Hebdomadis	1	200	Mice, goats, sheep, cattle, donkeys, chickens, hippo
Pyronenes	1	200	Rats, goats, donkeys, cattle, pigs, chickens
Tarassovi	1	200	Pigs, cattle

Biggs HM, et al. Am J Trop Med Hyg 2011; 85: 275-81

Leptospira studies in animals

- ***lipL32*** **qPCR of kidney positive**
  - **Cattle 18 (6.0%) of 300**
  - **Goats 2 (5.0%) of 40**
  - **Rodent 0 (0%) of 124 positive**

- ***Leptospira*** **culture**
  - **Animals: two isolates from cattle kidney**

Human leptospirosis prospective cohort study

- 20 Feb 2012 through 30 May 2014
- 1,415 participants
- Detailed risk factor questionnaire and geospatial mapping to the household level
- Pair sera 991 (70.0%)
- Shipping to reference laboratory Sep 2014

Invasive non-typhoidal *Salmonella*

- **Typhoidal *Salmonella***
  - ***Salmonella enterica* serovars Typhi, Paratyphi**
  - **Human host restricted**
  - **Febrile illness, bloodstream infection: typhoid and paratyphoid (enteric) fever**

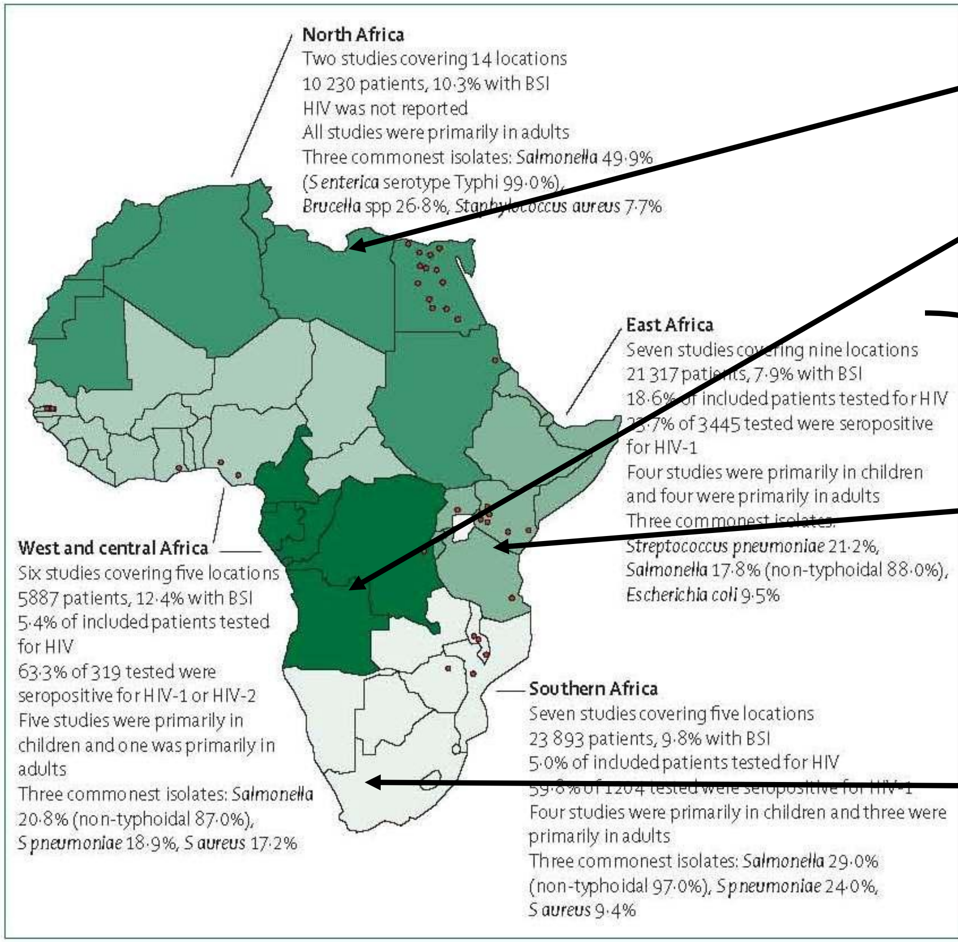
- **Non-typhoidal *Salmonella***
  - **>2,500 serovars e.g., Typhimurium, Enteritidis**
  - **Non-human animals: generalist, host adapted, host restricted**

Non-typhoidal *Salmonella* infection in humans

- **Industrialized countries**
  - **Foodborne transmission**
  - **Common cause of self-limited diarrhea**
  - **Occasionally invasive disease: infants, elderly, immunocompromised**

- **Sub-Saharan Africa**
  - **Transmission routes poorly understood**
  - **Uncommon cause of moderate to severe diarrhea**
  - **Leading community-acquired bloodstream infection**

Community-acquired bloodstream infections, Africa



North Africa (n=10,230)
- ***Salmonella enterica*** **50% (99% Typhi)**
- ***Brucella*** **spp** ***27%***
- ***Staphylococcus aureus*** **8%**

West and central Africa (n=5,887)
- ***Salmonella enterica*** **21% (87% NTS)**
- ***Streptococcus pneumoniae*** **19%**
- ***Staphylococcus aureus*** **17%**

East Africa (n=21,317)
- ***Streptococcus pneumoniae*** **21%**
- ***Salmonella enterica*** **18% (88% NTS)**
- ***Escherichia coli*** **17%**

Non-typhoidal *Salmonella* serovars
- **Typhimurium***
- **Enteritidis***
- **Stanleyville**
- **Dublin**
- **Isangi**

Southern Africa (n=23,893)
- ***Salmonella enterica*** **29% (97% NTS)**
- ***Streptococcus pneumoniae*** **24%**
- ***Staphylococcus aureus*** **9%**

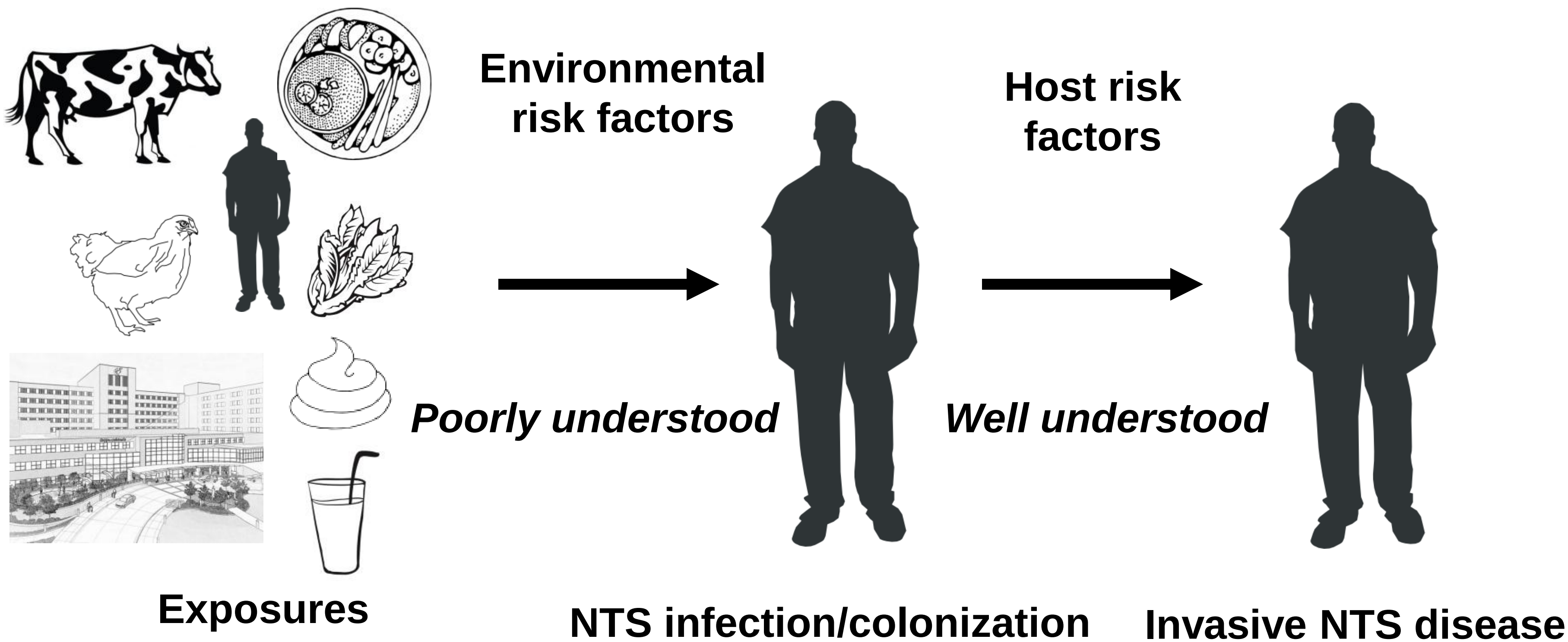
Reddy EA, et al. Lancet Infect Dis 2010; 10: 417

