

CD Alert

Monthly Newsletter of National Centre for Disease Control,
Directorate General of Health Services, Government of India

May - July 2009

Vol. 13 : No. 1

SCRUB TYPHUS & OTHER RICKETTSIOSES

RICKETTSIAL DISEASES

These are the diseases caused by rickettsiae which are small, gram negative bacilli adapted to obligate intracellular parasitism, and transmitted by arthropod vectors. These organisms are primarily parasites of arthropods such as lice, fleas, ticks and mites, in which they are found in the alimentary canal. In vertebrates, including humans, they infect the vascular endothelium and reticuloendothelial cells. Commonly known rickettsial disease is Scrub Typhus.

The family Rickettsiaceae currently comprises of three genera – Rickettsia, Orientia and Ehrlichia which appear to have descended from a common ancestor. Former members of the family, *Coxiella burnetii*, which causes Q fever and *Rochalimaea quintana* causing trench fever have been excluded because the former is not primarily arthropod borne and the latter is not an obligate intracellular parasite, being capable of growing in cell-free media, besides being different in genetic properties. Scrub typhus will be dealt in detail.

SCRUB TYPHUS

CAUSATIVE AGENT

Scrub typhus (Chigger borne typhus, Tsutsugamushi fever) is caused by *Orientia tsutsugamushi*. *Orientia* is a small (0.3 to 0.5 by 0.8 to 1.5 μm), gram negative bacterium of the family *Rickettsiaceae*. It differs from the other members in its genetic make up and in the composition of its cell wall structure since

it lacks lipopolysaccharide and peptidoglycan and does not have an outer slime layer. It is endowed with a major surface protein (56kDa) and some minor surface protein (110, 80, 46, 43, 39, 35, 25 and 25kDa). There are considerable differences in virulence and antigen composition among individual strains of *O.tsutsugamushi*. *O.tsutsugamushi* has many serotypes (Karp, Gillian, Kato and Kawazaki).

GLOBAL SCENARIO

Geographic distribution of the disease occurs within an area of about 13 million km² including-Afghanistan and Pakistan to the west; Russia to the north; Korea and Japan to the northeast; Indonesia, Papua New Guinea, and northern Australia to the south; and some smaller islands in the western Pacific. It was first observed in Japan where it was found to be transmitted by mites. The disease was, therefore, called tsutsugamushi (from tsutsuga meaning dangerous and mushi meaning insect or mite). This is found only in areas with a suitable climate, plenty of moisture and scrub vegetation. Recently, rickettsioses has been an emerging disease along the Thai Myanmar border. There are reports of emergence of scrub typhus in Maldiv Islands and Micronesia.

INDIAN SCENARIO

In India, scrub typhus has been reported from Rajasthan, Jammu & Kashmir and Vellore. In addition, few cases have been tested positive for IgM antibodies for scrub typhus from Sikkim,

Darjeeling, Nagaland & Manipur (unpublished data). In a study conducted from July through October 2004 in Himalayas, among several cases of acute febrile illness of unknown origin, *O.tsutsugamushi* was identified as causative agent by microimmunofluorescence and PCR. In an entomologic study in Himachal Pradesh, vector species *Leptotrombidium deliense* and *Gahrliepia spp.* were recorded.

DISEASE TRANSMISSION

Scrub typhus is transmitted by the mite *Leptotrombidium deliense*. The vector mites inhabit sharply demarcated areas in the soil where the microecosystem is favourable (mite islands). Human beings are infected when they trespass into these mite islands and are bitten by the mite larvae (chiggers). The mite feeds on the serum of warm blooded animals only once during its cycle of development, and adult mites do not feed on man. The microbes are transmitted transovarially in mites. Scrub typhus normally occurs in a range of mammals, particularly field mice and rodents.

