

Rickettsia, it is time to give more attention to...

What is Rickettsia?

Rickettsia is a genus of non-motile, non-spore forming, obligate intracellular, gram negative bacteria that can present as cocci, rods, or thread-like. Rickettsia can only live in eukaryotic host cells (e.g. endothelial cells, tissue or embryo cultures). Serologically, Rickettsia is classified into three groups (spotted fever, typhus, and scrub typhus). This grouping has since been confirmed by DNA sequencing. However, recently, the scrub typhus group has been reclassified into a new genus, *Orientia*.

The taxonomical order of Rickettsia is:

Phylum : Proteobacteria
Class : Alphaproteobacteria
Order : Rickettsiales
Family : Rickettsiaceae
Genus : Rickettsia, *Orientia*
Species : to name a few: *R. prowazekii*, *R. typhi* (typhus group), *R. rickettsii*, *R. akari* (spotted fever group/SFG), *Otsutsugamuchi* scrub typhus group)

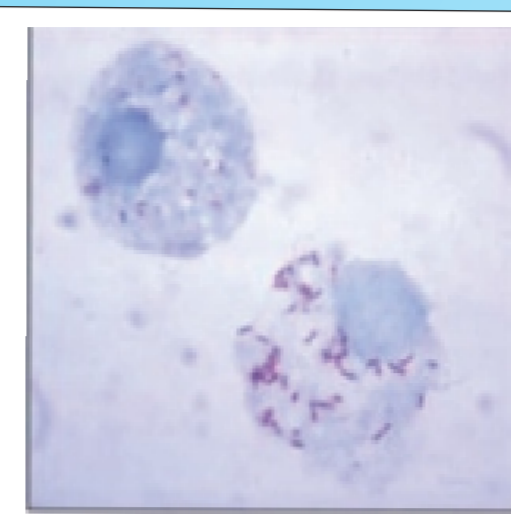


Figure 1 Gimenez stain of rickettsial infection in tick hemolymph cells. R rickettsii are shown as dark pink bodies. (Source: Wikipedia)

The confusion between typhus fever & typhoid fever

Typhus and typhoid fever were two diseases that people (including health care workers) often confuse and use interchangeably. To clarify, here are the differences.

	Typhus Fever	Typhoid Fever
about the terminology	Typhus fever was introduced by Boisser de Savvages in 1769 to explain 'confusional state or delirium' [Greek 'Typhos', meaning 'smoke', mist or fog].	Typhoid fever [mimicking typhus fever] was introduced by Pierre Charles Alexander Louis in 1829 to describe the clinical and pathological aspects of the disease with mental foginess.
Etiologies	The first species of Rickettsia, which caused epidemic fever, was found by Rocha Lima, a Brazilian physician and named it 'Rickettsia prowazekii', after his friend Stanislaus von Prowazek and American bacteriologist, Howard Ricketts, who ironically died from this disease.	Salmonella enterica serovar typhi was found in 1880 by Karl Joseph E. Berth. The genus 'Salmonella' was named after a US veterinary pathologist, Daniel E. Salmon.
Transmission	vector bites	Food contamination (fecal-oral)
Diagnostics assays	<ul style="list-style-type: none">Bacterial culture in animals, embryonated eggs or cell linesPCRIFA and ELISA	<ul style="list-style-type: none">Blood Culture, PCRTubex Rapid tests or other brandsWidal, ELISA
Treatment	Doxycycline, Fluoroquinolones, Chloramphenicol	Azithromycin, Ceftriaxone, Ciprofloxacin, Chloramphenicol, Amoxicillin, Trimethoprim/Sulfamethoxazole

Hosts, vectors, and transmission

Each species of rickettsia usually has its own hosts and vectors. Humans are the usual host for *R. prowazekii*, which is spread through lice (*Pediculus humanis corporis*) to other humans. Cats, rodents, and opossums are the hosts for *R. typhi* and *R. felis* which are spread to other hosts or humans by fleas (*Xenopsylla cheopis*). Mites/chiggers (*Leptotrombidium delicense*), which feeds on rodents, are both the natural host and vector of *Otsutsugamuchi*. House mice is the host of *R. akari*, and transmitted by a different kind of mites (*Liponyssoides sanguineus*).