Invasive non-typhoidal *Salmonella* death estimates in context

Condition	Year	Source	Deaths
Enteric NTS	2009	Majowicz SE	155,000
Typhoid and paratyphoid fevers	2010	GBD	190,200
Invasive NTS	2010	Ao TT	681,316
Protein-energy malnutrition	2010	GBD	599,800
Malaria	2010	GBD	1,169,500
HIV	2010	GBD	1,465,400

Majowicz SE, et al. Clin Infect Dis 2010; 50: 882–889
Ao TT, et al. Emerg Infect Dis 2014; in press
Lozano R, et al. Lancet 2012; 380: 2095-128

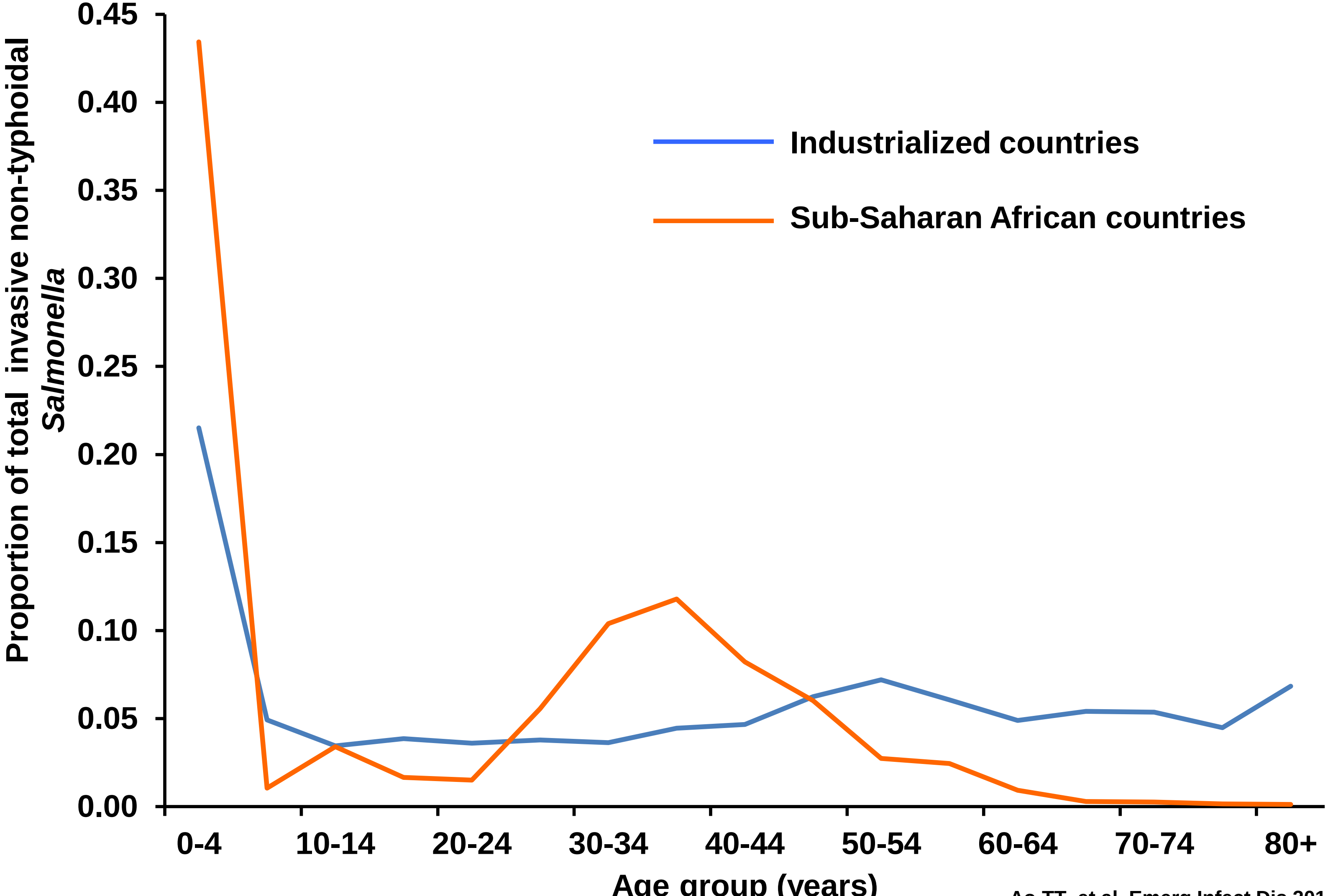
Pathway to invasive NTS in sub-Saharan Africa



Host risk factors

- **Age**
  - **Infants and young children**
- **Malaria**
  - **Recent malaria > current malaria**
  - **Severe malarial anemia**
- **Malnutrition**
  - **Severe acute malnutrition**
- **HIV**
  - **CD4-positive T-lymphocyte count <200 cells/mm^3**

Proportion invasive NTS by age, industrialized and sub-Saharan African countries



Ao TT, et al. Emerg Infect Dis 2014; in press

Environmental risk factors

- **Industrialized country paradigm**
  - **Food animal products**
  - **Feces contaminated produce and water**
  - **Direct and indirect animal contact**

- **Sub-Saharan African country challenges**
  - **Little epidemiologic research**
  - **Understanding infection/colonization**
  - **Genomic data suggest human host adaptation**
  - **Poultry challenge study *Salmonella* Typhimurium ST313 not human host restricted**

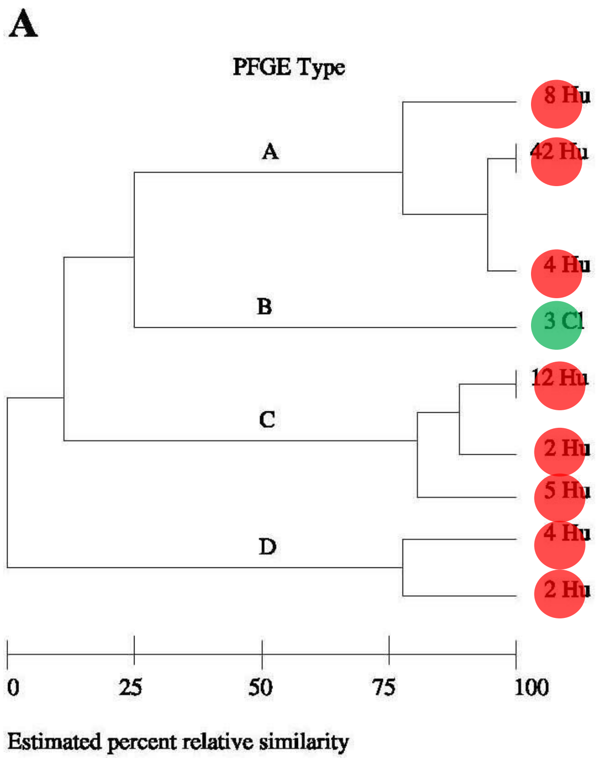
Kingsley RA, et al. Genome Res 2009; 19: 2279-87
Parsons BN, et al. PLoS Neglect Trop Dis 2013; 7: e2487

