Rickettsioses: Diagnosis & Epidemiology

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Introduction

- Scrub typhus, Murine typhus and Spotted fever group rickettsioses re-emerging causes of acute febrile illness in south and southeast Asia

- Obligate intracellular bacteria that comprise a large group of tick-, mite-, and flea borne zoonotic infections that are caused by closely related rickettsiae

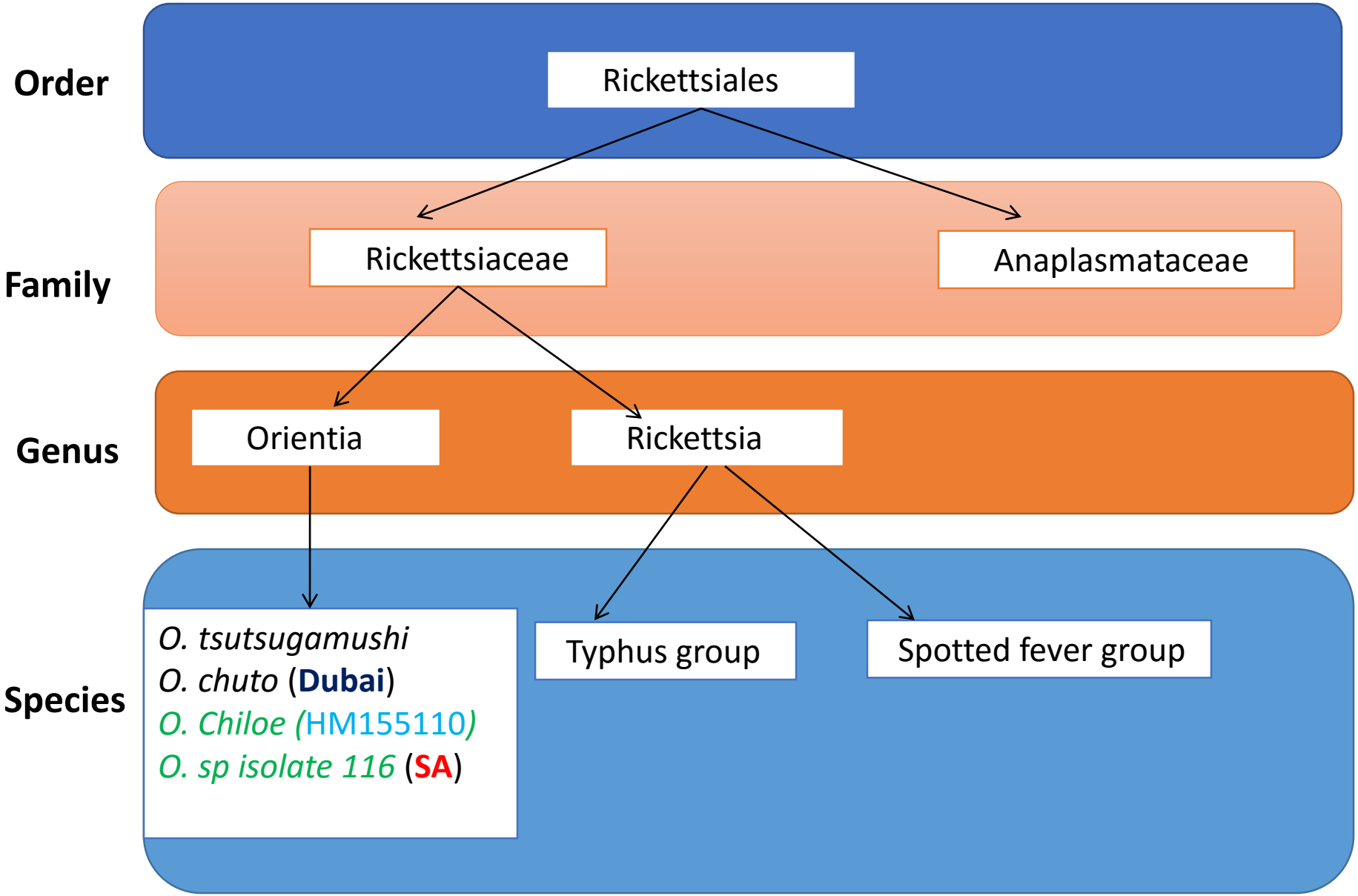
- **High morbidity and mortality: ST>SF>MT**
  - low index of suspicion
  - underdiagnosis
  - untreated cases

Generic Characteristics Used for Classification of Rickettsiae

Vector	Disease	Geography & Impact
• Louse-borne • Flea-borne • Mite-borne • Tick-borne	• Typhus • Spotted fever • Scrub typhus	• Epidemic • Endemic • Sporadic

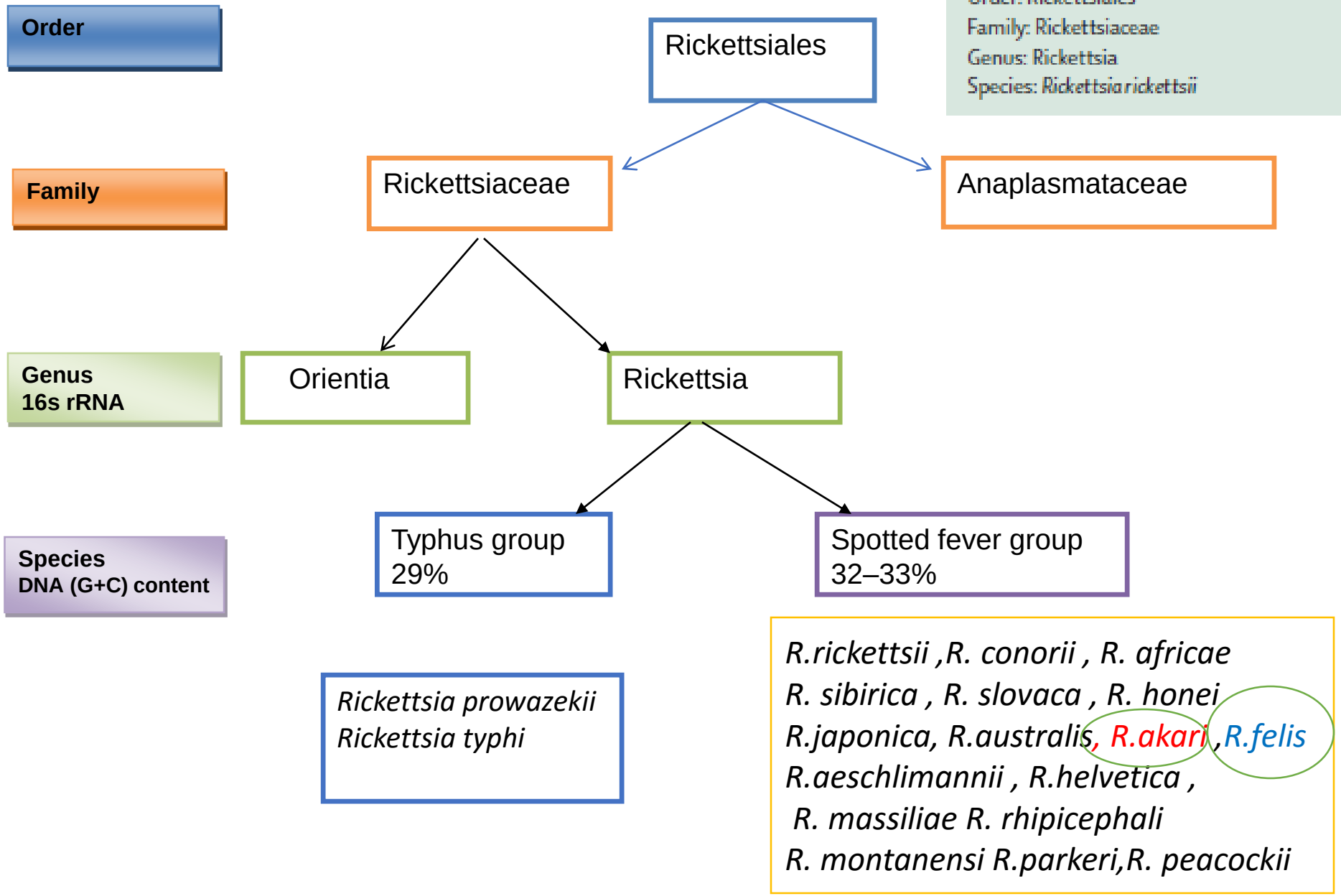
Syndromic classification

Taxonomy


Order
Rickettsiales
Family
Rickettsiaceae
Anaplasmataceae
Genus
Orientia
Rickettsia
Species
O. tsutsugamushi
O. chuto (Dubai)
O. Chiloe (HM155110)
O. sp isolate 116 (SA)
Typhus group
Spotted fever group

Taxonomy

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Family: Rickettsiaceae
Genus: Rickettsia
Species: *Rickettsia rickettsii*



Laboratory diagnosis: Rickettsioses

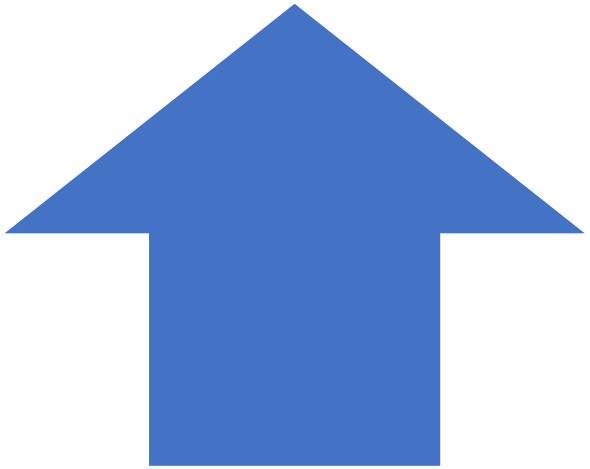
Laboratory Diagnosis

Presence of the typical clinical findings, epidemiological background, raise the suspicion of the disease, while awaiting laboratory confirmation.

Early diagnosis of Rickettsioses

Chiefly on clinical suspicion

No reliable diagnostic test is available on the early phase of the illness.



Clinical clue for diagnosis

Eschar:

- Not always present (1-97%)
- Covered areas of the body
- Single / multiple
- Detection of eschars:

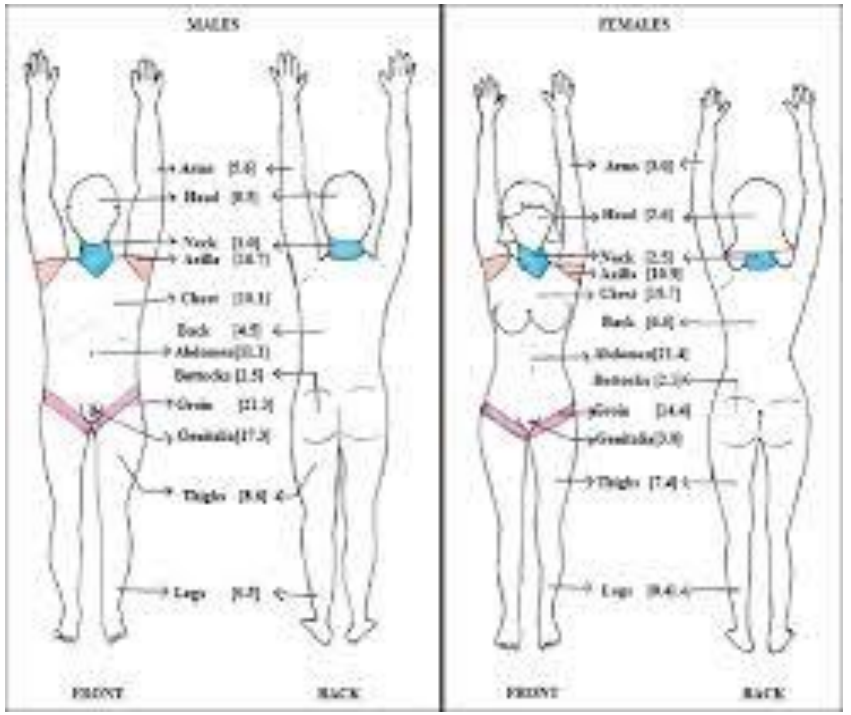
Eschar

- Painless (cigarette burn)
- Not always present (7-97%)
- **Thorough examination: Needed**
- Our eschar rate: **≈60%**
- When present: Appears before disease manifests

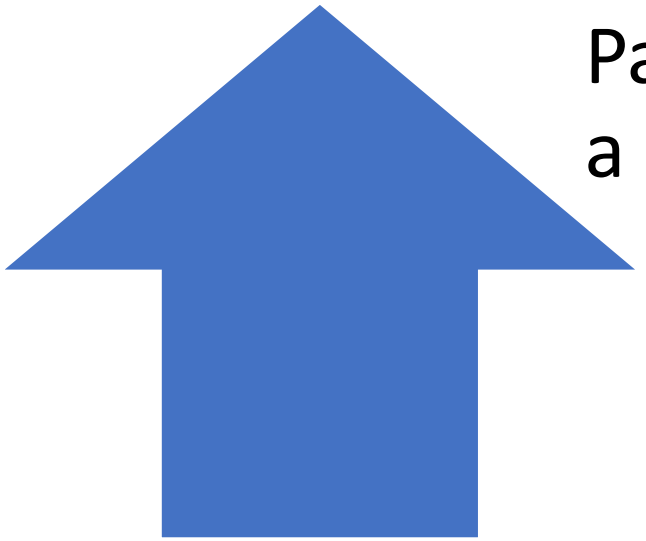
qjmed.oxfordjournals.org

- Other conditions with eschar
- Spotted fever
- Spider bites
- Insect bites
- Scab due to trauma
- **Inexperienced: Skin infections**
- **Note: When in doubt consult the dermatologist**

(Kundavaram AP 2013)