The *L. deliense* group of vector mites are widely distributed all over the country coexisting primarily with rodents and other small mammals. On the body of small mammalian hosts, the chiggers attach in clusters on the tragus of the ear, the belly and on the thighs. The *Leptotrombidium* mites, on the rat host, may appear orange or pink. The typical vector *L. deliense* is generally found associated with either established forest vegetation or secondary vegetation after clearance of forest areas. This species is generally abundant on grasses and herbs where bushes are scarce. Sentinel animals can also be used for collection of trombiculid mites from the field. These animals are generally white laboratory mice or rats kept in small cages containing food and water and placed in the field overnight to attract chiggers. Chiggers can also be collected from field directly on human beings, by walking in the field after wearing stockings. The following

morning, chiggers can be collected from the body of the sentinel animals. The mites can be preserved in 70% alcohol till they reach laboratory for identification. Chigger index (average number of chiggers infesting a single host) of ≥ 0.69 (critical value) is an indicator for implementation of vector control measures.

Habitats favorable for disease transmission

Scrub typhus, originally found in scrub jungles, has also been identified in a variety of other habitats, such as sandy beaches, mountain deserts and equatorial rain forests.

Incubation Period

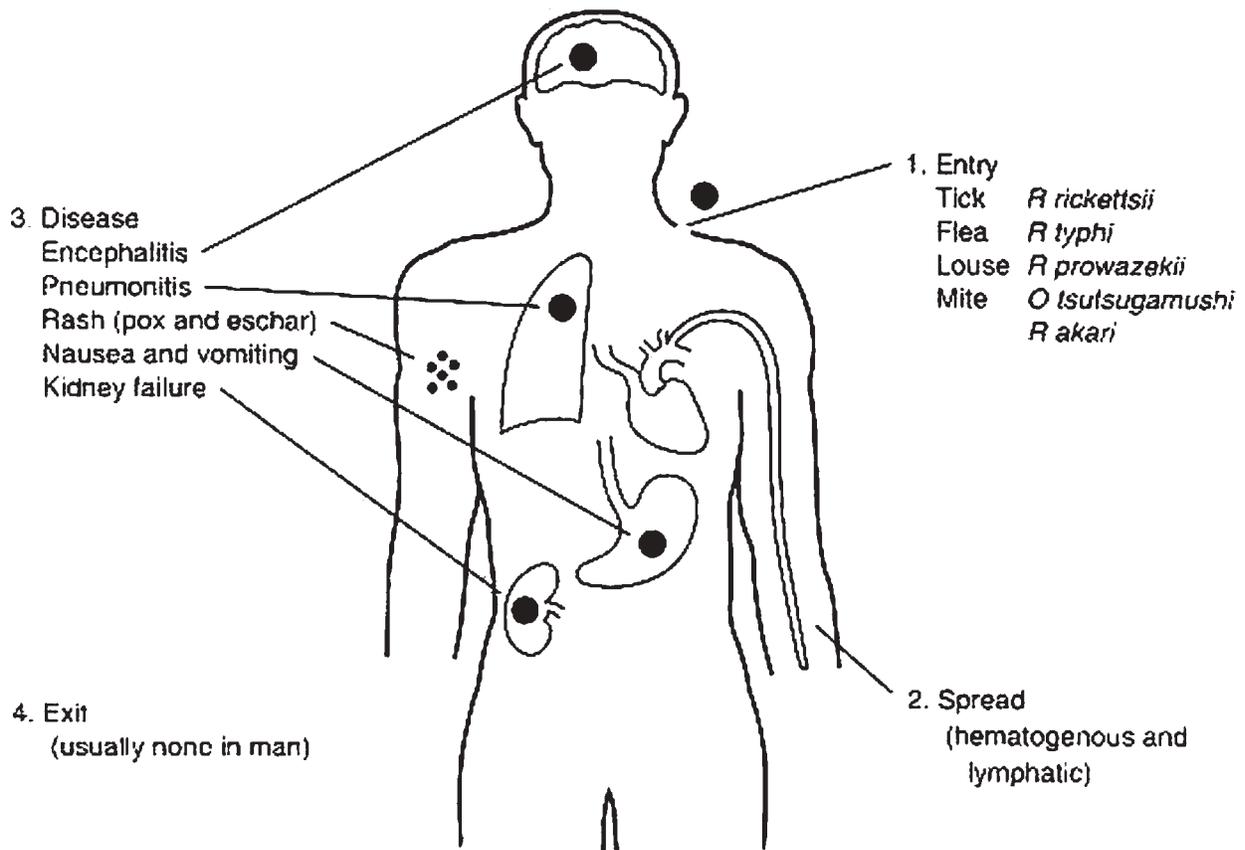
Incubation Period: 1 – 3 weeks.