New research

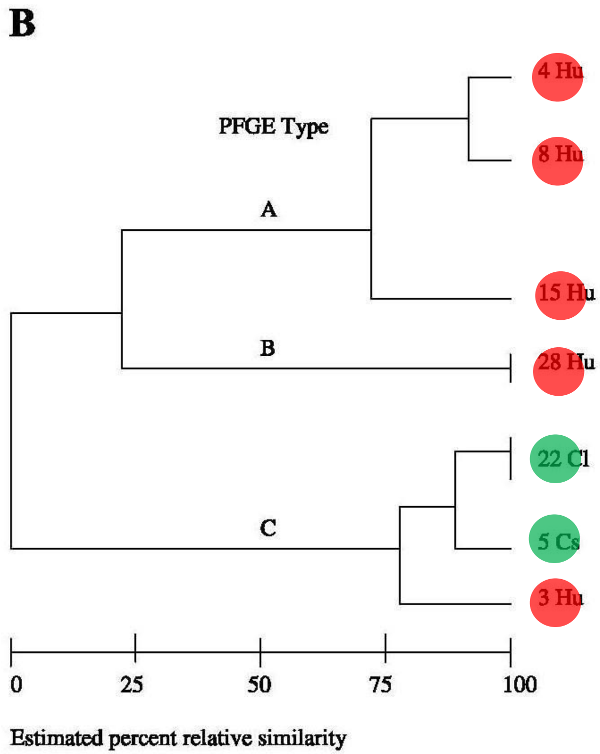
- **Hazards Associated with Zoonotic Enteric Pathogens in Emerging Livestock systems (HAZEL)**
  - **Use modular process risk model (MPRM) approach to describe non-typhoidal *Salmonella* and *Campylobacter* meat pathways from poor farmers through abattoirs to meat retailers in northern Tanzania**
  - **Links to regional invasive non-typhoidal *Salmonella* surveillance**
  - **Collaboration between Massey University, University of Otago, University of Glasgow, Kilimanjaro Christian Medical Centre, Sokoine University of Agriculture**

Molecular relatedness of NTS isolated from humans and their peridomestic animals

Salmonella Typhimurium



Salmonella Enteritidis



Kariuki S, et al. FEMS Immunol Med Microbiol 2002; 33: 165

Burden of illness and death

- **Global burden of disease (WHO, IHME, other)**
  - **Typhoid and paratyphoid fever**
  - **Enteric non-typhoidal *Salmonella***
  - **No estimate for invasive non-typhoidal *Salmonella***

Invasive non-typhoidal *Salmonella*

- **Incidence**
  - **Systematic review for population-based incidence studies (n=10)**
  - **Extrapolation based on national HIV seroprevalence and malaria population at risk percent**
  - **United Nations Population Division 2010 population**

- **Case fatality ratio**
  - **Case series**
  - **Expert opinion**

Global deaths invasive NTS, 2010

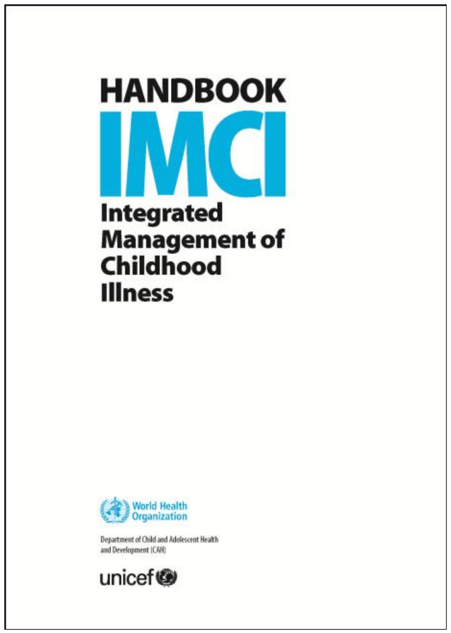
Case fatality ratio	Estimated number of annual deaths
3%	102,197
5%	170,329
10%	340,658
20%	681,316
30%	1,021,974
40%	1,362,632
50%	1,703,290

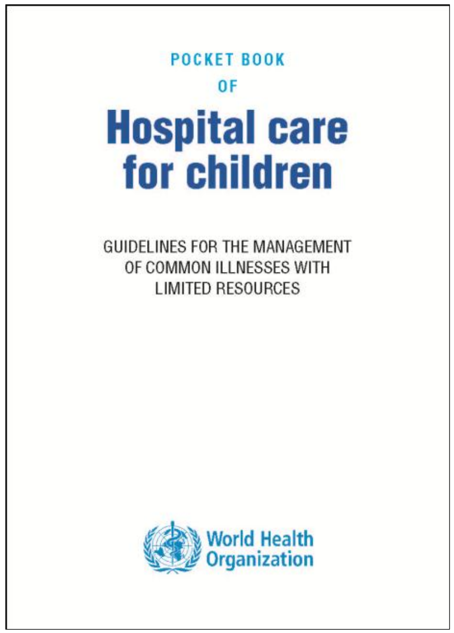
Ao TT, et al. Emerg Infect Dis 2014; in press

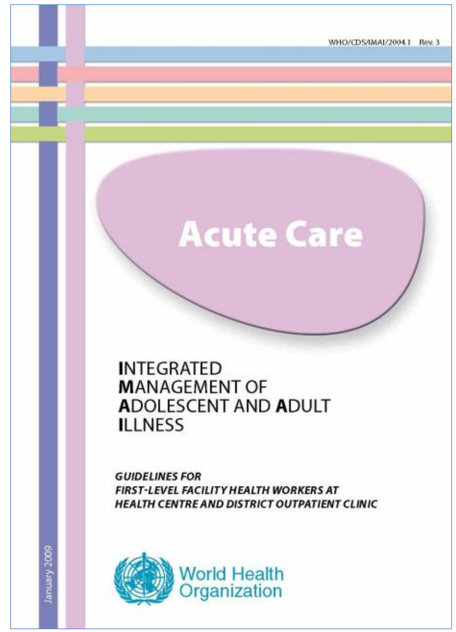
‘Western medicine’ in Africa

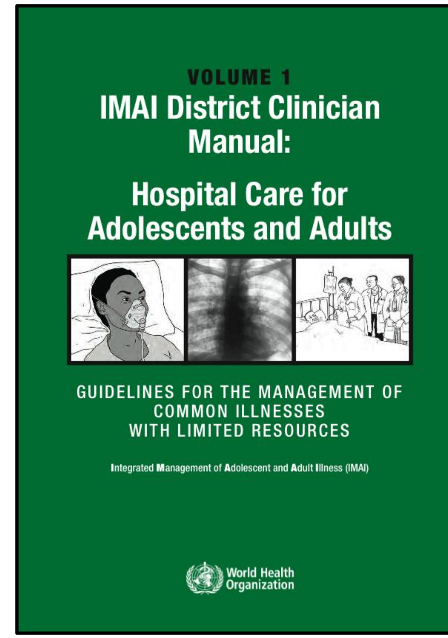
- Colonial period
  - European health: tropical medicine, fever, ‘scramble for African diseases’
  - Health of indigenous peoples: diseases preventing development
- Post-colonial period
  - Extend primary healthcare services to rural areas
  - Adoption of syndromic patient management
  - De-emphasis of laboratory and pathology services