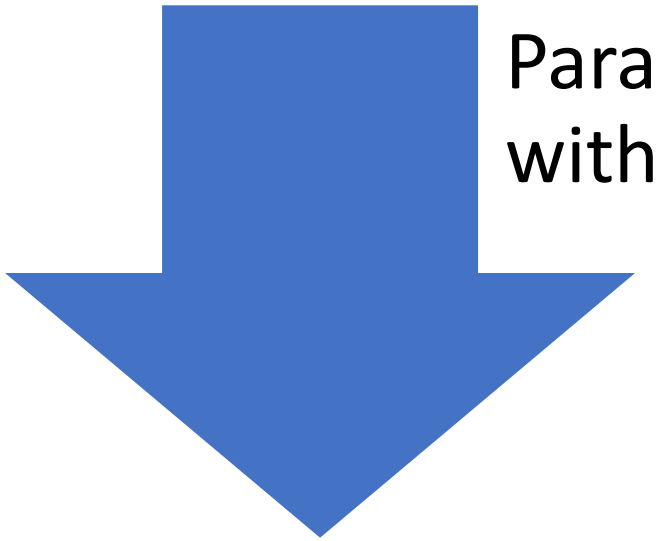


Clinical clue for diagnosis



Parameters compatible with a diagnosis

- Eschar
- Regional lymphadenopathy
- Total fever ≥8 days

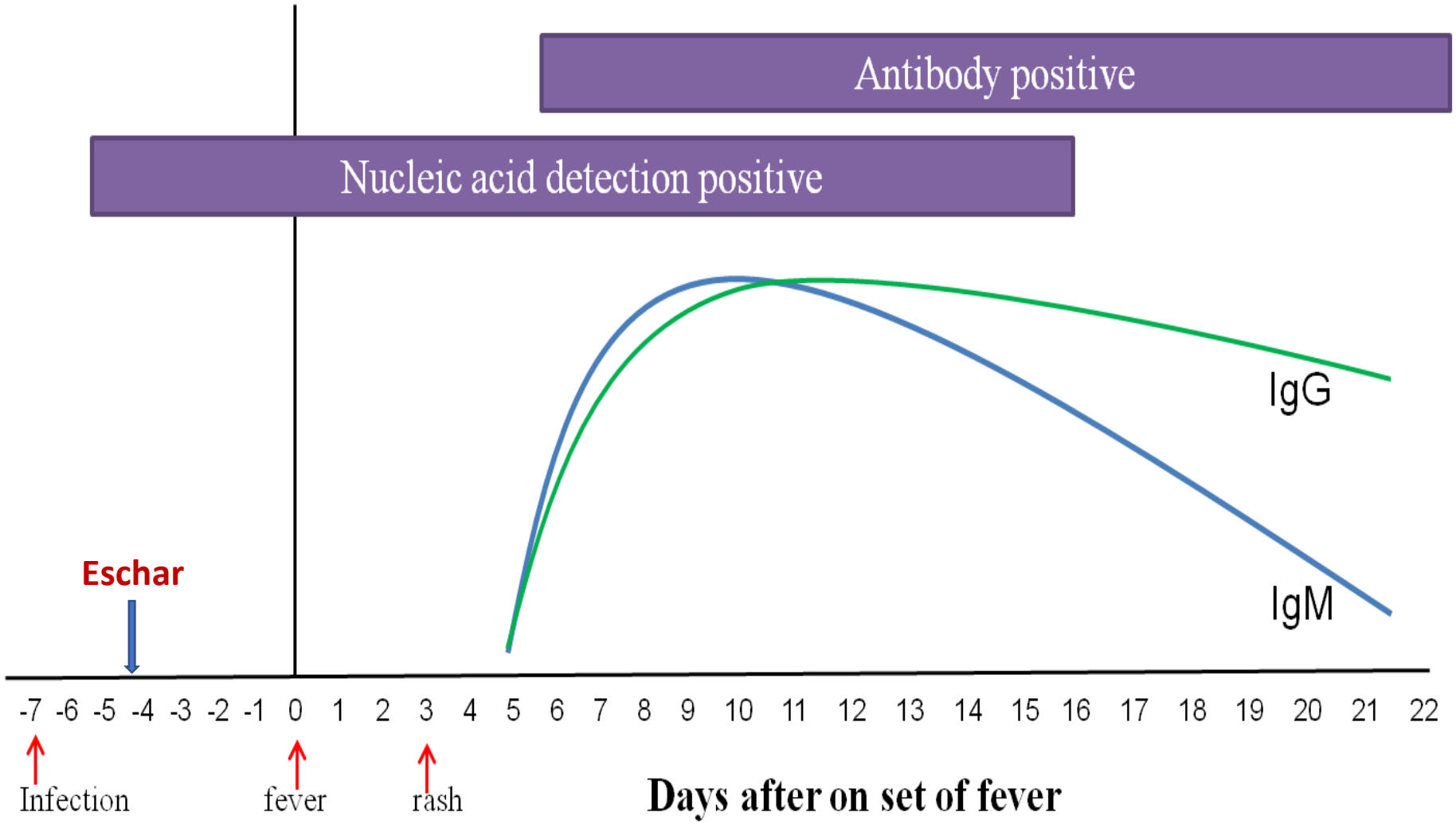


Parameters incompatible with a diagnosis

- Bone pain (dengue)
- Bleeding manifestations (dengue)
- Loose stools (enteric fever)

Chao CC & Ching WM,
NMRC, MD, USA

Time course of rickettsial infection



Confirmation of diagnosis

- Definitive methodS
- Isolation of agent: Cell culture or animals
- Antigen detection
- Molecular assays: PCR & LAMP
- Serological assays:
- Four fold rise in titre
- Seroconversion

Alternate: More practical

- Acute undifferentiated febrile illness

  (Malaria negative & blood culture negative)

- **Eschar/rash**
- Defervescence of fever within 48 hours of

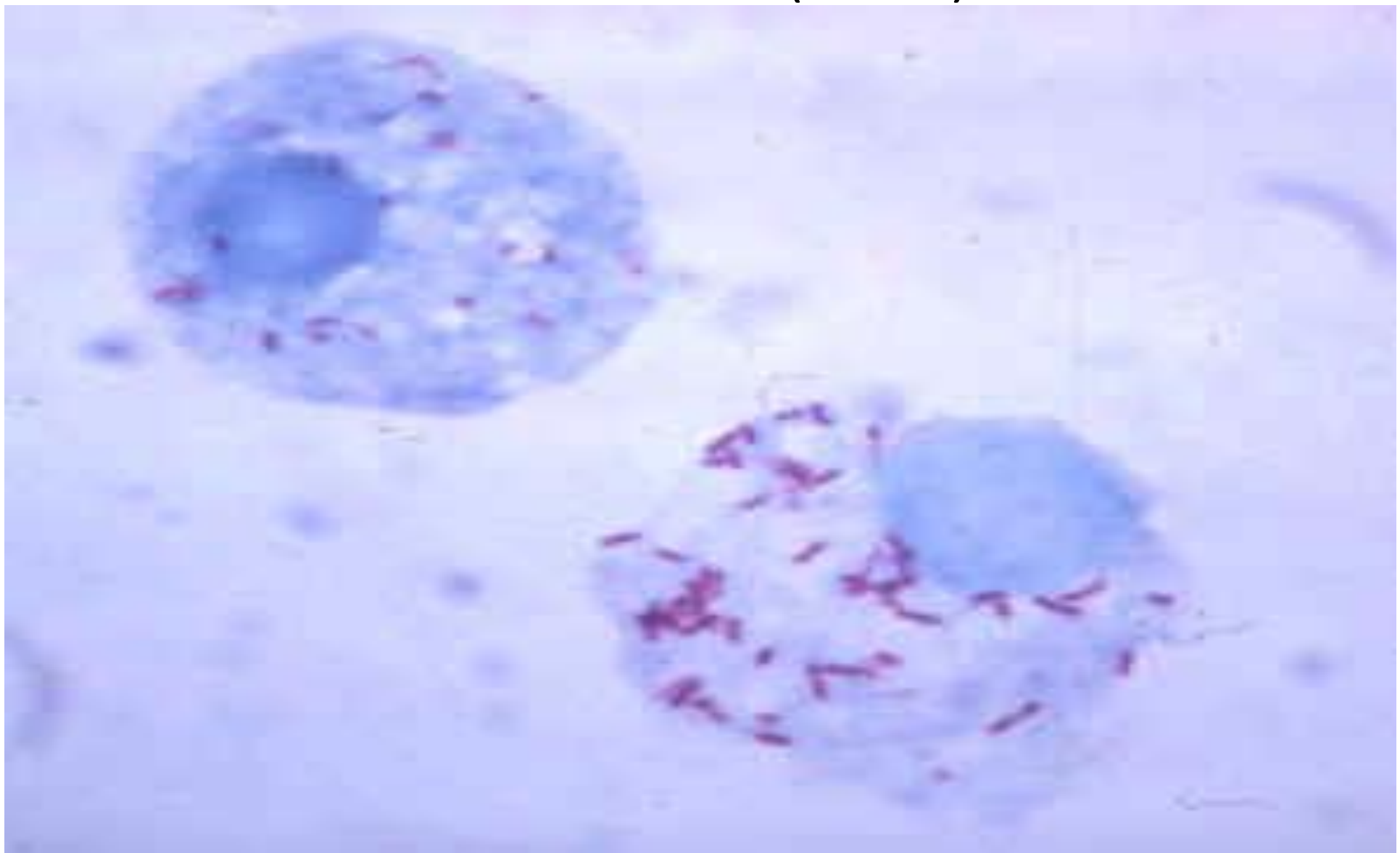
  therapy

  and

- Detection of significant level of IgM antibodies: **ELISA/IFA /RDT**

Stains

Giminez stain – rickettsia (CDC)

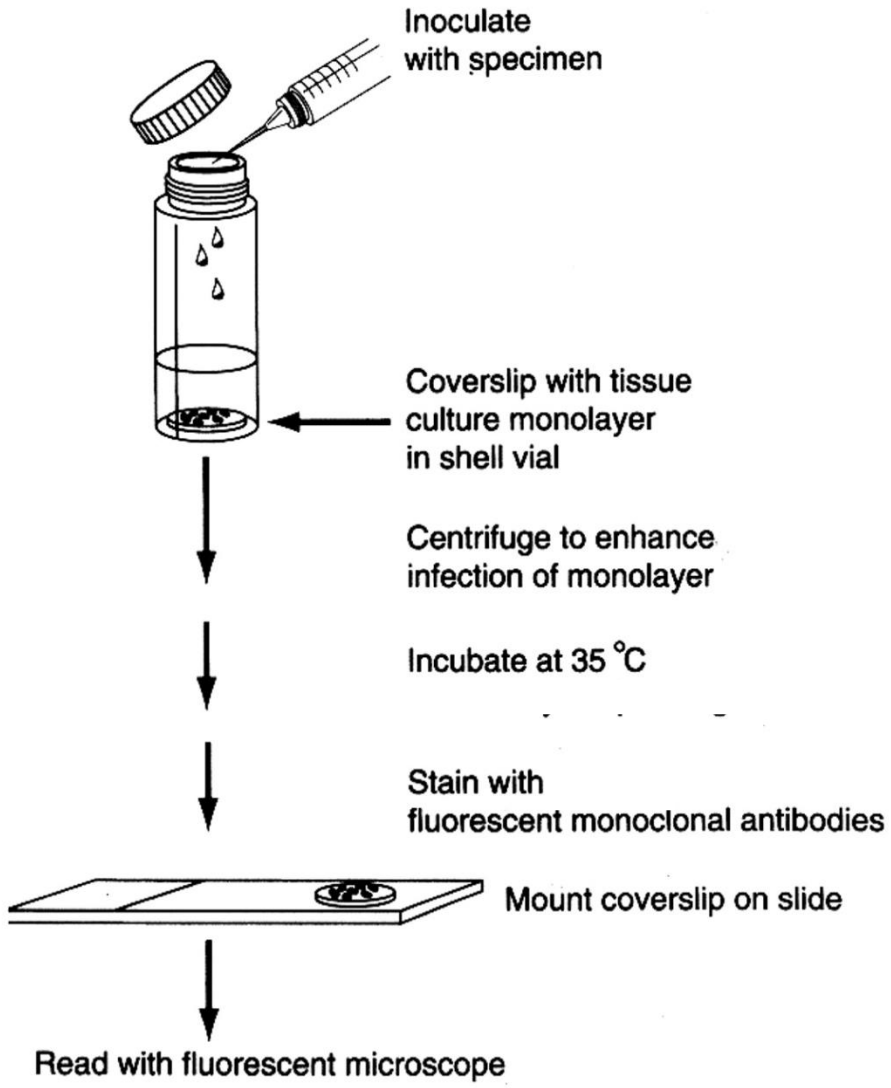


Culture

- Most definitive diagnostic method: **Gold std**
- **BSL III facilities required: Under query by experts**
- Cell lines: Vero, HEL, MRC5, L929
- Embryonated egg: Yolk sac inoculation
- Animals: mice/ guinea pigs
- Culture: Reference labs
- Time to positivity: days to weeks/months
- Note: Ultimate method for diagnosis & detection of new species

Shell vial culture

- Specimens: Human blood & Skin biopsy
- Detection of the rickettsiae - cell staining and immune and molecular Detection
- Faster isolation of rickettsiae using L929 and Vero cells in comparison to HEL or MRC5
- Centrifugation determines sensitivity
  Sensitivity - 35-50%
  Time taken: 48-72 hours
- Drawback: Non-viable on subsequent passage