Figure 2 lice (*Pediculus humanis corporis*) (Source: Insect Stock Photo - B. Thomas Photo Research)



Figure 3 fleas (*Xenopsylla cheopis*) (Source: Insect Stock Photo - B. Thomas Photo Research)



Figure 4 mites (*Liponyssoides sanguineus*) (Source: <https://identify.us.com>)



Figure 5 mites (*Leptotrombidium delicense*) (Source: <http://www.vetbook.k.org>)

The vectors of *R. prowazekii*, *R. typhi*, *R. akari*, and *Otsutsugamuchi*

The Clinical Manifestations

On the infected skin, a sore covered by black scar (eschar) often appeared and nearest lymph nodes are then swollen. The bacteria then infect the cells lining small blood vessels, causing the blood vessels to become inflamed or blocked or to bleed into the surrounding tissue. Symptoms will develop based on how the body responds, commonly flu-like symptoms, undistinguishable with other infectious diseases. Most patients have fever, malaise and severe headache. Rash and swollen lymph nodes may appear after several days of infections. In severe form, patients may experience severe weakness, confusion, cough, breathing difficulty, vomiting, gangrene, liver and spleen enlargement, kidney failure, shock and death.

Laboratory detection of Rickettsia

Diagnosis is done either by detecting the bacteria (the antigen) or detecting antibody expressed by infected individuals from patient's blood.

Antigen-based detection can be done by culturing the bacteria in lab animals, embryonated chicken eggs, and cell lines (Vero, C6/36, L929, S2), or by detecting the rickettsia-specific gene in polymerase chain reaction (PCR).

Antibody-based detection (serology) includes enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent assay (IFA).

Ideally rickettsial infection is confirmed by both assays. However, results depend on the time the blood specimens are collected. Antigen can be detected on the first week after onset of fever, whereas antibody on the second week. Consequently, acute and convalescent specimens should be collected. The time points of the clinical manifestations, antigen and antibody detection are shown in figure 6.

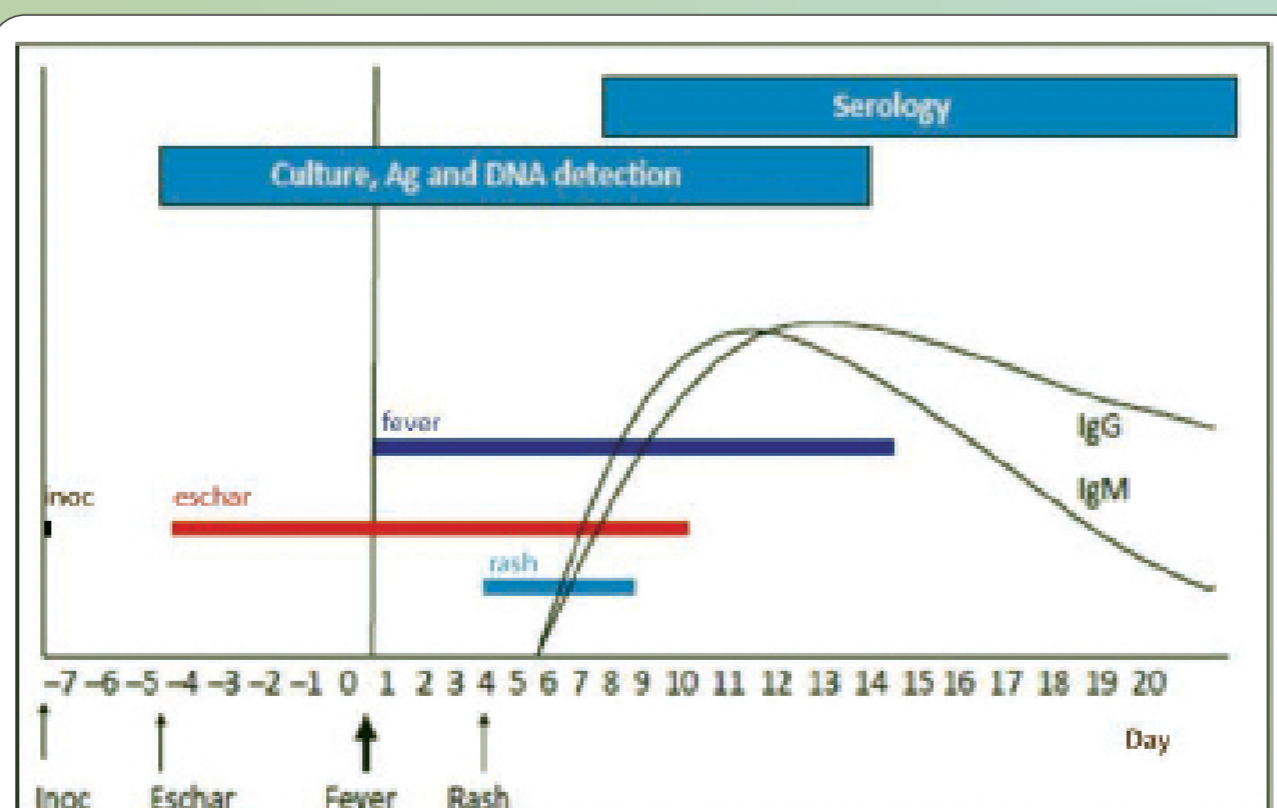


Figure 6. Time points of rickettsia infection in relationship with detection and diagnosis.