CLINICAL PICTURE

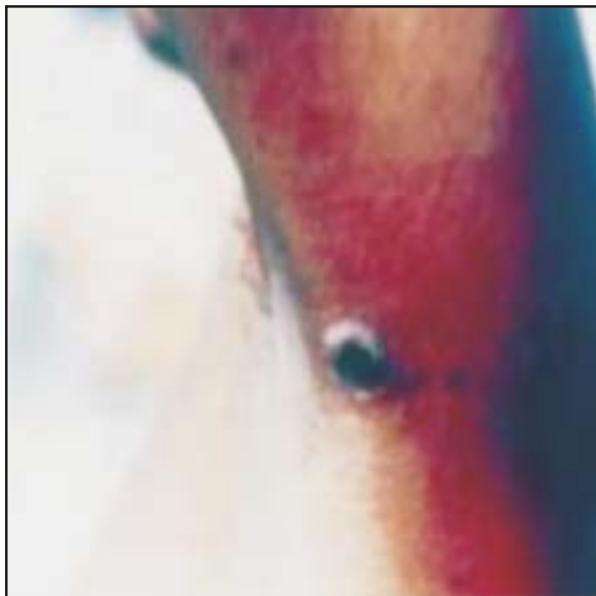
- A Papule develops at the site of inoculation.
- The papule ulcerates and eventually heals with development of a black eschar.
- General symptoms are sudden fever ($>40^{\circ}\text{C}$ [104°F]) with relative bradycardia, severe headache, apathy, myalgia, generalized lymphadenopathy, photophobia and a dry cough.
- Approximately one week later, a spotted and then maculopapular rash appears first on the trunk and then on the extremities and blenches within a few days.
- Complications are interstitial pneumonia (30 to 65% of cases), meningoencephalitis and myocarditis.
- Symptoms generally disappear after two weeks even without treatment.
- In severe cases with pneumonia and myocarditis, the mortality rate may reach 30%.

DIAGNOSIS

Routine laboratory tests are unlikely to be diagnostic for any rickettsial diseases.



Common clinical manifestations of the Rickettsial Diseases



Scrub Typhus axillary eschar



Maculopapular rash on back of a case of scrub typhus

However, investigation may reveal early lymphopenia with late lymphocytosis. Albuminuria is a common laboratory finding. Thrombocytopenia is observed in more than half of the patients with epidemic typhus.

Laboratory diagnosis

Scrub typhus may be diagnosed in the laboratory by:

- (i) isolation of the organism
- (ii) serology
- (iii) molecular diagnosis (PCR).

Collection, storage & transportation of specimen

The collection, transportation and storage of specimens are extremely vital steps in laboratory diagnosis and hence, must be undertaken with utmost care.

Specimen

- Serum
- Blood collected in tubes containing EDTA or Sodium citrate
- Blood clot

Blood collection in tubes and vials

- Aseptically collect 4-5 ml of venous blood.
- Allow blood to clot at room temperature, centrifuge at 2000 rpm to separate serum.
- Collect the serum in sterile dry vial.
- Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.
- Use adhesive tape marked with pencil, indelible ink, or a typewritten self adhesive label to identify the container. The name of the patient, identification number and date of collection must be indicated on the label.

Do's/Don'ts while collecting specimen:

- Collect sufficient quantity of specimen
- Avoid contamination by using sterile equipment and aseptic precautions.
- Despatch the specimen immediately to laboratory at 2-8°C (ice box) as soon as possible.
- Don't freeze whole blood as haemolysis may interfere with serology test results.
- In case the delay is inevitable, keep the specimen at + 4°C in a refrigerator.

- Label all specimens accurately and send all pertinent information to laboratory which will help in better interpretation of the laboratory findings.

Isolation of the organism

As rickettsiae are highly infectious and have caused several serious and fatal infections among laboratory workers, it comes under Risk Group 3 organisms. Isolation should be done in laboratories equipped with appropriate safety provisions preferably Biosafety level-3 laboratory following strict biosafety precautions.