Shell vial culture

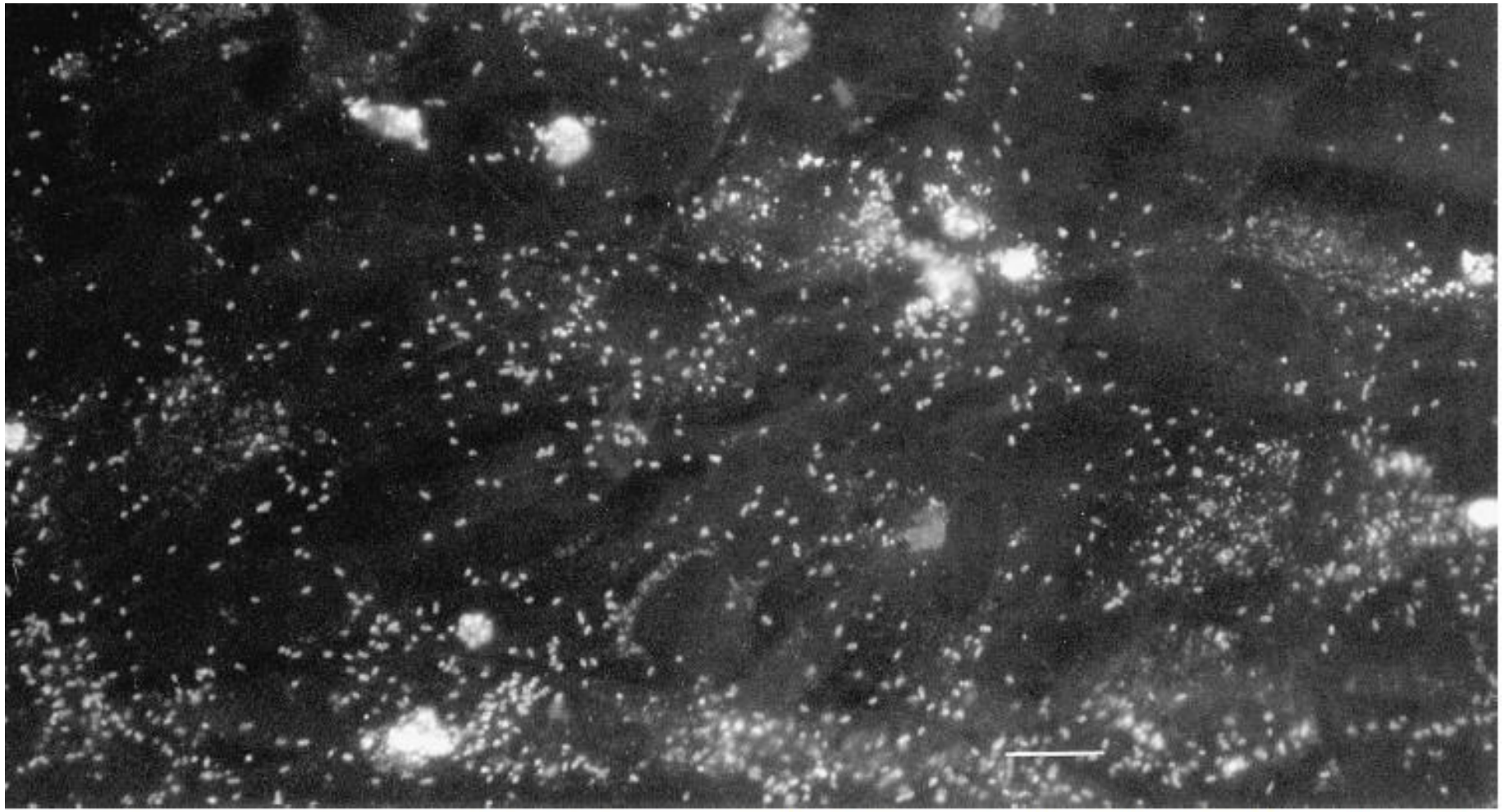
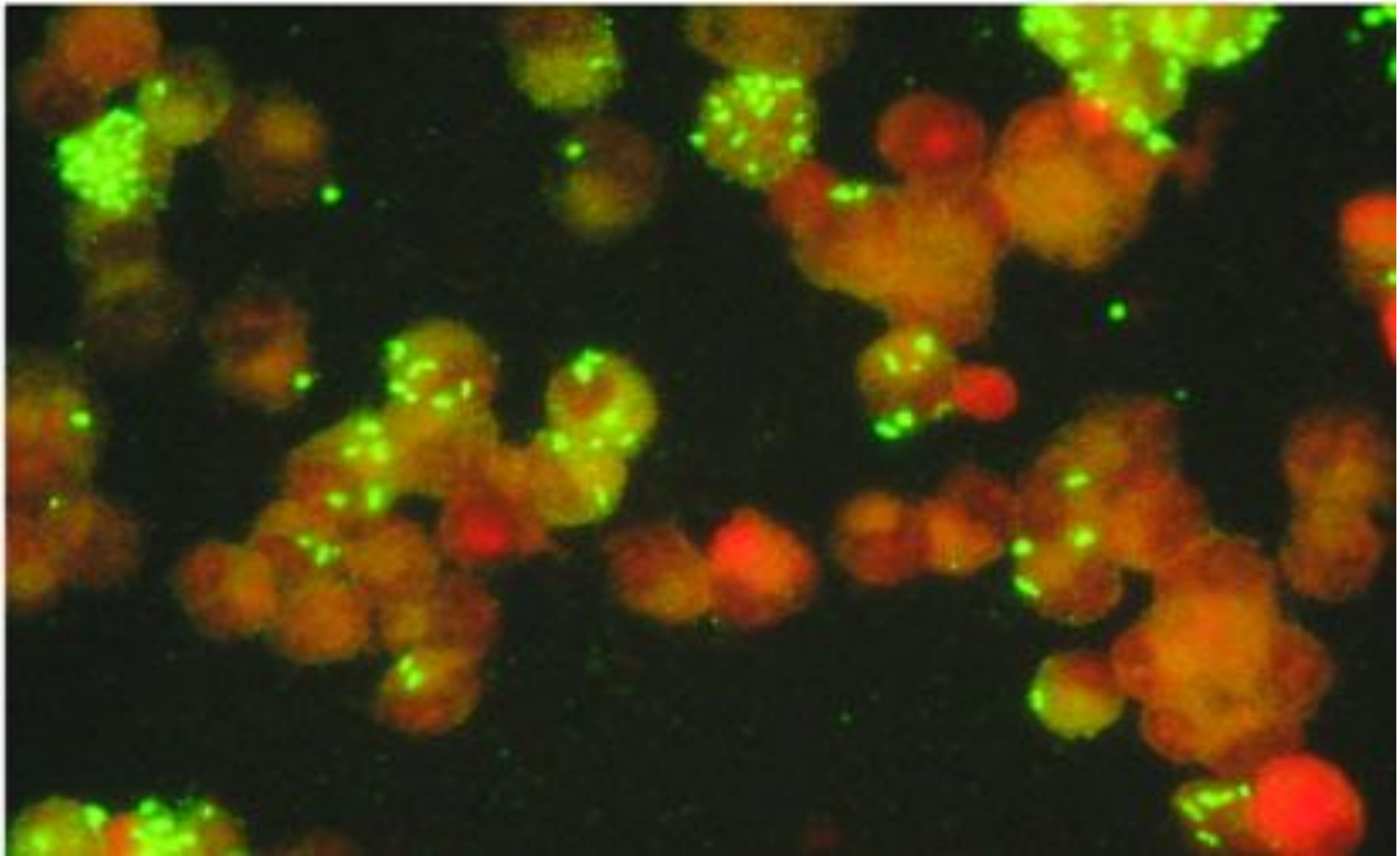


FIG. 3. Indirect immunofluorescence staining of *R. conorii* in HEL cells by using the shell vial assay. Bar, 4 μm. Magnification, ×400.

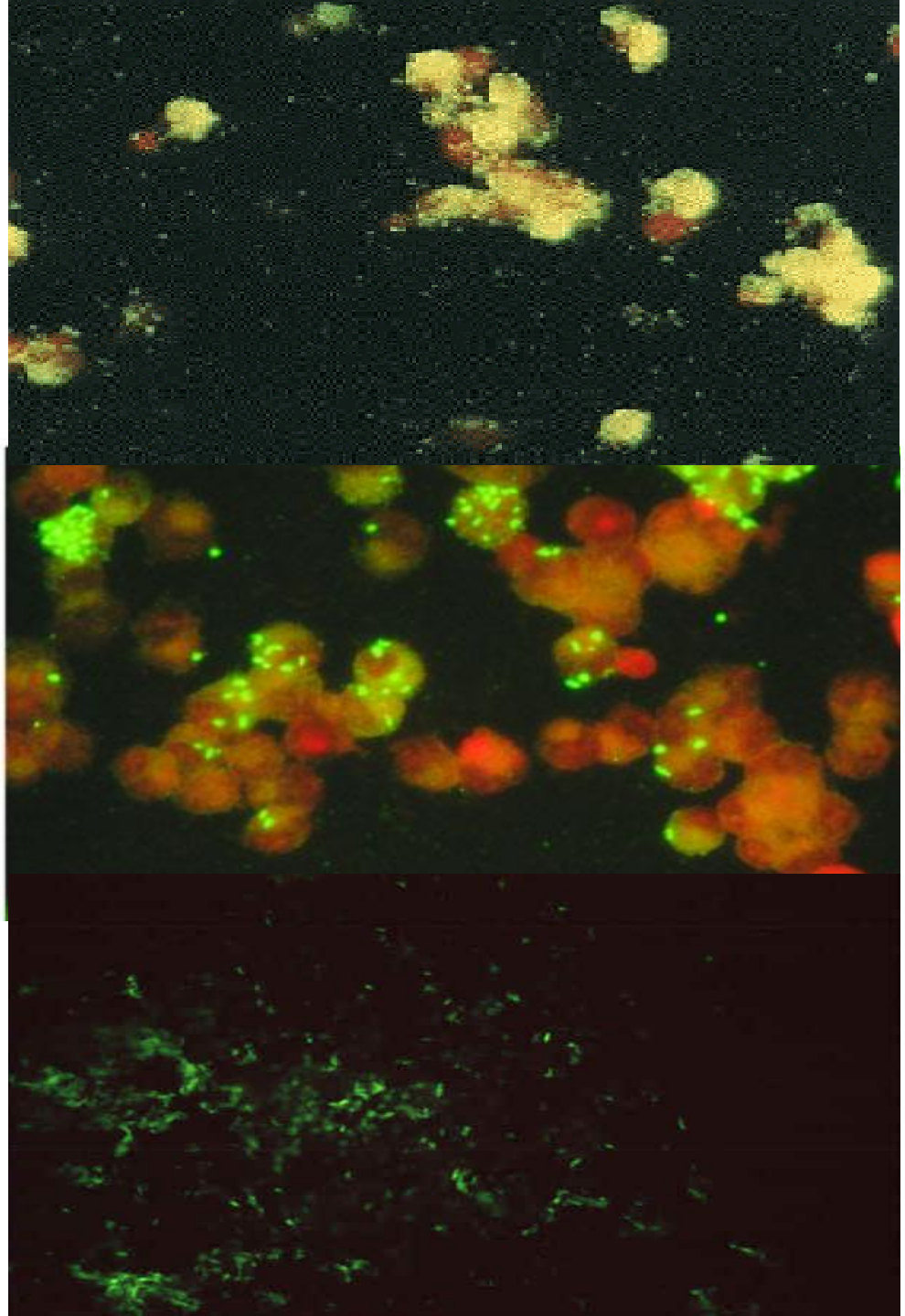
IFA



IFA

- Antigen source:
- Ref labs/commercial
- Commercial kits:
- Very expensive

- Results:
- Subjective
- COV
- No consensus
- **True accuracy:**
- **Imperfect**



IgM ELISA

- Advantages:
- Less expensive
- Reliable automation available
- Values objective (OD)
- Uses recombinant antigens: **Cocktail**
- Large numbers of sera can be screened

- Disadvantages:
- Cut off value has to be determined
- Sero-conversion more reliably demonstrated
- Cross reactions
- Commercially obtained

RDTs

- Rapid
- POCT
- Currently more expensive than ELISA **(Cost/test)**

Test	n	Sens (%)	Spec (%)	Reference
IgM ICT	161	73	97	Lim C 2015
IgM Dot EIA	129	100	94	Prakash JA 2006

STIC (Scrub typhus Infection criteria)

- Proposed and used by Blacksell, Paris, et al

- **STIC components**
- *O tsutsugamushi* isolation
- Two of three PCRS positive (56 kDa, 47 kDa & *groEL*)
- Admission IFA titre is ≥12,800
- Rise in titre (4 fold admission vs convalescence)
- STIC positive: Any one criteria positive

Assessment of STIC & components

- Lim C 2015
- Low specificity of STIC due to low specificity of IFA IgM
- Bayesian LCM analysis:
- STIC & IFA IgM: Poor reference standards

- Estimation of accuracy of diagnostic tests
- Bayesian LCM recommended: Why

- **Note**: **Eschar & scrub typhus: Sens: 43%; Spec: 99%**

Scrub typhus diagnostics summary (Prakash JA 2017)

Type of assay	Sensitivity (%)	Specificity(%)
Cell culture	**5 - 56**	**100**
Antigen detection	65- 100	100
IgM IFA	70-100	84-100
IgM ELISA	70-100	87-100
IgM ICT	47-99	95- 100
56 kDa	Conventional: 0-96 Nested: 16-100 qPCR: 65-73	Conventional: 100 Nested: 88-100 qPCR: 100
47 KDa	Conventional: 3-7 Nested: 81-85 qPCR : 63-81	Conventional: 100 Nested:100 qPCR: 90-100

Co-infections described in scrub typhus

(Prakash JA, 2017)