ELISA measures the expression of immunoglobulin G (IgG) and immunoglobulin M (IgM) against rickettsiae. An ELISA plate reader measures the fluorescence intensity, where higher fluorescent level (visible deeper color, Figure 7A) corresponds to higher antibody titer. Rickettsial infection is confirmed when fourfold rise of IgG between sample taken at the acute period of infection and during the convalescent time points (at least 14 days apart) is observed; or, if there is a significant increase in IgM titer. However, the current 'gold standard' of rickettsial detection is Immuno-fluorescence assay (IFA). The presence of rickettsia-specific antibody is shown as green fluorescent bodies (Figure 7B). Since antibody develops at least five days after symptoms appear, confirmation of rickettsial infection by ELISA or IFA may not be used to guide treatment.

A

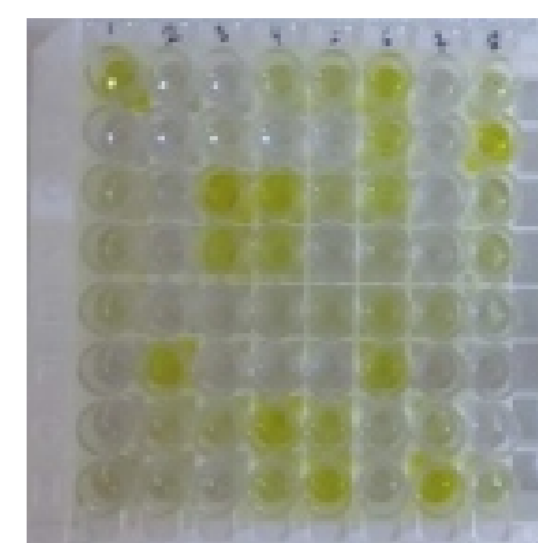


Figure 7A. Rickettsia ELISA plate, deeper color corresponds with higher antibody titers.

B

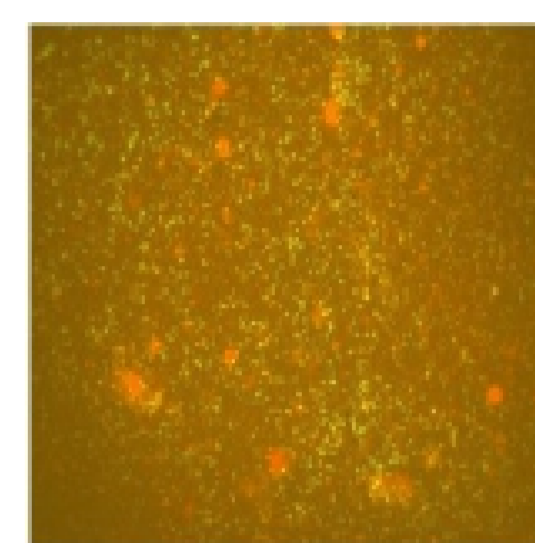


Figure 7B. Murine typhus IgM antibodies were detected by IFA (400X enlargement).

Antigen-based detection (PCR) fills in this gap because bacterial multiplication has started even before symptoms appear (Figure 6). Polymerase chain reaction (PCR) detects rickettsiae in clinical specimens by amplifying a portion of the bacterial DNA multiple times. Detection of fluorescence during PCR cycles confirms the presence of rickettsial DNA. More copies of DNA (more bacteria) correspond with lesser values of Ct (cycle threshold). PCR is advantageous compared to serological assay in terms to confirm rickettsial infection in the early stages of disease, such that patients can obtain appropriate treatment. It is also highly sensitive (theoretically it can detect down to 1 DNA copy), which unfortunately makes it prone to cross-contamination leading to false positive result.