Rickettsia may be isolated in male guinea pigs or mice; yolk sac of chick embryos; vero cell line or MRC 5 cell lines from patients in early phase of the disease. Egg and animal inoculation methods have been replaced by faster and more sensitive cell cultures. Rickettsiae grow well in 3-5 days on Vero cell and MRC 5 cell coverslip cultures and can be identified by immunofluorescence using group and strain specific monoclonal antibodies.

Serological diagnosis

Diagnosis of the etiology of rickettsial diseases can be accomplished most easily and rapidly by demonstrating a significant increase in antibodies in the serum of the patient during the course of infection and convalescence. Several serological tests are currently available for the diagnosis of rickettsial diseases like Weil-Felix Test (WFT), Indirect Immunofluorescence (IIF), Enzyme linked Immunosorbent assay (ELISA) etc. Although many techniques have been used successfully for rickettsial sero diagnosis, relatively few are used regularly by most laboratories. BSL-3 Lab is not required for performing serological tests.

Weil-Felix Test (commonly used test)

The Weil-Felix test is helpful in establishing presumptive diagnosis in diseases caused by members of typhus and spotted fever groups of Rickettsiae. The interpretation of Weil-Felix test

is given in table 1.

Table 1 : Weil-Felix Test

	OX 19	OX 2	OXK
Epidemic typhus	++++	+	0
Brill Zinsser disease	++++	+	0
Murine typhus	++++	+	0
Scrub typhus	0	0	+++
RMSF	++++	+	0
Other tick borne infection	+	++++	0
Indian tick typhus	+	++++	0

The sensitivity and specificity of the Weil-Felix test is reported to be low as compared to the specific serological tests for detection of IgM antibodies. However, comparative evaluation of Weil-Felix test and IgM ELISA for diagnosis of Scrub Typhus carried out at NCDC, showed that Weil Felix test is equally sensitive with specificity of 89%.

Indirect Immunofluorescent antibody (IFA) test

IFA is used as a reference technique; however, availability and cost are major constraints and is not available in most of the laboratories.

Enzyme linked Immunosorbent Assay (ELISA)

ELISA techniques, particularly immunoglobulin M (IgM) capture assays, are probably the most sensitive tests available for rickettsial diagnosis, and the presence of IgM antibodies, indicate recent infection with rickettsial diseases. In cases of infection with *O.tsutsugamushi*, a significant IgM antibody titer is observed at the end of the first week, whereas IgG antibodies appear at the end of the second week.

Molecular diagnosis – PCR

For PCR, blood sample is collected in tubes containing EDTA or sodium citrate. However, blood clot, whole blood or serum can also be used for the detection of *O.tsutsugamushi*, *R.rickettsii*, *R. typhi* and *R.prowazekii* organisms by PCR test.

Facilities for laboratory diagnosis of Rickettsial diseases are available at National Centre for Disease Control, Delhi where samples can be sent for confirmation.

TREATMENT

Prompt institution of effective antibiotic therapy against rickettsiae is the single most effective measure for preventing morbidity and mortality due to rickettsial diseases. Anti rickettsial therapy improves the outcome of all rickettsioses, with the occasional exception of fulminate or complicated cases of RMSF, epidemic typhus and scrub typhus where the illness is no longer susceptible to intervention. If the illness is severe, the cardiac, pulmonary, renal, and central nervous systems should be assessed and additional measures instituted to prevent complications.

Tetracyclines and chloramphenicol remain the only proven therapy for the rickettsial diseases. Doxycycline in a dose of 100 mg twice daily for 7-15 days or Chloramphenicol 500 mg four times a day PO for 7-15 days (for children 150 mg/kg/day for 5 days) is recommended. Tetracyclines may cause discoloration of teeth, hypoplasia of the enamel, and depression of skeletal growth in children; the extent of discoloration is directly related to the number of courses of tetracycline therapy received. Therefore, tetracycline should not be used for children under 8 years of age and for pregnant women.