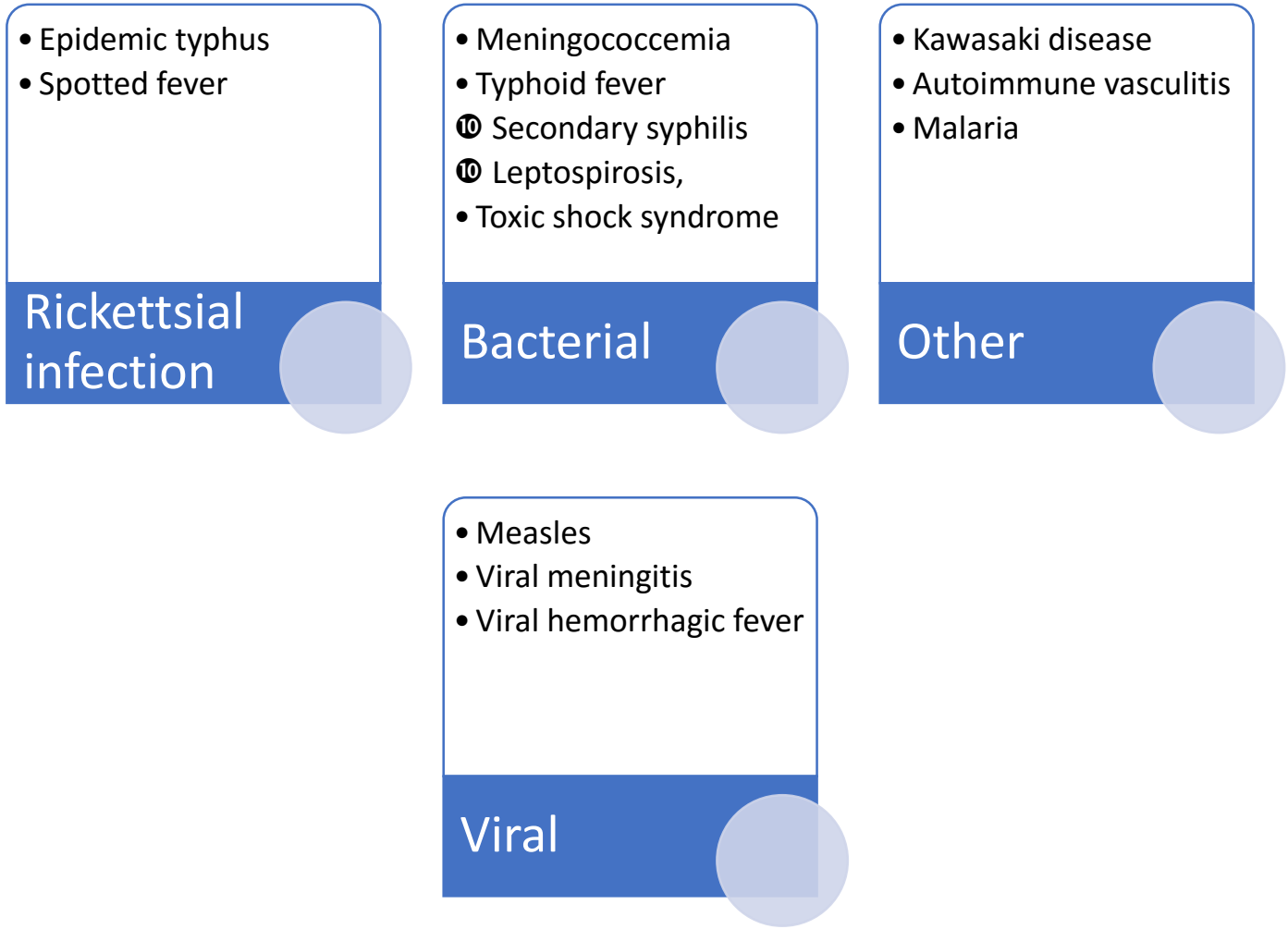
Grade	Tests	Sensitivity	Specificity	Diseases / pathogens described
1	Culture, NAATs & antigen detection	Poor	Best	TB, Malaria, leptospirosis, dengue, murine typhus, spotted fever & typhoid,
2	Seroconversion Rise in titre WB positive	Good	Good	Leptospirosis, dengue, JEV & Q fever
3	Single serological value >COV	Best	Poor	Malaria (smear) Dengue (NS1 Ag) Leptospira (MAT) Spotted fever (IFA) Murine typhus (IFA)

Differential diagnosis: Spotted fever

Bacterial diseases	Viral diseases	Other condition
✓ Meningococcemia	✓ Measles	✓ Kawasaki disease
Leptospirosis	✓ Dengue	✓ Vasculitis
✓ Typhoid fever	Chikungunya	✓ Thrombotic thrombocytopenic purpura
Secondary syphilis	✓Infectious mononucleosis	✓ Adverse drug reactions
✓ Infective endocarditis	✓Varicella	✓ Steven- Johnson syndrome
✓ Scrub typhus	Viral haemorrhagic fever	✓ Henoch- schonlein purpura
	Protozoal diseases Malaria	

Modified from MMWR / May 13, 2016 Centers for Disease Control and Prevention

Differential diagnosis: Murine typhus



Vellore case definition: Scrub typhus

ST case: If any one of the following observed

- ST qPCR (47kDa) positive
- Eschar positive and ST IgM ELISA positive (OD≥1.0)
- Eschar negative, ST IgM ELISA positive (OD≥1.0) and defervescence of fever within 72 hours of initiation of therapy.

- Sensitivity & specificity: 100 % (BLCM analysis)

- Note:
- ST IgM ELISA pos ± eschar + Rx response detects 90% cases
- ST IgM ELISA positive after 5^{th} day
- ST qPCR positives: Majority before 10^{th} day (whole blood)

Spotted fever diagnostic algorithm: Vellore

- **Suspected case**:
- AUFI with rash ± eschar; fever ≤ 20 days
- **Probable case**:
- AUFI + rash ± eschar
- Negative for drug rash & viral exanthem
- **Confirmed case**: Either one of the following
- Probable case + ompA qPCR positive
- Probable case + SF IgM ELISA positive with fever defervescence (≤ 72 days)
- Note: **This is the current algorithm is subject to change with new knowledge**

Epidemiology of Rickettsioses

Epidemiology (Luce-Fedrow A *et al* 2018)

Current epidemiology (Jiang & Richards- 2018)

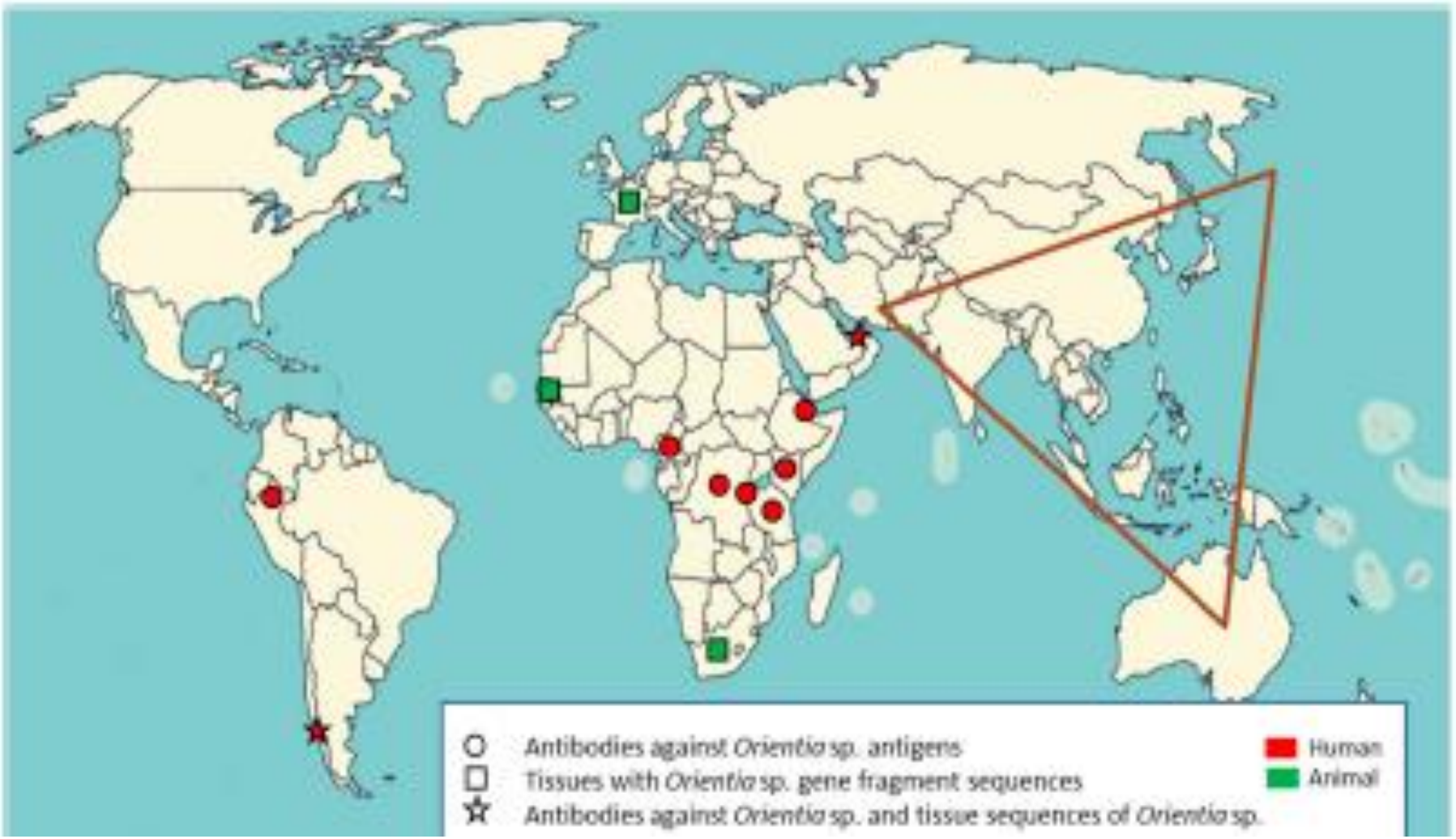
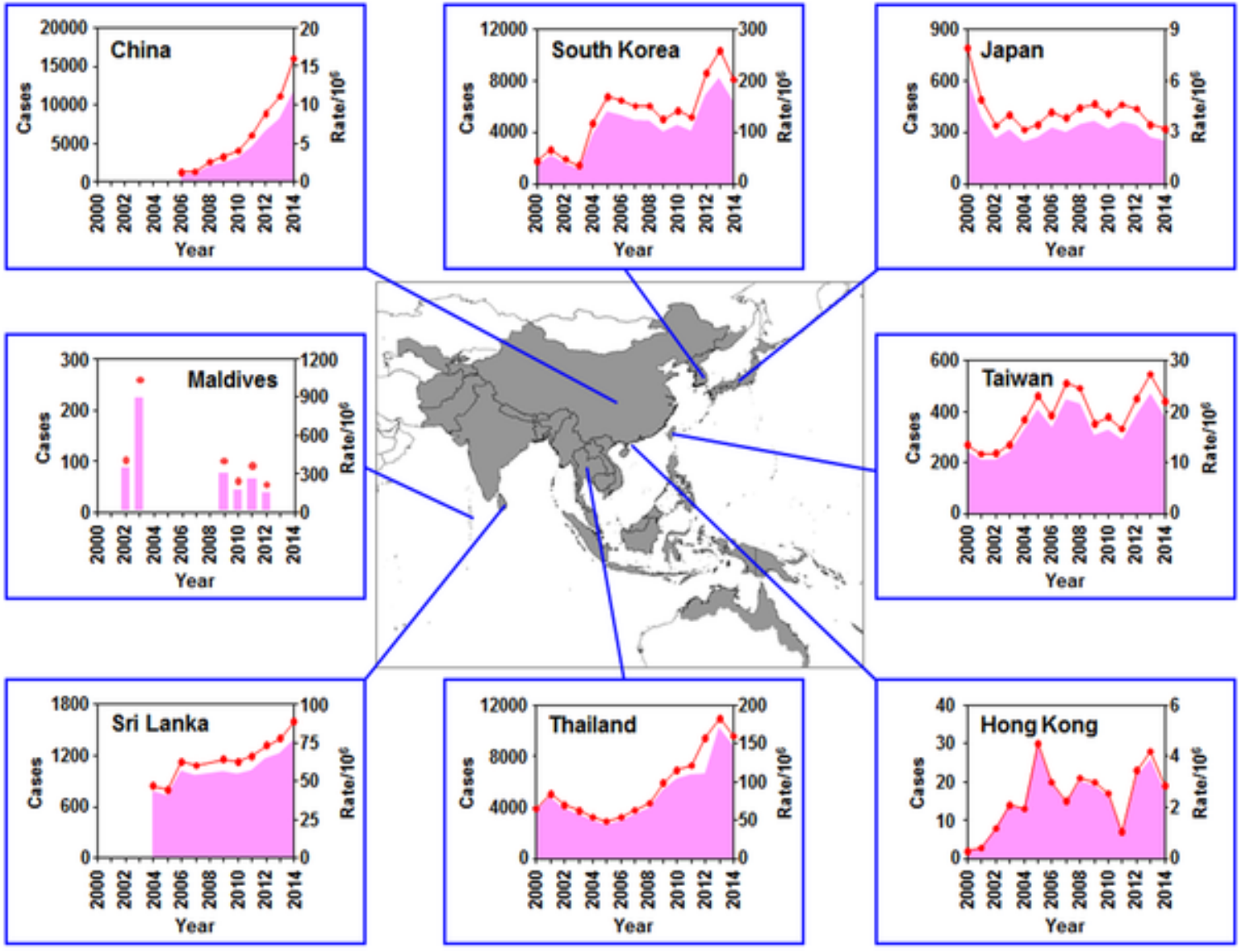


Fig 1. Epidemiological trends of scrub typhus incidence in several endemic countries from 2000 to 2014.