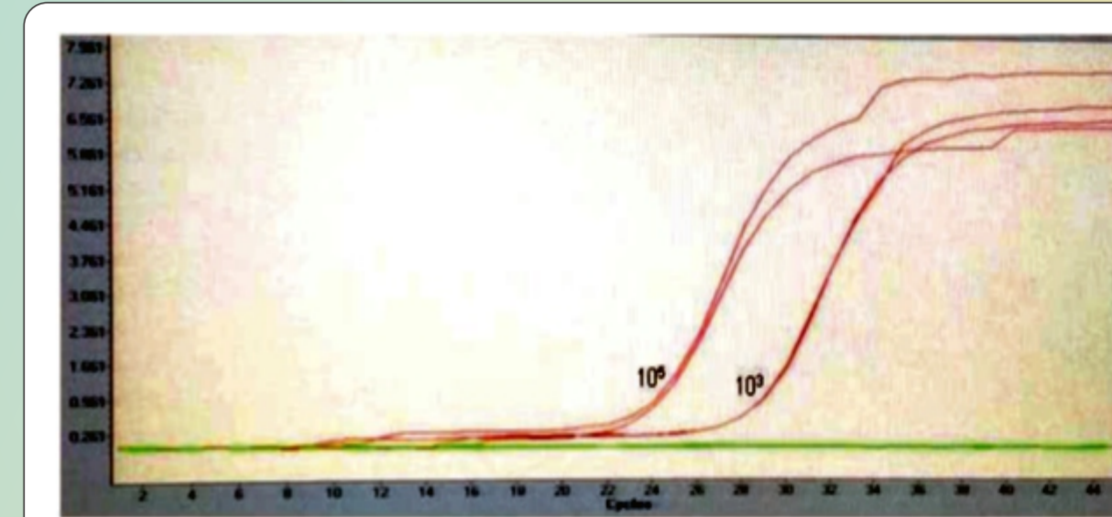


Figure 8. Realtime PCR detection of *O. tsutsugamushi*. Earlier Ct detection means there are more DNA (bacteria) within the sample, 4 X 10⁵ DNA copies were detected at Ct 23 while 4 X 10³ DNA copies were detected at Ct 28.

Laboratory confirmation tests for rickettsial infection are not available at the hospitals in Indonesia for the following several reasons: the need of living media and high biosafety level space for culture; the delicate nature and costly materials/instruments of PCR, ELISA or IFA; the needs for high-skilled technicians to perform these assays; and the lack of accurate rapid diagnostic tests. Consequently, rickettsia is rarely diagnosed as the cause of infectious diseases. It is reflected in INA-RESPOND's acute fever requiring hospitalization (AFIRE) study where more than 5% of acute fever were confirmed rickettsial infections.

The treatment and prevention

Early treatment with appropriate antibiotics (first week of illness) is highly effective. Fever usually disappears in 1-3 days after treatment. If fever remains, other diagnosis should be reconsidered. The drug of choice is doxycycline which is preferred over other tetracyclines. Chloramphenicol may be used as an alternative. However, due to the side effect of bone marrow depression, it is rarely used. Fluoroquinolones (ciprofloxacin, ofloxacin) may also be effective. Sulfonamides is contra-indicated as it may stimulate the growth of the organisms.

To prevent the infection by rickettsia bacteria, we need to avoid getting bitten by the vectors (tick, fleas, mites, chiggers) by covering our skin from exposure and using insect repellent when doing outdoor activities. At home, we need to keep our home clean of rats and to ensure that our pets are free from fleas.

The situation in Indonesia and future steps

In Indonesia, the endemicity of the three biotypes has been reported. The circulation of *R. felis* and other member of SFG, as well as the causative agent of murine typhus *R. typhi*, have been observed within the *X. cheopis* fleas in East Java, West Java, East Kalimantan, and North Sulawesi. Seroreactivity either to murine typhus (*R. typhi*), SFG (*R. rickettsii* and *R. conorii*), or scrub typhus (*O. tsutsugamushi*), was shown in rodent population of Jayapura (Papua) as well as in human population of Malang (East Java) and Gag Island (Papua). Human cases have been confirmed serologically by two studies in Semarang and molecularly by several returning travelers. Our study confirmed that Rickettsia is prevalent in Indonesia as Rickettsia genomes and antibodies have been detected in a number of subjects from the AFIRE study, indicating that Rickettsia must not be neglected anymore. Rickettsioses must be one of the differential diagnoses of patients hospitalized with fever, and hospital laboratory should be equipped with capacities to perform the assays. Study on Rickettsia should be conducted to have a better estimate of the burden of this disease nationally.

Further Reading

Richards, A.L., et al. 1997. Am J Trop Med Hyg 57(1); Andersson, S.G.E. et al. 1999. Mol Biol Evol 16(7); Richards, A.L. et al. 2002. Am J Trop Med Hyg 66(4); Richards, A.L. et al. 2003. Am J Trop Med Hyg 68(4); Jiang, J. et al. 2006. Emerging Infectious Diseases 12(8); Jiang, J. et al. 2006. Emerging Infectious Diseases 12(8); Eremeeva, M & Dasch, G. 2008. In Heyman, D.L. Control of Communicable Diseases Manual, 19th ed.; Todor, K. http://textbookofbacteriology.net/Rickettsia_2.html; Barbara, K.A. et al. 2010. J Med Entomol 47(6); Richards, A.L. 2012. FEMS Immunol Med Microbiol 64