Syndrome-based clinical management





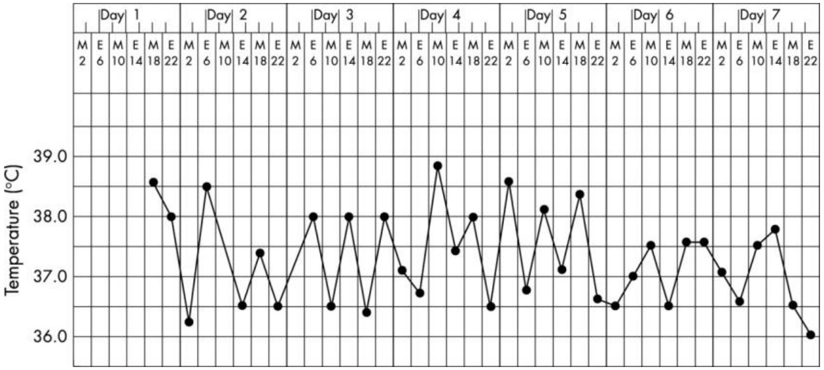




Integrated Management of Childhood Illness (IMCI)

Integrated Management of Adolescent and Adult Illness (IMAI)

Diagnostic challenge



World Health Organization

GUIDELINES
FOR THE TREATMENT
OF MALARIA

'The signs and symptoms of malaria are nonspecific. Malaria is clinically diagnosed mostly on the basis of fever or history of fever.'

World Health Organization. Guidelines for the treatment of malaria. 2006

9 789241 546942

Severely patients hospitalized with fever, Tanzania

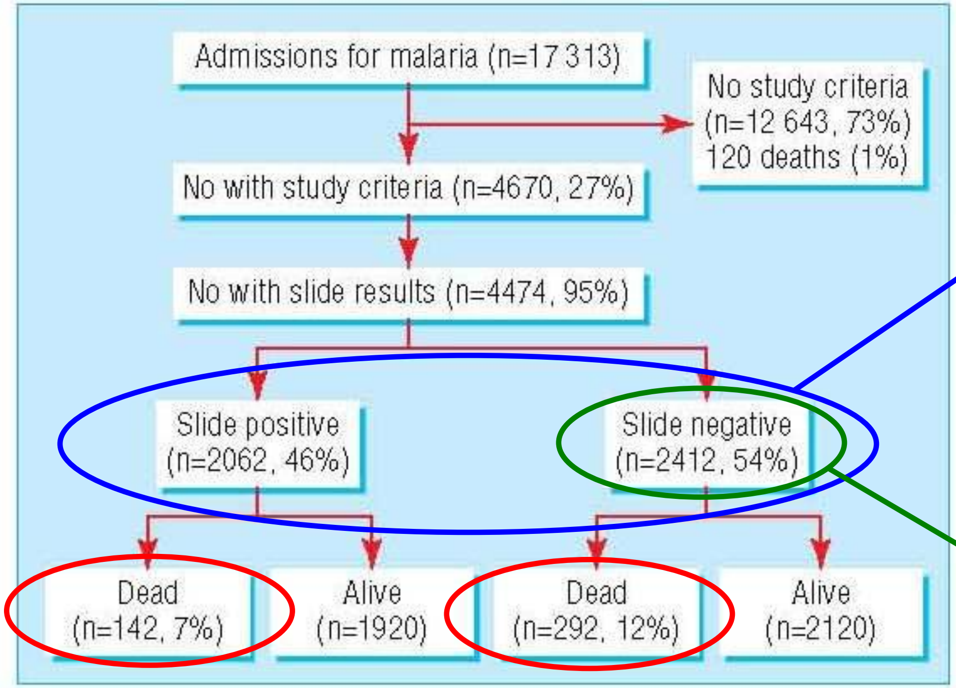


Fig 1 Patients admitted to 10 hospitals with diagnosis of malaria over one year by outcome, presence of any *P falciparum* asexual parasites on the research blood slide, and case fatality

95% treated with quinine

66% received antibacterial

Reyburn H, et al. Brit Med J 2004; 329: 1212

World Health Organization

GUIDELINES
FOR THE TREATMENT
OF MALARIA

Second edition

'Prompt parasitological confirmation by microscopy or by rapid diagnostic test is recommended in all patients suspected of malaria before treatment is started.'

'Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible.'

World Health Organization. Guidelines for the treatment of malaria. 2010

THE "SILENT HIGHWAY"-MAN.

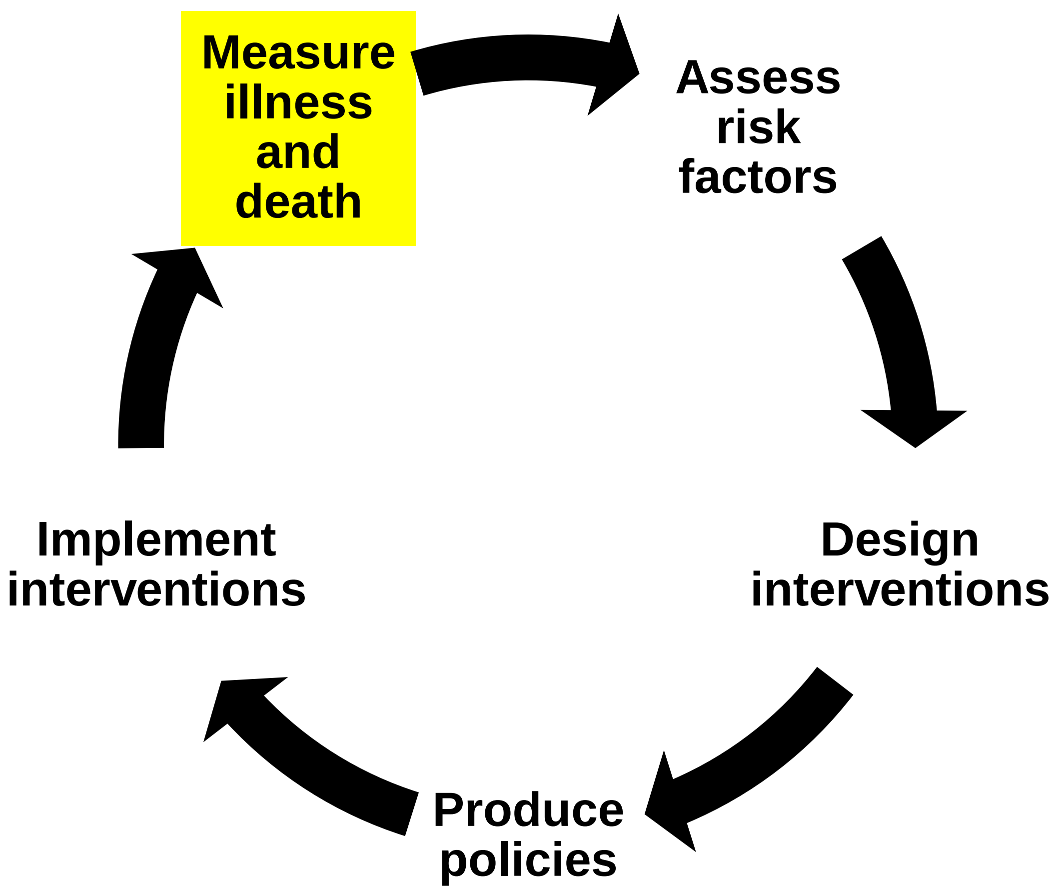
"Your MONEY or your LIFE!"

Your final contribution

- Your cause of death
  - Or ‘what you died with’

- Most people in the world cannot make this contribution
  - Inadequate vital event registration systems
  - Lack of laboratory and pathology services
  - ‘The uncounted count for nothing’

Improving health



Cause of death

- Two-thirds of the world’s population
  - Live in countries that lack reliable system for issuing medical death certificates
  - Majority of premature global deaths occur in these countries

- Verbal autopsy
  - Assigns probable cause of death based on interview of families about symptoms of deceased
  - Death with fever in a malaria-endemic country = malaria death

GLOBAL HEALTH

Verbal autopsy methods questioned

Controversy flares over malaria mortality levels in India.