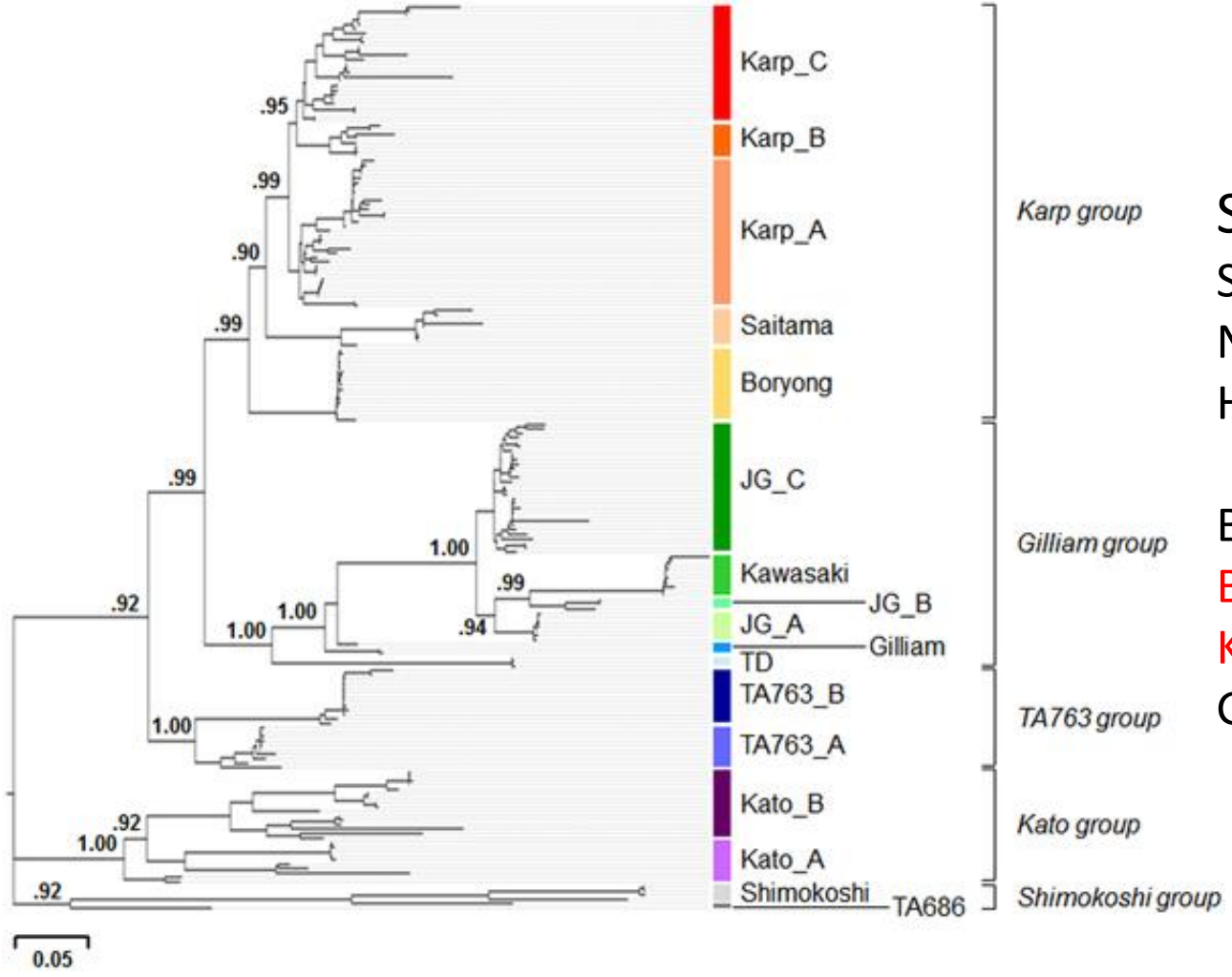
Vellore Prevalence (Plos NTD 2019)

30 to 130/100,000

Kim G, Ha NY, Min CK, Kim HI, Yen NTH, et al. (2017) Diversification of Orientia tsutsugamushi genotypes by intragenic recombination and their potential expansion in endemic areas. PLOS Neglected Tropical Diseases 11(3): e0005408. https://doi.org/10.1371/journal.pntd.0005408
https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005408

PLOS | NEGLECTED TROPICAL DISEASES

Fig 2. Phylogenetic analysis of 206 tsa56 genes and their classification into genotypes and genogroups.



ST phylogeny: 56 kDa (GMV etal EID 2015)

S India: Kato: 78 %, Karp:19%

NE India: Kt 57%; Kp 23%

HP: Kt: 43% & Kp: 43%

Biswal 2018 Chandigarh

Boryong: 63%

Kp; 23.6%

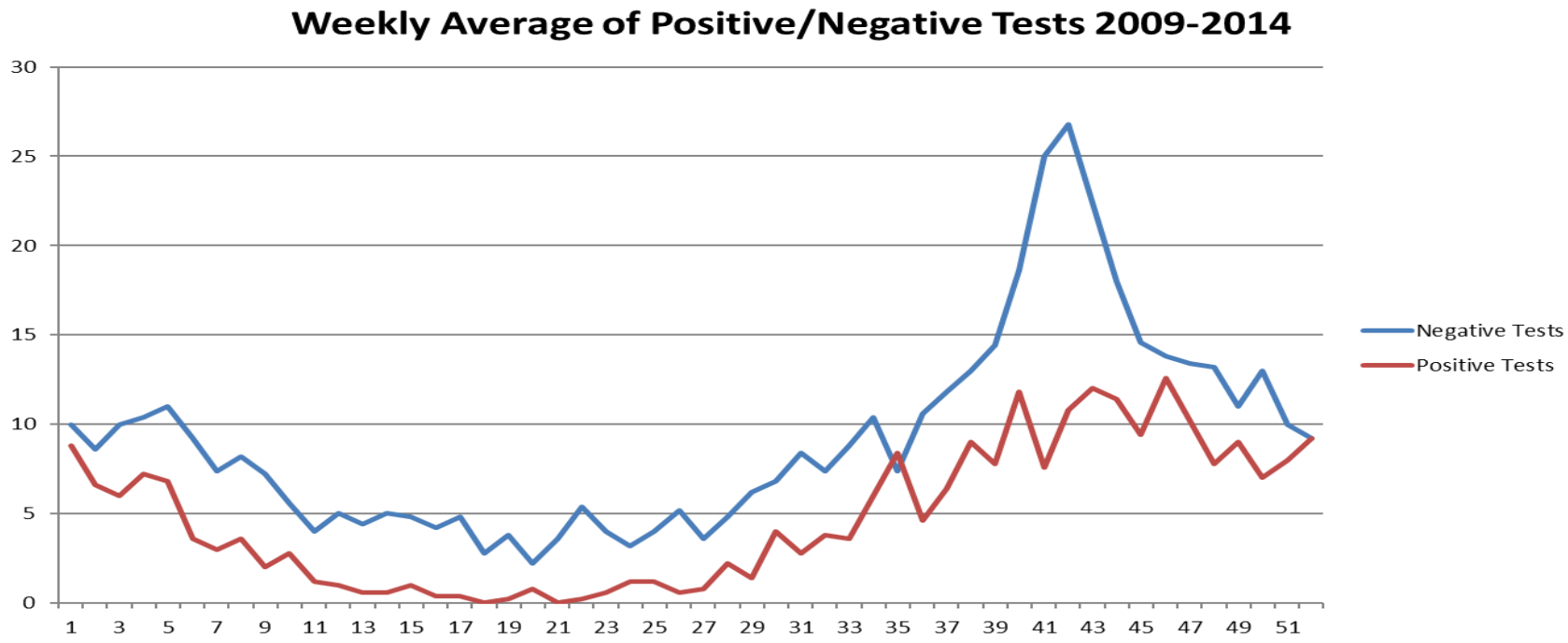
Gilliam: 12%

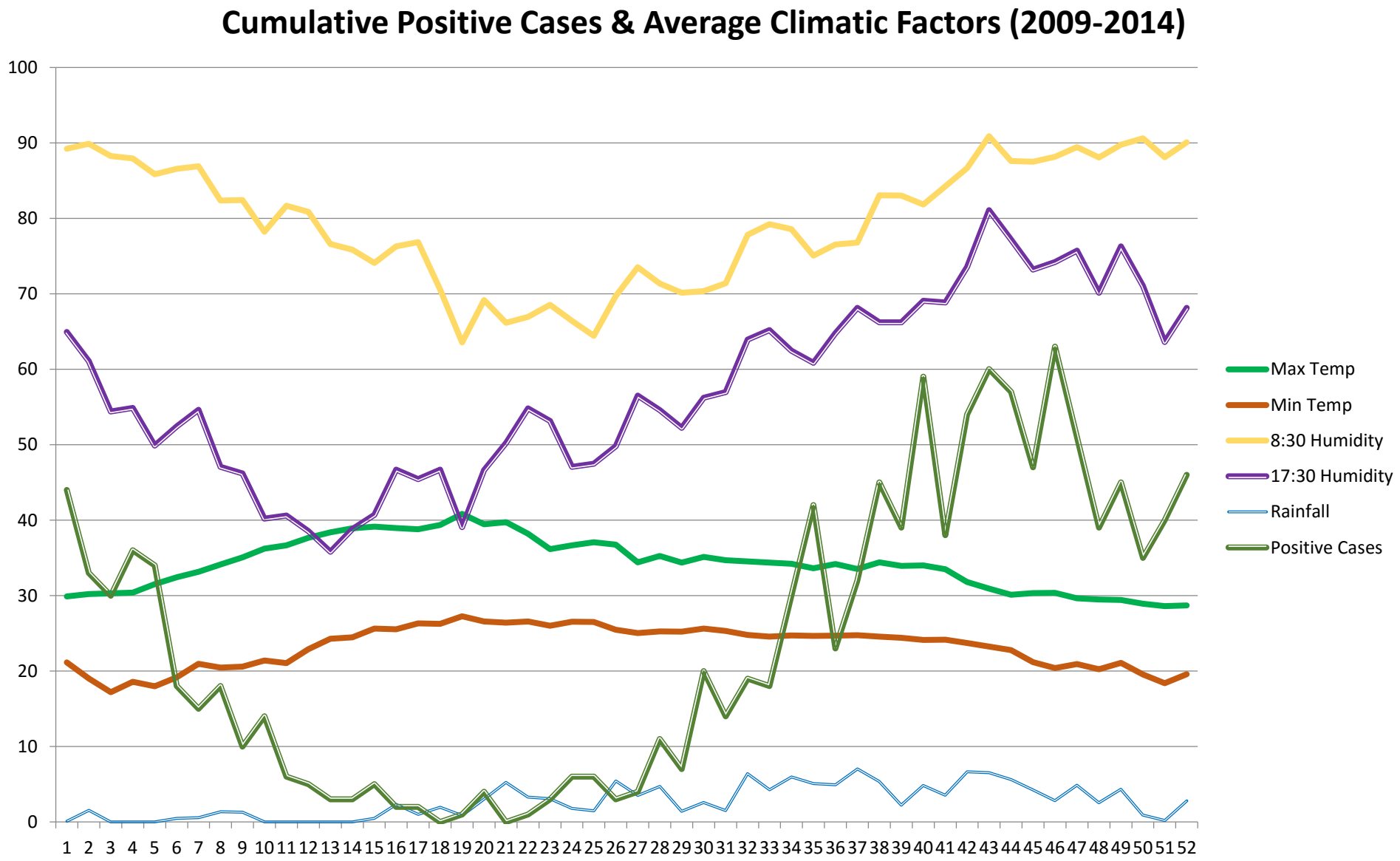
Kim G, Ha NY, Min CK, Kim HI, Yen NTH, et al. (2017) Diversification of Orientia tsutsugamushi genotypes by intragenic recombination and their potential expansion in endemic areas. PLOS Neglected Tropical Diseases 11(3): e0005408. https://doi.org/10.1371/journal.pntd.0005408
https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005408

PLOS | NEGLECTED TROPICAL DISEASES

Rickettsial epidemiology: India

- Evidence of disease present (India & TN)
- Seasonal variation well documented (Mathai E)
- In TN: Cooler months (August-February)

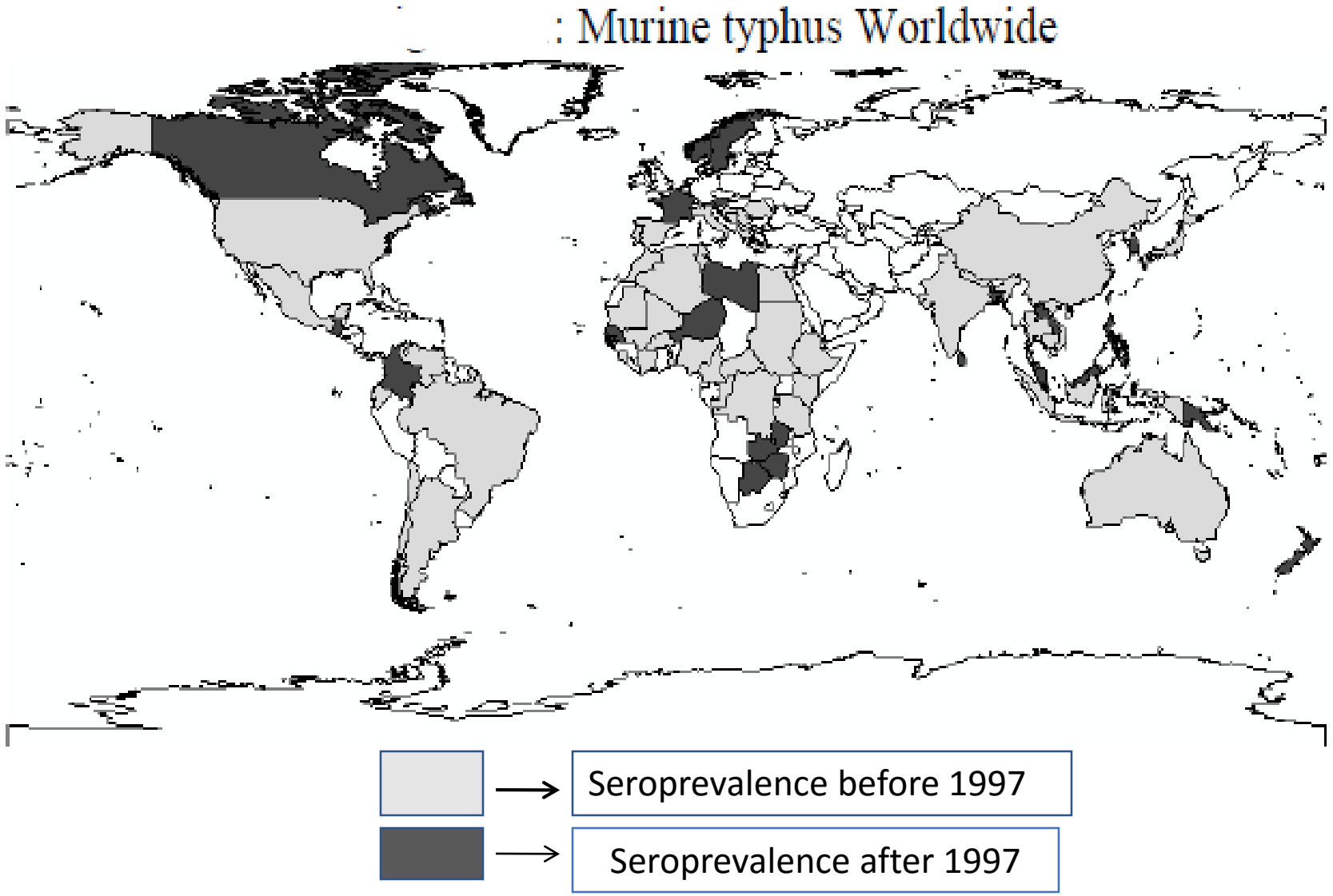



Cumulative Positive Cases & Average Climatic Factors (2009-2014)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
Max Temp
Min Temp
8:30 Humidity
17:30 Humidity
Rainfall
Positive Cases