BY DECLAN BUTLER

More than two-thirds of the world's population lives in countries that lack a reliable system for issuing medical death certificates, leaving the true scale and distribution of disease in serious doubt. The main tactic for filling that gap is verbal autopsy, which assigns a probable cause of death based on interviews with families about the deceased's symptoms.

But the reliability of the technique is under fresh scrutiny after a paper published in *The Lancet* last week[1] used verbal autopsy to calculate that 125,000–277,000 people in India die from malaria every year (see 'Malaria mortality'). That is an order of magnitude larger than the 30,000 deaths per year that the World Health Organization (WHO) estimates.

The *Lancet* paper used the most common form of verbal autopsy, in which physicians assign the cause of death. But statisticians argue that probabilistic computer models can do a better job than doctors. The WHO also argues that verbal autopsy can be poor at differentiating malaria from other diseases that cause fever symptoms, which include septicaemia, viral encephalitis and pneumonia. Although the WHO has accepted the use of verbal autopsy to monitor malaria deaths and other diseases, Christopher Dye, a senior WHO official, says the method can easily give misleading results.

Brian Greenwood, a malaria epidemiologist at the London School of Hygiene and Tropical Medicine, who performed some of the earliest verbal autopsies for malaria in Africa, says that malaria deaths in India are probably underestimated to some extent, but shares the WHO's concern about the "very poor" performance of the technique on fever symptoms.

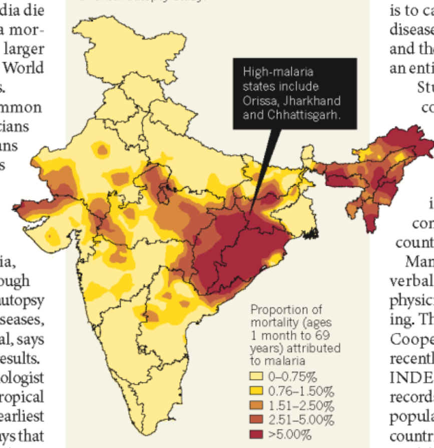
Greenwood is also concerned that as physicians in the study were familiar with the Indian states that they reviewed case reports from, the survey had a built-in bias. As any medic in India probably knows the most malarious states, this could lead to "a temptation to ascribe febrile cases to malaria" in such states, says Greenwood.

Prabhat Jha, an epidemiologist at the Centre for Global Health Research at the University of Toronto, Canada, and a co-author of the study, vigorously defends the results, arguing that physicians were given clear guidelines to carry out differential diagnosis to exclude malaria as the cause. The "total assignment of malaria deaths is not as biased as might be first believed", he says.

"We didn't blind as we thought it was important that coders knew where the case report came from," he adds. "It gave contextual information. If it smells like malaria, looks like malaria, and you see it in malarious regions then it probably is malaria."

MALARIA MORTALITY

In 2001–03, malaria death rates in India were far higher than previously thought, according to a verbal-autopsy study.



SOURCE: REF. 1

But Gary King, a statistician at Harvard University in Cambridge, Massachusetts, notes that the different pairs of physicians that looked at each case in the *Lancet* paper often disagreed on the cause of death. "The error rates between the experts account for half the malaria deaths estimated," he says.

Bob Snow, a malaria epidemiologist at the Kenya Medical Research Institute–Wellcome Trust Research Programme in Nairobi, says that whatever the limitations of the study, its estimates are "closer to the truth than the WHO figures", and that its findings are consistent with the spatial and temporal epidemiology of malaria in India. Snow notes that the paper is in line with his own team's findings that the WHO has underestimated the clinical incidence of malaria in India by a similar order of magnitude[2].

THE NEEDS OF THE MANY

Verbal autopsy is increasingly being questioned by statisticians, says Edward Fottrell, an epidemiologist at Umeå University in Sweden. Until now, verbal autopsy has been dominated by physicians, whose clinical background means that they tend to believe that diagnosing individual cases is key for accuracy, he says.

But the ultimate goal of verbal autopsy is not to make clinical diagnoses of individual cases, Fottrell points out. It is to estimate the distribution of causes of deaths, known as cause-specific mortality fractions (CSMFs), which are crucial to setting health-system and research priorities, and to monitoring the effectiveness of disease-control measures.

Pigeonholing cases into a single, accurate cause of death can amplify the errors in the CSMFs, says King. A better approach, he says, is to calculate the probabilities that various disease symptoms are associated with a death, and then aggregate those probabilities across an entire set of cases[3].

Studies show that these probabilistic computer models can give CSMFs as good as or better than physician review, but are far faster and cheaper[4]. They also overcome the issue of physician subjectivity, providing a standardized method that makes results more comparable between different studies and countries.

Many researchers are reluctant to embrace verbal-autopsy models that dispense with physician review, but attitudes may be changing. The Swedish International Development Cooperation Agency, based in Stockholm, recently recommended that the international INDEPTH surveillance network, which records births, deaths and disease within large population cohorts in 17 African and Asian countries, adopts a probabilistic verbal-autopsy model. Fottrell predicts that computer models will eventually prevail over physician review.

The ultimate goal, however, is to ensure that verbal autopsy is no longer needed, says Dye, and the WHO is helping all countries to eventually implement the gold standard of a systematic medical death certification. "That is the end point that the WHO is working towards." ■

1. Dhingra, N. *et al. Lancet* doi:10.1016/S0140-6736(10)60831-8 (2010).
2. Hay, S. I. *et al. PLoS Medicine* **7,** e1000290 (2010).
3. King, G., Lu, Y. & Shibuya, K. *Popul. Health. Metr.* **8,** 19 (2010).
4. Fottrell, E. & Byass, P. *Epidemiol. Rev.* **32,** 38–55 (2010).

Butler D. Nature 2010; 467: 1015

Universities and global health

- Those in low-resource areas
  - Lack of knowledge fuels illness and death
  - Research impacts on health
  - Develop researchers to address health questions

- For universities in wealthier countries
  - Global health is multi-disciplinary
  - Cross-disciplinary work expected and sought
  - Global health as a platform for work within and between institutions

John and Alice Crump
Ulu, Bismarck Archipelago, c 1897

TREPHINING IN THE SOUTH SEAS.

BY THE REV. J. A. CRUMP.

[PRESENTED MARCH 12TH, 1901. WITH PLATES XII, XIII.]

ABOUT eighteen months ago I wrote a short article on "Native Surgery in New Pommern" (New Britain) to the small monthly periodical issued by the Missionary Society of which I am an agent. That article has excited so much interest in the colonies—and even in Europe—that perhaps I am right in assuming that a more detailed account, containing the results of my further research, may be found of value to the cause of science and acceptable to the Anthropological Institute.

My previous inquiry was limited to New Britain itself, and in that part of the district the operation of trephining is practised on the skull solely in cases of fracture.

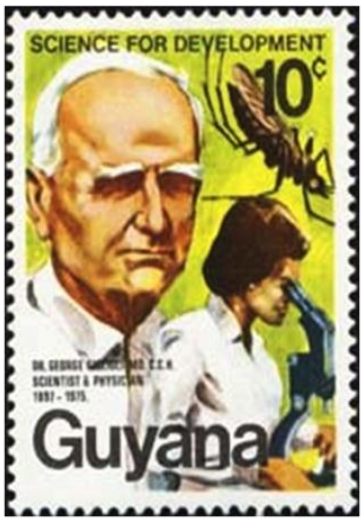
In the native fights the sling is the most formidable weapon used, a smooth stone as large as a pullet's egg being thrown with moderate accuracy but considerable force. A blow from a sling-stone is generally the cause of the fracture for which the operation is found necessary; the depressed portions of bone or hæmorrhage beneath the skull causing compression, and death almost invariably results if the injury is not attended to. Injury caused by the stone-headed club is almost instantly fatal, but the flat two-edged club is not so deadly and permits of an occasional operation.

The man who performs the operation is the wizard or "tena-papait" of the tribe or district, using a piece of shell or a flake of obsidian for a trephine.

An incision is made over the seat of the fracture generally in the shape of a Y or V, and then perhaps some loose fragment is picked out with the finger nail, and while assistants hold back the scalp, the fractured bone is scraped, cut and picked away, leaving the brain exposed to the size of half-a-crown. Then, all loose pieces having been removed, the scalp is carefully laid down and the wound bandaged with strips of the banana stalk about 4 inches wide. These strips are when dry of a spongy nature, the water which formerly filled the cells being replaced by air. Moreover the inner surface is silky to the touch and forms an admirable dressing for tender surfaces. It is astringent in its action and non-absorbent, all discharge escaping below the bandage. Sometimes a few bruised leaves are applied before bandaging. The patient is generally insensible from the time of the injury, and, if consciousness returns during the operation, soon faints away again.

In five or six days the bandages are renewed and in two or three weeks a complete recovery is the result. The number of deaths is about 20 per cent., most

Crump JA, et al. J R Anthropol Inst 1901; 31: 167-72



Section of Tropical Diseases and Parasitology.

[*November* 7, 1929.]

Paratyphoid C an Endemic Disease of British Guiana: A Clinical and Pathological Outline. *B. paratyphosum* C as a Pyogenic Organism: Case Reports.

By GEORGE GIGLIOLI, M.D.

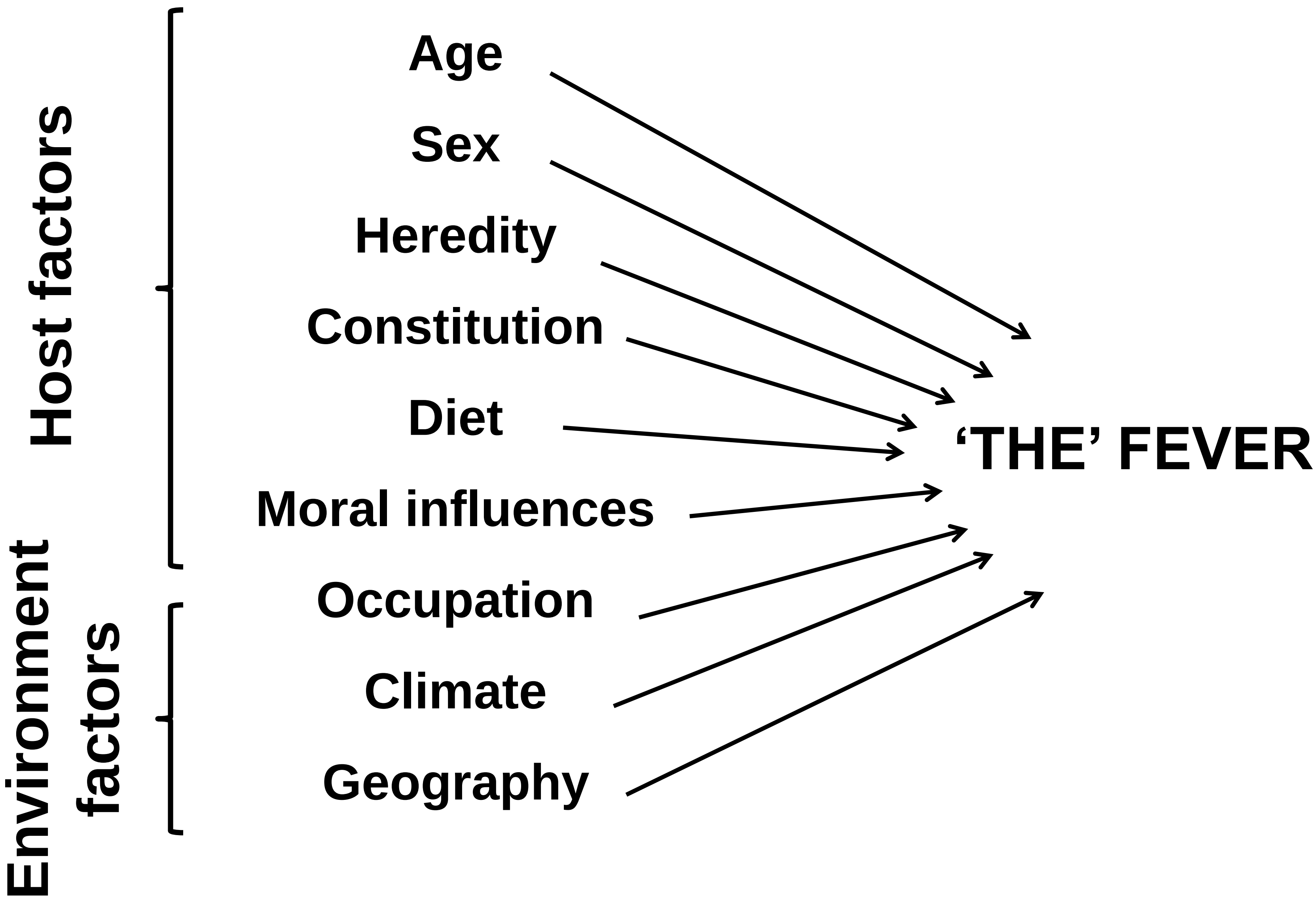
of which 80 were confirmed bacteriologically by isolation of the specific organism from the blood during life, or from the spleen post mortem. The disease appears to be endemic in the Colony, with a tendency to occasional epidemic outbursts, two of which were observed in 1924 and 1926 respectively, in coincidence with important malarial outbreaks.

DEC.—TROP. DIS. 1

Giglioli G. Proc R Soc Med 1929; 23: 165-177

AGUE & FEVER.

‘The’ fever as a single disease



‘The’ fever as a single disease

Host

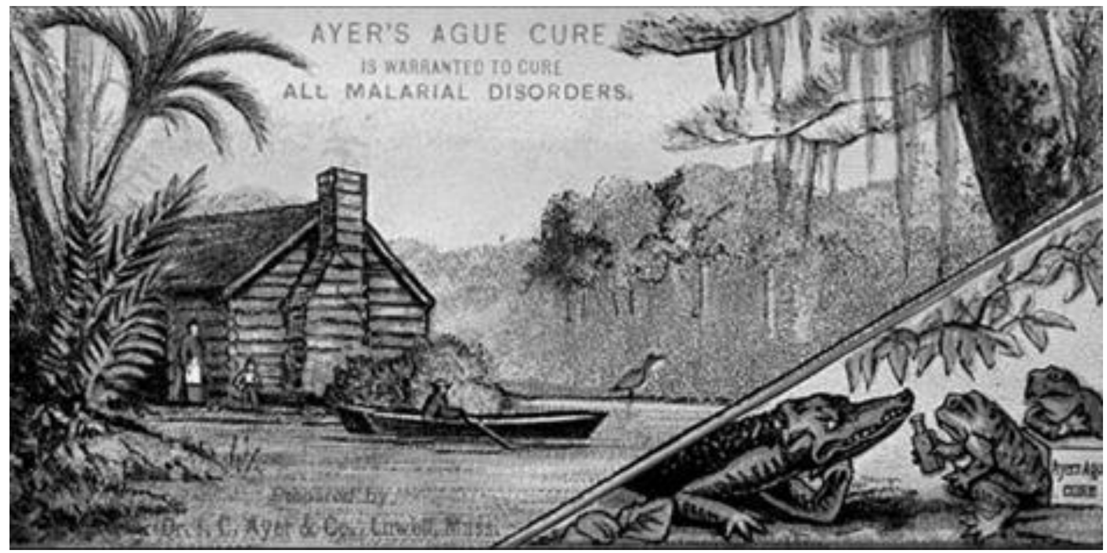
‘THE’ FEVER

Environment
‘miasma’

FARADAY GIVING HIS CARD TO FATHER THAMES;

And we hope the Dirty Fellow will consult the learned Professor.