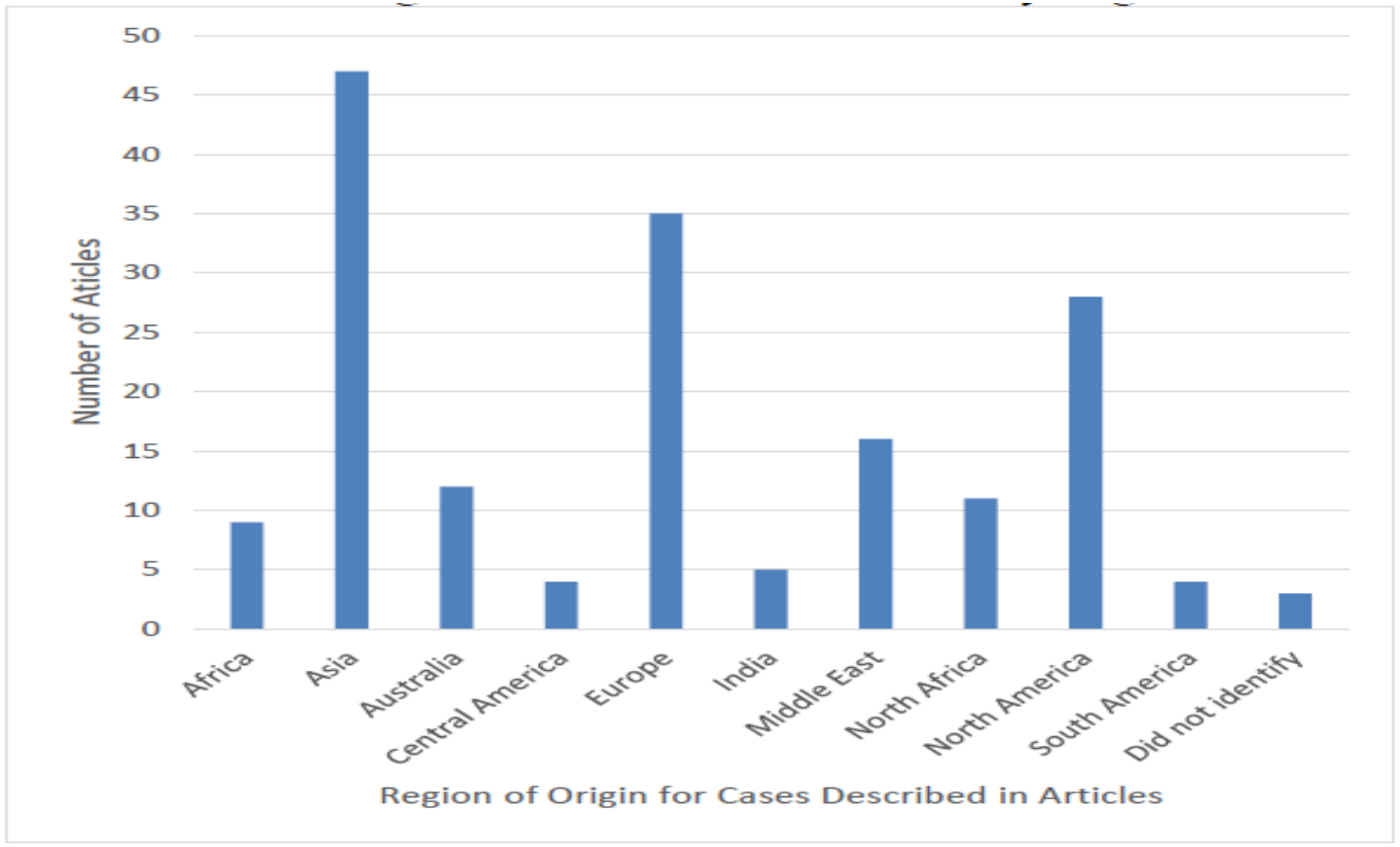
Murine typhus -2014

Manson tropical disease pg no 285 -Courtesy of the Department of Entomology, London School of Hygiene and Tropical Medicine

Epidemiology



Epidemiology - India



Thimothy et al systematic review of murine typhus - 2015

Spotted fever group rickettsia (SFGR)

- Currently 30 *Rickettsia* species in nomenclature
  (21 of them are pathogenic in humans)
- **Genus level**

➢ **16S rRNA gene (rrs)**

➢ Citrate synthase gene (gltA)

➢ **17 kDa protein gene**

Murine typhus: R. typhi

- **Species level**

➢ OmpA protein gene(ompA)

➢ **OmpB protein gene (ompB)**

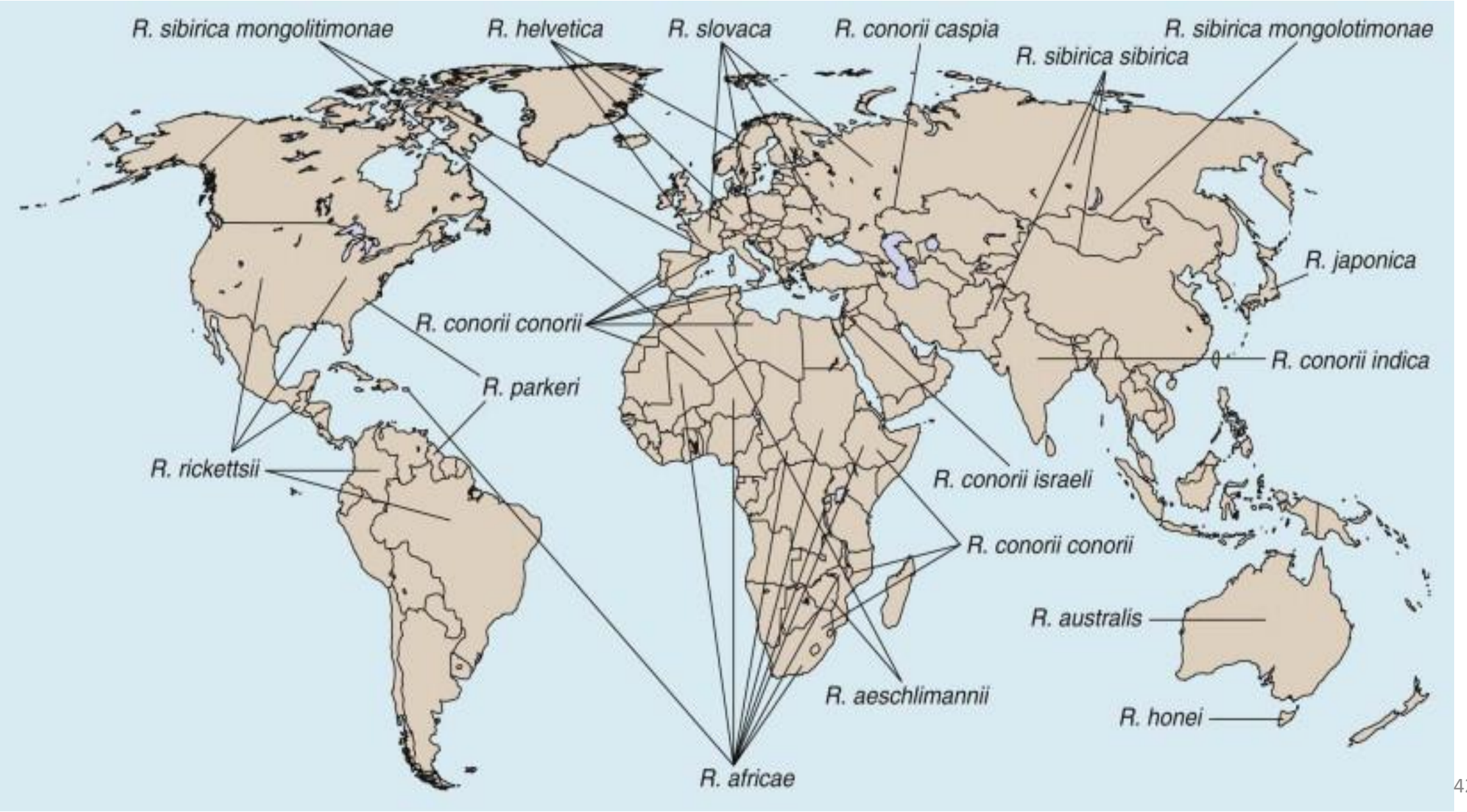
➢ Sca4 protein gene (sca4)

- **Isolate level**

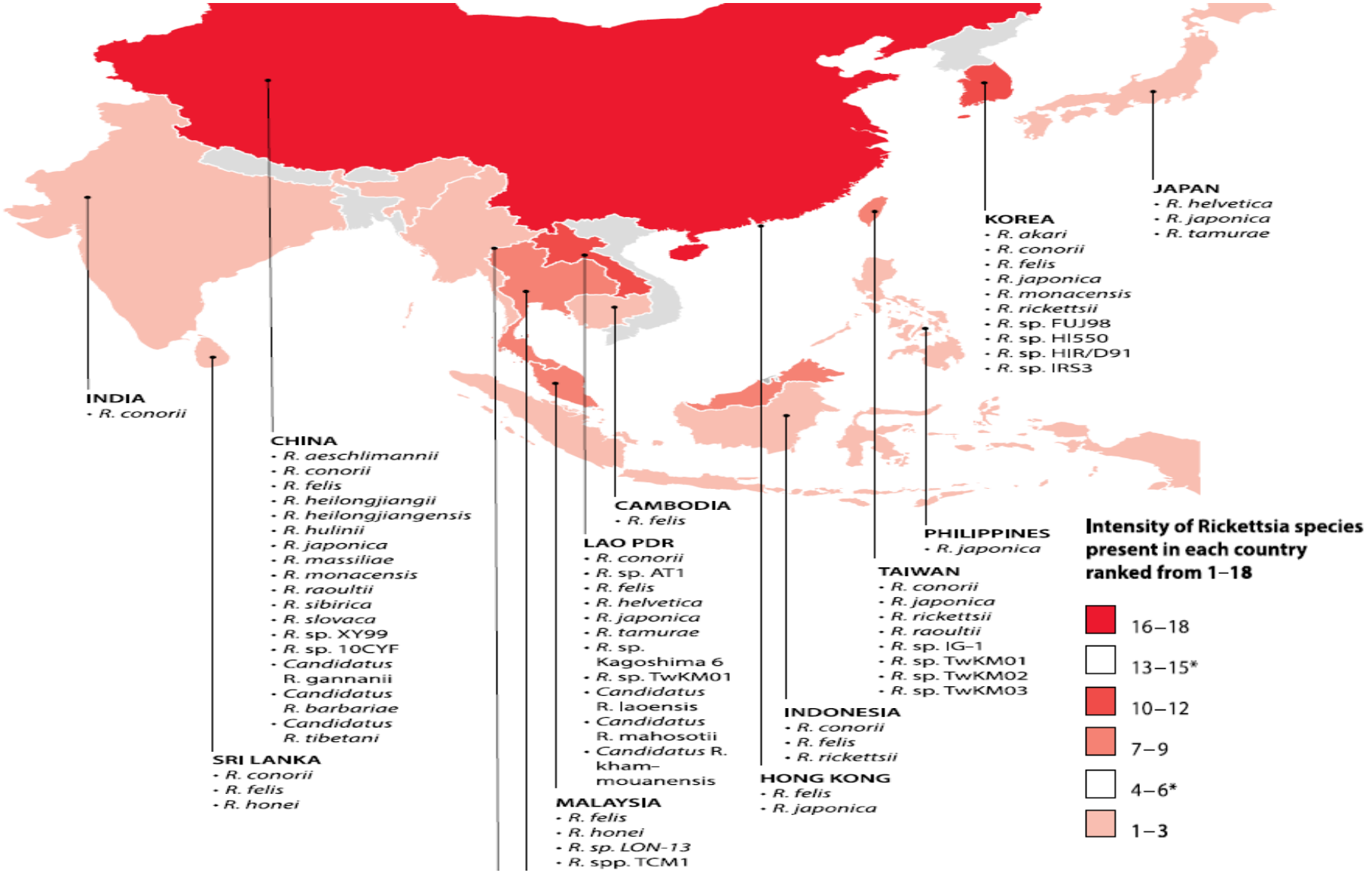
➢ Intergenic regions

(http://www.bacterio.net).