DISMAL SWAMP
(SHE PADDLED HER OWN CANOE)
CHILL & FEVER TONIC
A GUARANTEED REMEDY FOR CHILLS & FEVERS.
DIRECTIONS
TEASPOONFUL IN LITTLE WATER EVERY 4 HOURS FOR THREE DAYS. THEN 3 TIMES A DAY UNTIL CONTENTS OF BOTTLE ARE TAKEN.
CONTAINS 10% ALCOHOL.
GUARANTEED BY THE GUI-A-COL MEDICINE CO INC. UNDER THE PURE FOOD AND DRUGS ACT JUNE 30, 1906. SERIAL NO. 34307.
PREPARED ONLY BY
THE GUI-A-COL. MEDICINE CO. INC.
PORTSMOUTH, VA.


AYER'S AGUE CURE
IS WARRANTED TO CURE
ALL MALARIAL DISORDERS.

‘The’ fever as a single disease

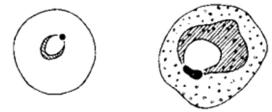
Host

‘THE’ FEVER

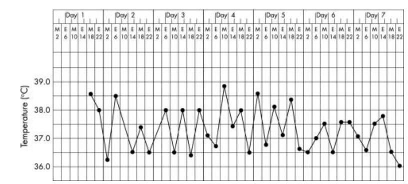
Environment
‘miasma’

Fever as a sign of many infections

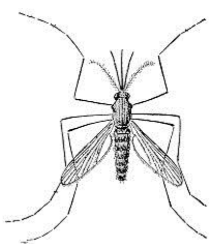
Plasmodium falciparum



Pathogen



Mosquito



Host ⟶ INFECTION

Fever

Myalgia

Other

Malaria

Environment

Mosquito breeding
tropical marshes

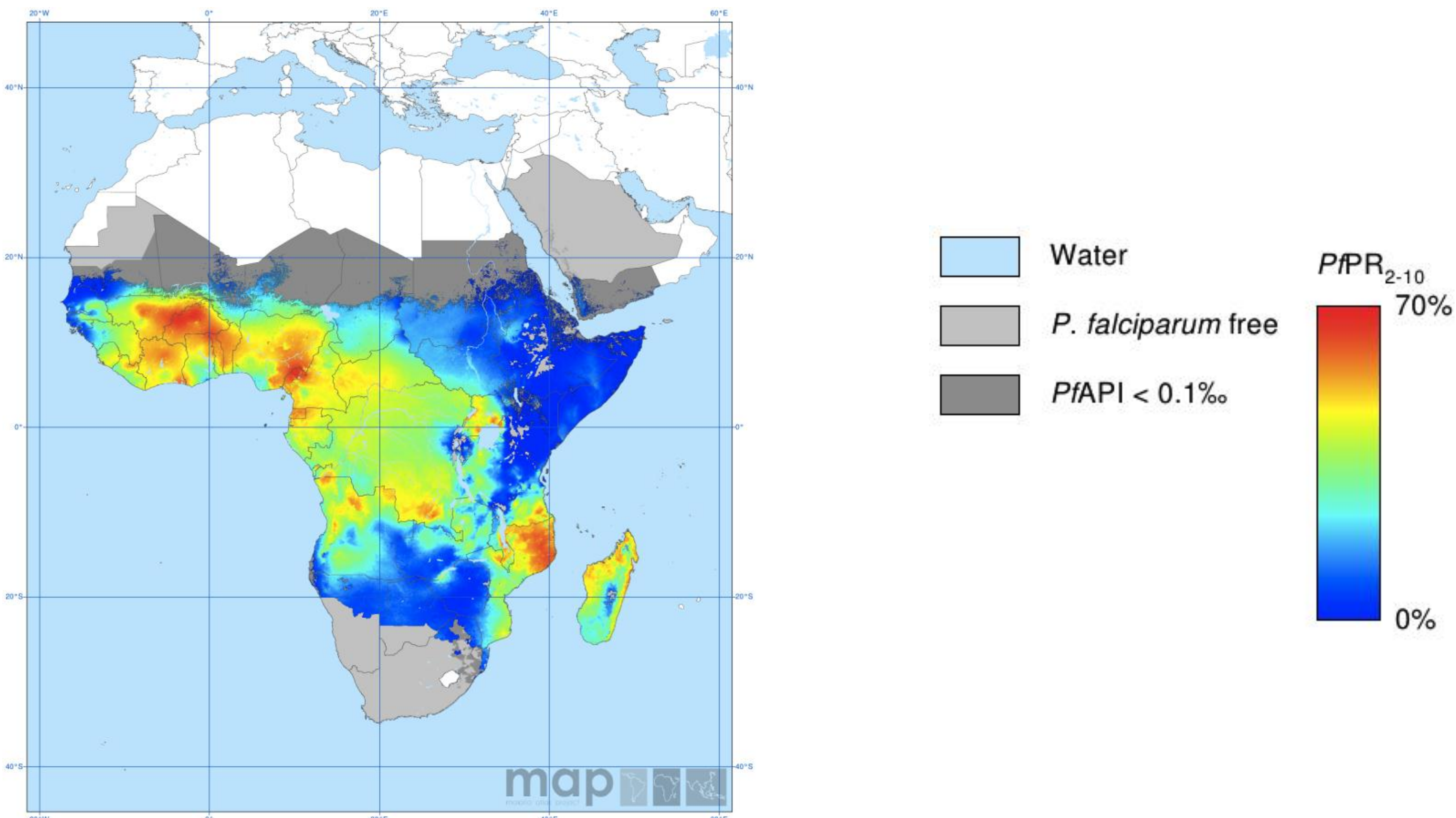
Progress with malaria control and treatment

- 1874: dichloro-diphenyl-trichloroethane synthesized
  - 1939: insecticidal property discovered

- 1934: chloroquine discovered
  - 1946: established as safe, effective antimalarial

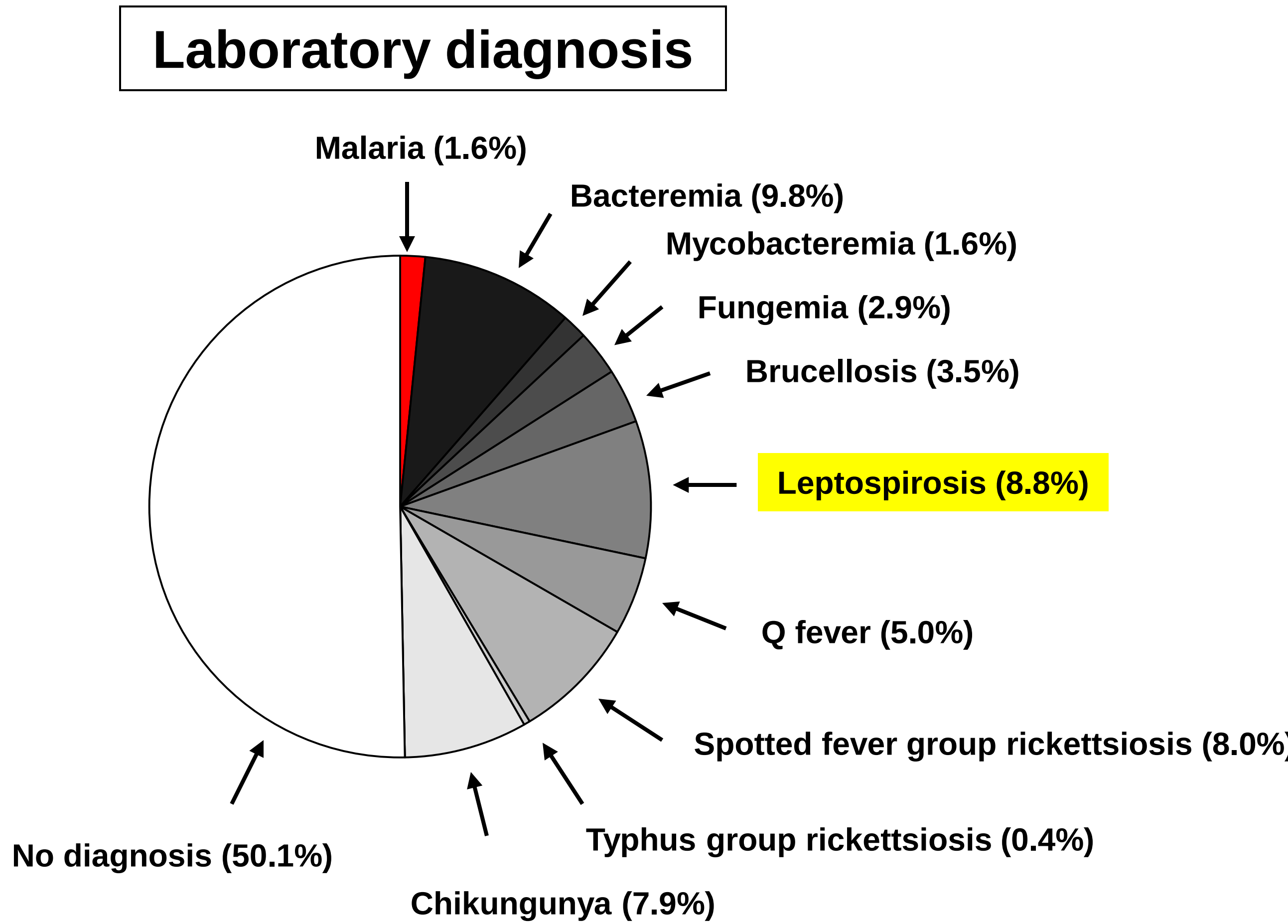
- 1955-78: Global malaria eradication
  - Residual insecticides
  - Antimalarial drug treatment
  - Surveillance
  - Succeeded in temperate zones and areas with seasonal malaria
  - Was not attempted in most of sub-Saharan Africa

Proportion of people with malaria, Africa, 2010



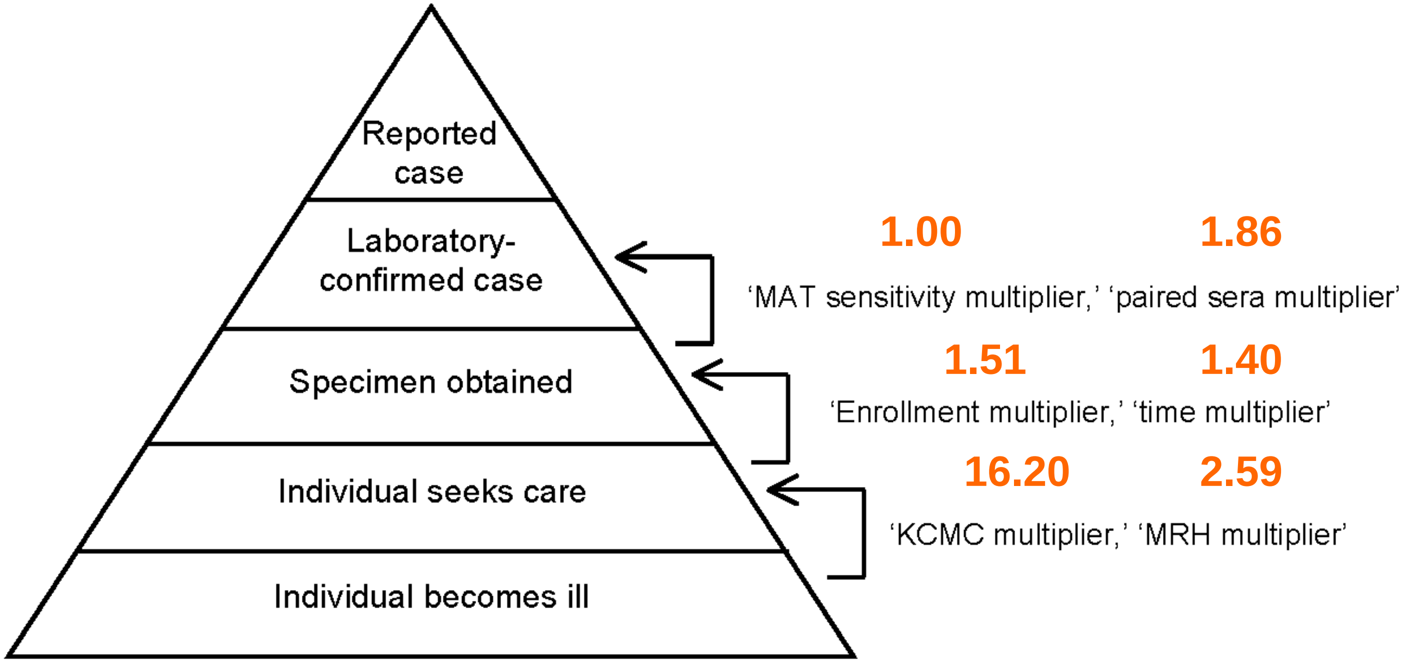
Hay, S.I. et al. PLoS Medicine 2009; 6: e1000048
www.map.ox.ac.uk

Laboratory diagnosis of febrile inpatients, northern Tanzania, 2007-8 (n=870)



Crump JA, et al. PLoS Neglect Trop Dis 2013; 7: e2324

Leptospirosis incidence ‘multiplier’ study



Leptospirosis incidence

- **Moshi Urban and Moshi Rural:**
  - **97 to 102 cases per 100,000 persons**

Clinical features

- **Febrile illness with sepsis**
  - Difficult to distinguish from other causes of fever
  - Clinical overlap with malaria and pneumonia
  - Usually not associated with diarrhea
  - Common cause of meningitis in endemic areas

- **Case fatality ratio**
  - 22-47% in hospital series

Antimicrobial susceptibility

- *Salmonella* Typhimurium
  - Ampicillin 3%
  - Chloramphenicol 100%
  - Trimethoprim-sulfamethoxazole 15%

- *Salmonella* Enteritidis
  - Ampicillin 34%
  - Chloramphenicol 39%
  - Trimethoprim-sulfamethoxazole 93%

- *Salmonella* Isangi

- Aminoglycosides

Reddy EA, et al. Lancet Infect Dis 2010; 10: 417

Global incidence of enteric and invasive NTS, 2009 and 2010

Region	Enteric NTS 2009			Invasive NTS 2010		
	Population (000s)	Cases	Incidence (/100,000)	Population (000s)	Cases	Incidence (/100,000)
North Africa/Middle East	410,800	563,000	140	446,721	3,617	<1
Africa	767,239	2,458,000	320	854,091	1,942,776	227
Asia/Oceania	1,628,815	53,610,000	3,280	1,693,046	13,920	<1
Southeast Asia	2,072,274	29,839,000	1,440	2,220,248	472,263	21
Europe	738,071	5,065,000	690	746,372	763,191	102
Americas	888,437	2,222,000	250	934,132	210,811	23
GLOBAL	6,511,638	93,757,000	1,140	6,894,610	3,406,579	49

Majowicz SE, et al. Clin Infect Dis 2010; 50: 882–889
Ao TT, et al. Emerg Infect Dis 2014; in press

Not a diagnostic challenge