Global epidemiology: Spotted fever (Didier Raoult, 2012)



Distribution of spotted fever group in Asia



Ref: Satjanadumrong J *et* al Distribution and Ecological Drivers of Spotted Fever Group Rickettsia in Asia – Ecohealth 2019

Rickettsioses India

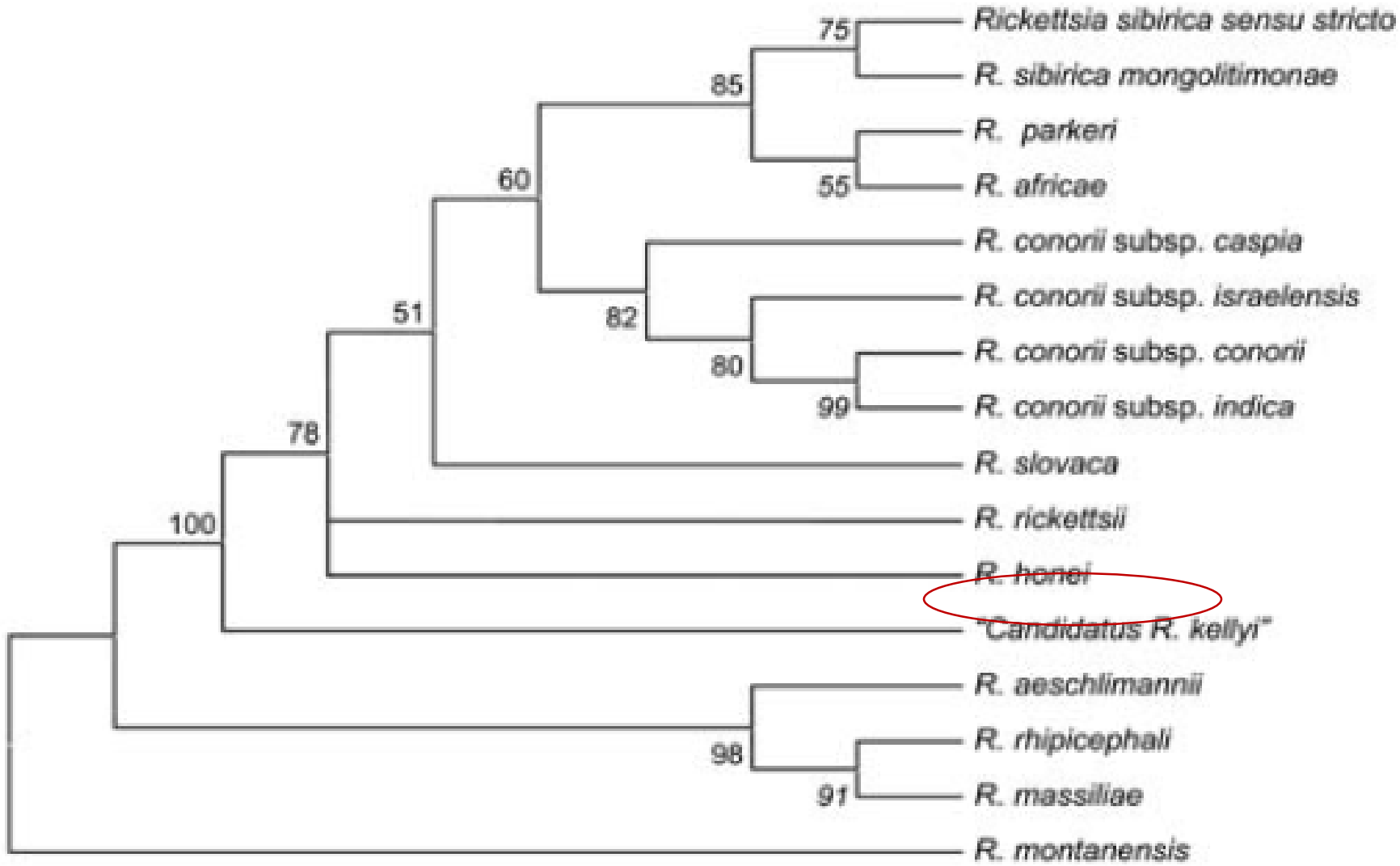
- E. Mathai et al (2001)
- PUO: 1996 - 1998, tested using the WF test
- N=475, 21 titre ≥1:160
- SF & MT: Each 6 & ST by IHAT
- Spot the spotted fever (1996-2000)
- Fever with rash: 57 children
- SF: 12 by WF test
- Somasekar *et* al (CMC, Vellore): November 2003 to November 2004
- N= 180; Initial screen by WF
- ST: 27; SF; 14 & MT: 2 (confirmed by IFA)
- **21 of 31 children: Afebrile with Doxy (≤24 hours)**

Potentially novel rickettsia

- “Candidatus Rickettsia kellyi," India (Rolain JM et al 2006)
- Reported in a 1-year-old boy with fever and maculopapular rash who presented to CMC, Vellore
- Diagnosis: Serology, PCR & immunohistochemicstry
- Skin biopsy specimen sent to Professor Didier Raoult at the Universite de la Mediterranee, Marseille, France

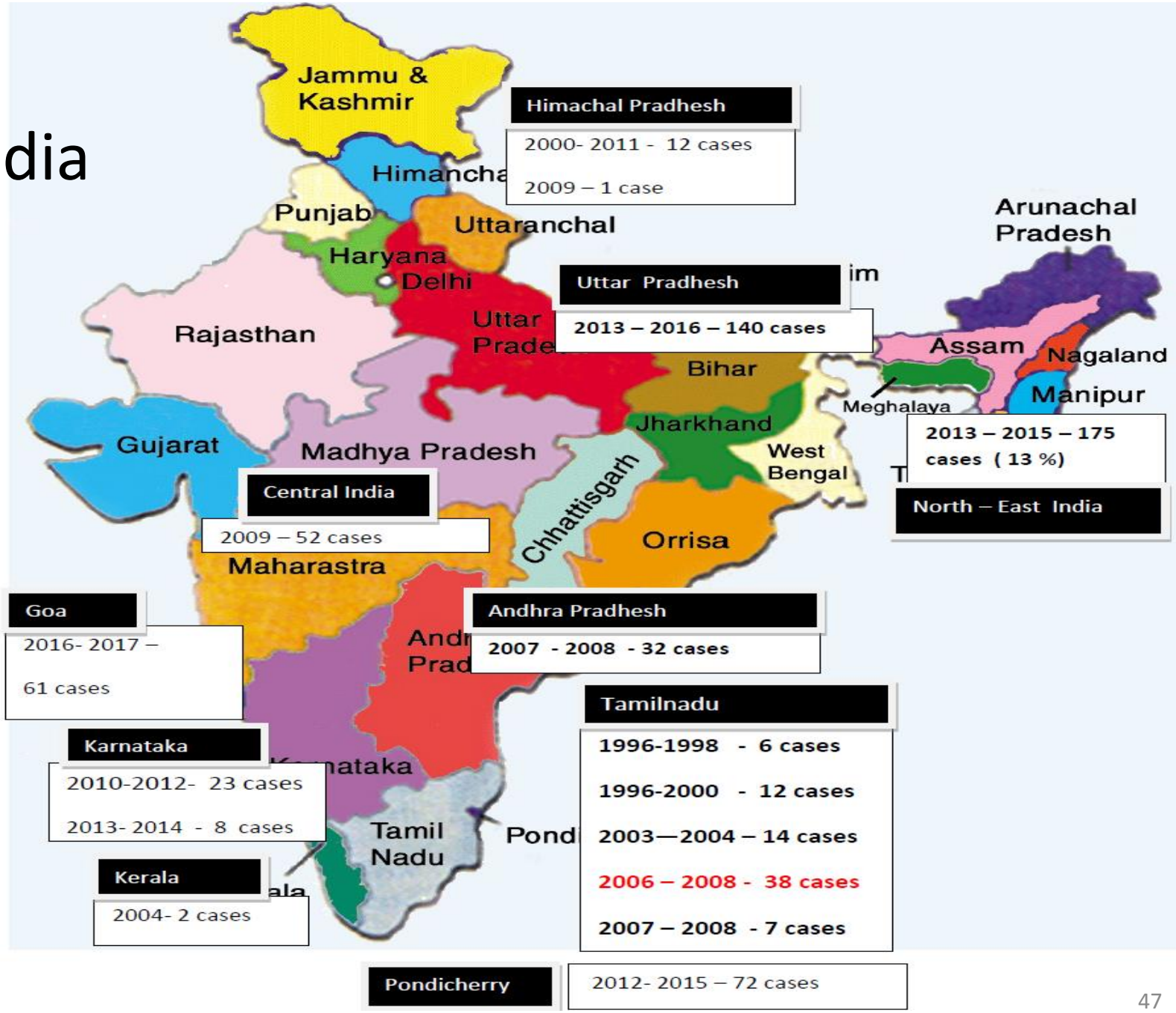
- Further evidence; *5 of 6 ompA genes similar to* “Candidatus Rickettsia kellyi," India (Prakash JA et al 2012)
- Not yet isolated in culture

Candidatus Rickettsia kellyi



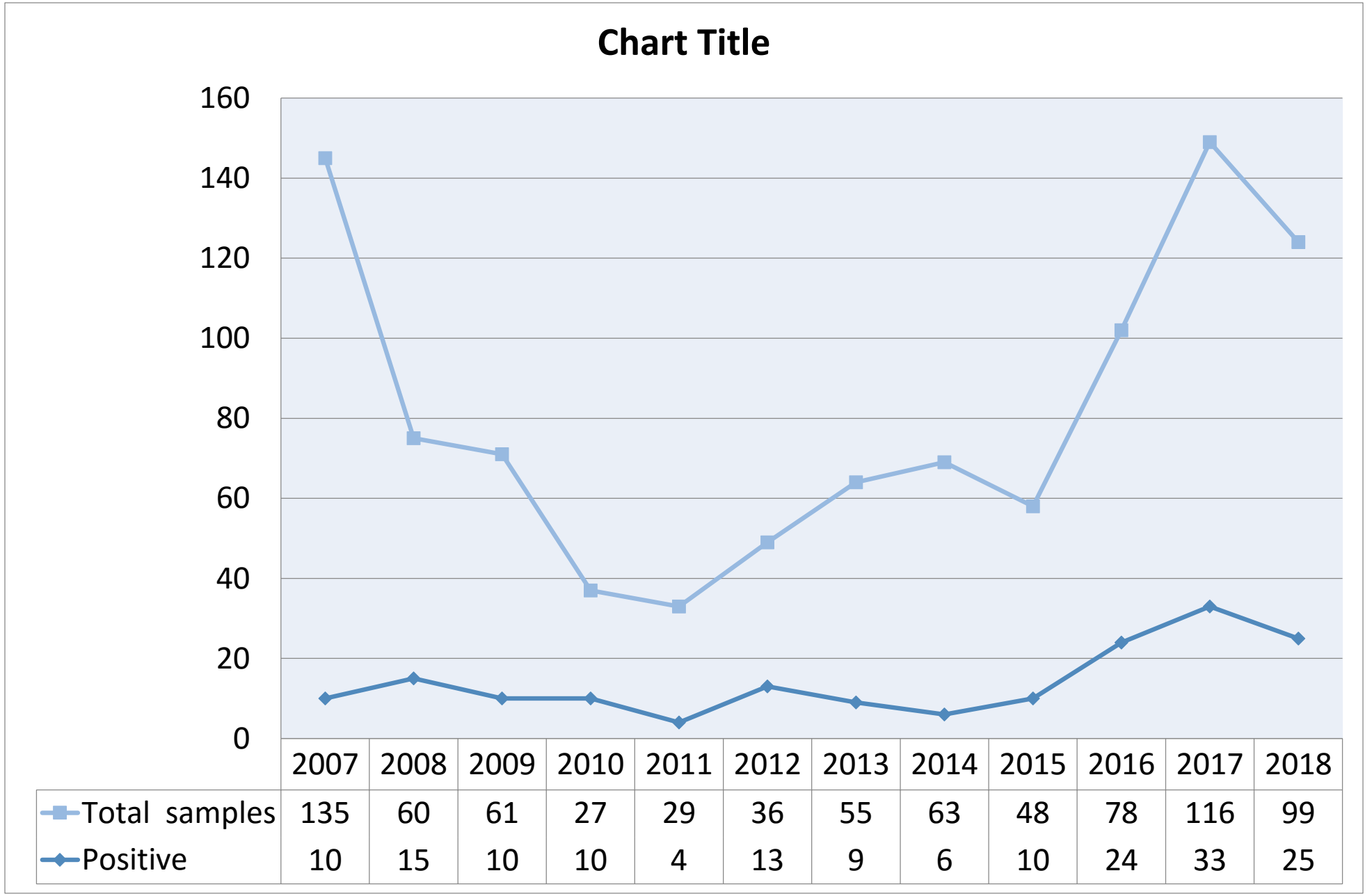
John A.J. Prakash and Didier Raoult- Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 3, March 2006

Spotted fever in India



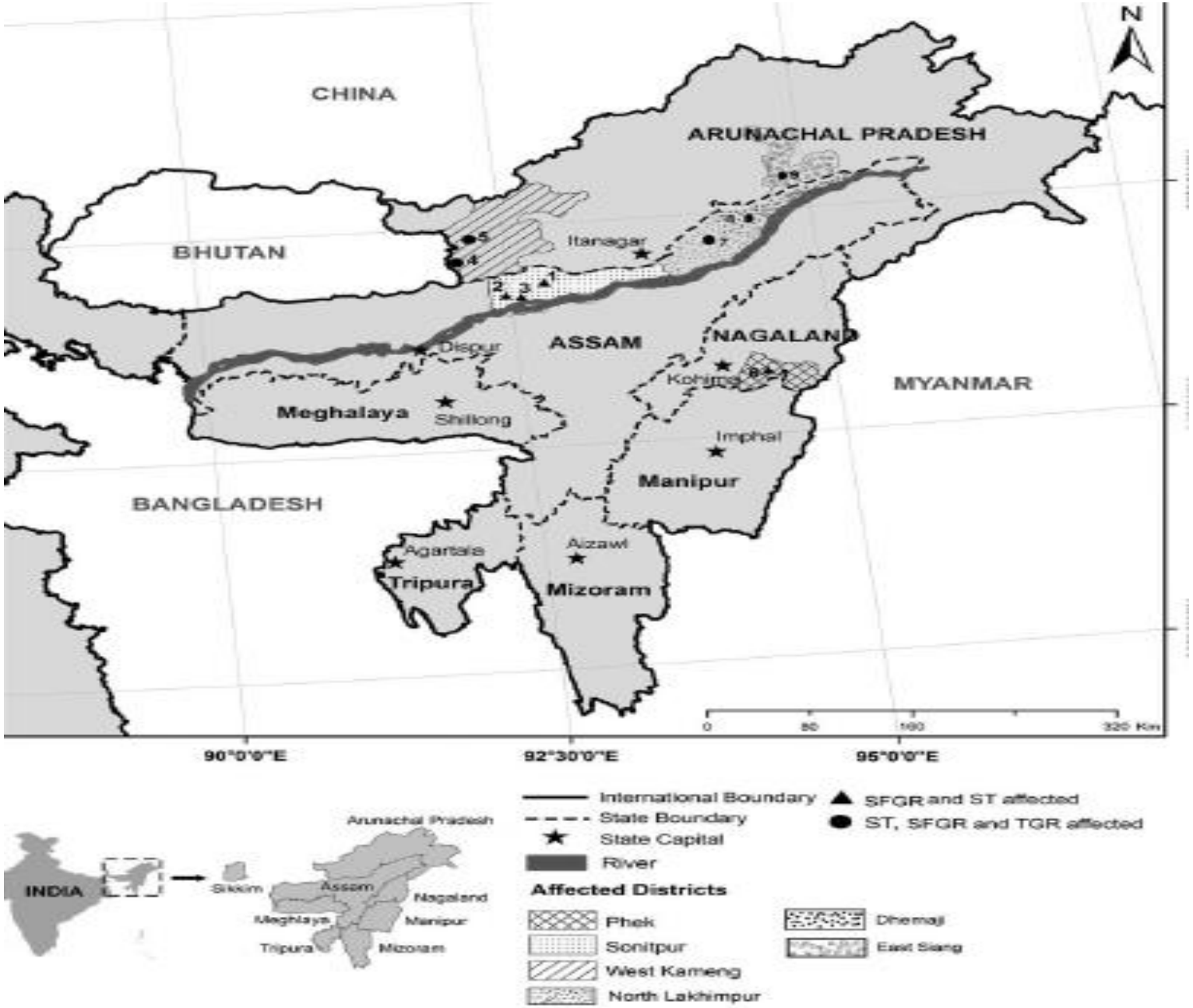
Pubmed – Advanced search data for spotted fever prevalence in India

CMC SF data from 2007-18



	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Total samples	135	60	61	27	29	36	55	63	48	78	116	99
Positive	10	15	10	10	4	13	9	6	10	24	33	25

NE India



30.8%- Scrub typhus
13.8% - SFGR
4.2% -Typhus group

S. A. Khan et al Trans R Soc Trop Med Hyg 2016; 1–8

MURINE TYPHUS : A FORGOTTEN CAUSE OF FEBRILE ILLNESS IN SINGAPORE

K C Loh, Y S Leo, M K Heng, B C Goh

ABSTRACT

We report 6 cases of murine typhus presented to us within a period of 3 months. The diagnoses were made based on the Weil-Felix reaction in the context of supportive clinical and epidemiological features, and response to appropriate antimicrobial therapy. This review serves to remind us that murine typhus is still an important cause of acute febrile illness in Singapore, especially among the migrant Indian workers.

Keywords: *endemic typhus, Rickettsia typhi, clinical features, evaluation, treatment.*

SINGAPORE MED J 1996; Vol 37: 39-43

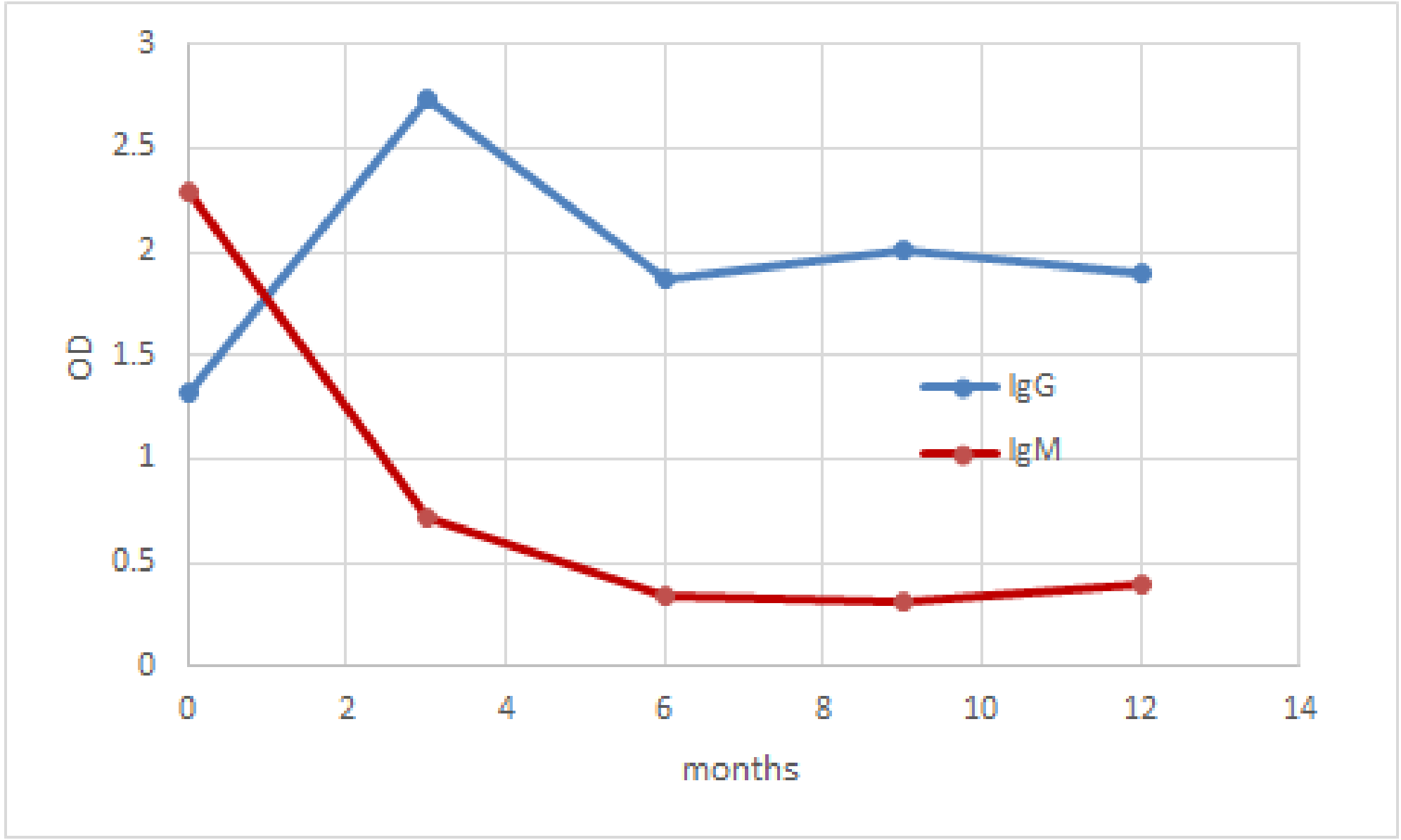
Murine Typhus in Returned Travelers: A Report of Thirty-Two Cases

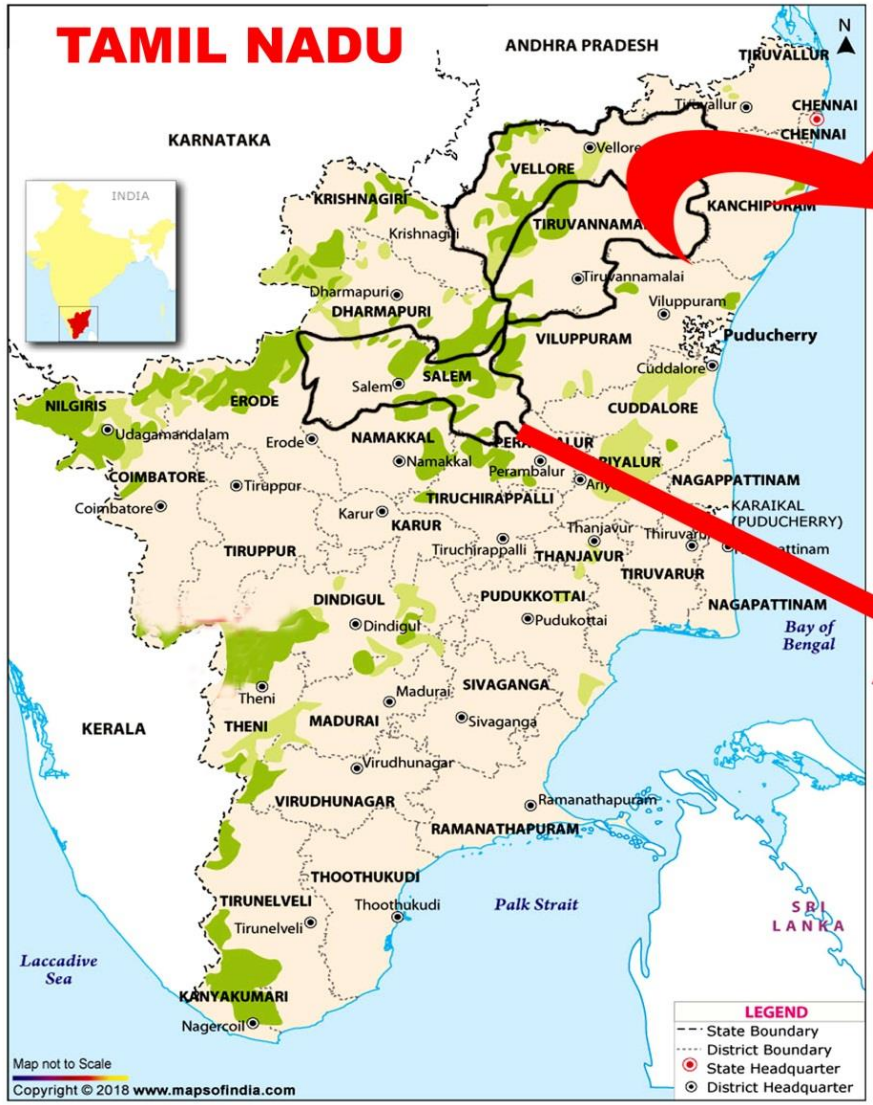
Gaëlle Walter, Elisabeth Botelho-Nevers, Cristina Socolovschi, Didier Raoult, and Philippe Parola*

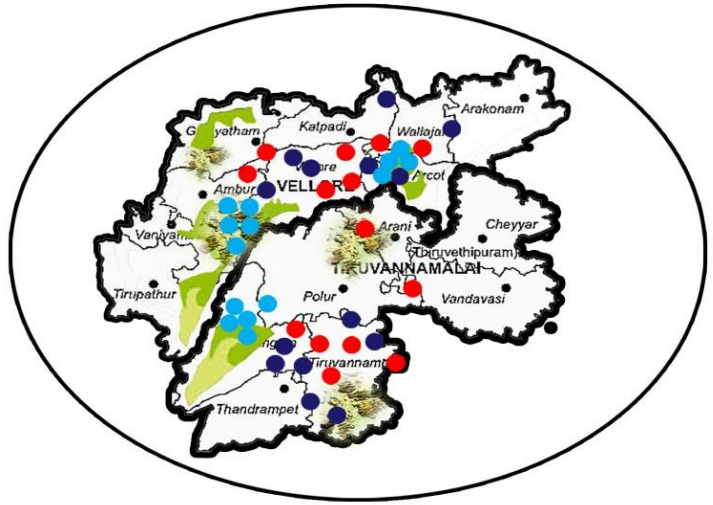
Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), UMR CNRS-IRD, WHO Collaborative Center for Rickettsial Diseases and Other Arthropod-borne Bacterial Diseases, Marseille, France; Service Maladies Infectieuses et Tropicales, Hôpital Nord, Marseille, France

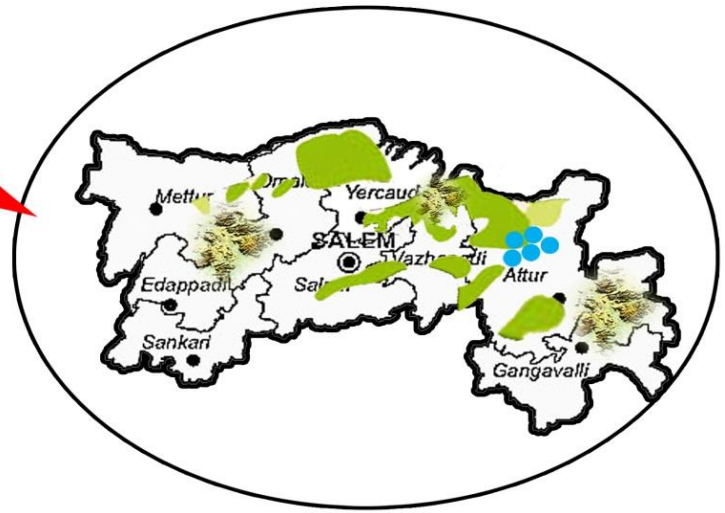
Abstract. Murine typhus, caused by *Rickettsia typhi* and transmitted mainly by the rat fleas, *Xenopsylla cheopis*, has emerged in the field of travel medicine. We analyzed retrospectively the epidemiological, clinical, and biological characteristics of the 32 murine typhus cases that were diagnosed during the past 3 years at the World Health Organization Collaborative Center for Rickettsial diseases, Marseille, France. All of the cases occurred in travelers and most of them had returned from Africa ($N = 13$ of 32) and South-east Asia ($N = 12$ of 32). Exposure to rats was reported only in a few ($N = 2$ of 32) patients. Almost half of the cases were diagnosed in August and September. Only four patients presented the classic triad: fever, rash, and headache. Moreover, we report the first known cases of a hemophagocytic syndrome associated with this disease. Murine typhus must be considered as an etiologic agent of febrile illness in returning travelers, particularly in those with unspecific symptoms.

Ped ST serology vs time


3
2.5
2
1.5
1
0.5
0
OD
0
2
4
6
8
10
12
14
months
IgG
IgM







URBAN

RURAL

PERI FOREST

RiQVest study: Prelim results (Serosurvey)

For exposure positive: IgG ELISA OD ≥1.5

Districts: Vellore & Tiruvannamalai (urban & rural)

Periforested: Palamathi, Jawadhi & Kalrayan Hills

Locality (n)	ST IgG ELISA (%)	MT IgG ELISA (%)	SF IgG ELISA (%)
Urban (427)	15.5	5.4	3.8
Rural (576)	28.7	8.7	12.7
Peri-forested (350)	13.7	0.0	14.9

	Scrub typhus	Spotted fever	Murine typhus
Etiological agent	*O. Tsutsugamushi* *O.chuto*	Spotted Fever group rickettsiae	*R.typhi*
Vector	Chigger (mite) borne	Mite/Tick/Flea borne	Flea borne
Prevalence	More common (*tsutsugamushi triangle)*	Less common	Least common
Clinical presentation	AFI with eschar ± Rash	AFI with Rash ± Eschar	AFI with Rash No eschar
Investigation	Weil felix- OX K Positive ST IgM ELISA qPCR—47 kda	WF (OX 2/19-positive) SF IgM ELISA qPCR- OMP A	WF OX 19- positive MT IgM ELISA qPCR-OMP B/gltA/17kDa
Prognosis (untreated)	Mortality rate - high(ARDS)	Mortality rate: moderate	Less

Lacunae in knowledge

- Community prevalence: Not clearly known
- Vectors: **Identified for scrub typhus only**
- Reservoir of infection: No definite idea
- Risk factors for rickettsioses: Needs further study
- Role of climate & environment: Not well studied

Thank you