PREVENTION AND CONTROL

The mite vectors of scrub typhus are especially amenable to control because they are often

found in distinct areas (Typhus Island).

- These foci can be eliminated by treating the ground and vegetation with residual insecticides, reducing rodent populations, and destroying limited amounts of local vegetation.
- Persons who cannot avoid infested terrain should wear protective clothing, impregnate their clothing and bedding with a miticide (e.g. benzyl benzoate) and apply a mite repellent, diethyltoluamide, to exposed skin. Chemoprophylaxis should also be considered.
- In a controlled trial, the weekly administration of 200 mg doxycycline decreased the incidence of clinical illnesses but not of inapparent infection.
- An effective vaccine for humans has not been developed till now, mainly due to serotypic heterogeneity of the organism.

OTHER RICKETTSIAE INFECTIONS

EPIDEMIC TYPHUS

Epidemic typhus (Louseborne typhus, Classical typhus, Gaol fever) has been one of the great scourges of mankind, occurring in devastating epidemics during times of war and famine. The disease has been reported from all parts of the world but has been particularly common in Russia and Eastern Europe. During 1917-1922, there were some 25 million cases in Russia, with about three million deaths. In recent times, the main foci have been Eastern Europe, Africa, South America and Asia. In India, the endemic spot is Kashmir.

The causative agent of epidemic typhus is *R.prowazekii*, named after von Prowazek. Humans are the only natural vertebrate hosts. Natural infection in flying squirrels has been reported from South-eastern USA. The human body louse, *Pediculus humanus corporis*, is the vector. The head louse may also transmit the

infection but not the pubic louse. The lice become infected by feeding on rickettsiaemic patients. The rickettsiae multiply in the gut of the lice and appear in the faeces in 3-5 days. Lice succumb to the infection within 2-4 weeks, remaining infective till they die. They can transmit the infection after about a week of being infected.

Transmission

Lice may be transferred from person to person. Being sensitive to temperature changes in the host, they leave the febrile patient or the cooling carcass and parasitise other persons. Lice defecate while feeding. Infection is transmitted when the contaminated louse faeces is rubbed through the minute abrasions caused by scratching. Occasionally, infection may also be transmitted by aerosols of dried louse faeces through inhalation or through the conjunctiva.

Incubation period: 5 - 15 days.

Clinical Presentation

- The disease starts with fever and chills.
- A characteristic rash appears on the fourth or fifth day, starting on the trunk and spreading over the limbs but sparing the face, palms and soles.
- Towards the second week, the patient becomes stuporous and delirious. The name typhus comes from the cloudy state of consciousness in the disease. The case fatality may reach 40% and increases with age.

In some who recover from the disease, the rickettsiae may remain latent in the lymphoid tissues or organs for years. Such latent infection may, at times, be reactivated leading to recrudescent typhus or Brill Zinsser disease. Brill noticed a mild, sporadic, typhus-like disease in New York among Jewish immigrants from southeastern Europe. Zinsser isolated *R.prowazekii* from such cases and proved that they were recrudesences of infections acquired many years previously.

ENDEMIC TYPHUS

Endemic typhus (Murine typhus) is a milder disease than epidemic typhus. It is caused by *R.typhi* which is maintained in nature as a mild infection of rats, transmitted by the rat flea, *Xenopsylla cheopis*. The rickettsia multiplies in the gut of the flea and is shed in its faeces. The flea is unaffected but remains infectious for the rest of its natural span of life. Humans acquire the disease usually through the bite of infected fleas, when their saliva or faeces is rubbed in or through aerosols of dried faeces. Ingestion of food recently contaminated with infected rat urine or flea faeces may also cause infection. Human infection is a dead end. Man to man transmission does not occur. **In India, endemic typhus has been reported from Pune, Lucknow, Mysore, Kolkata, Golkunda, Karnal, Rewari and Kashmir.**

Clinical presentation

- Endemic typhus resembles many other illnesses and very few patients are provisionally diagnosed correctly.
- Initially, the patient can present with headache, fever and rash. This is seen only in 12% of cases.
- Rash develops in 54% of patients some time during the course of illness.
- Nausea, vomiting, diarrhoea and abdominal pain suggest gastrointestinal diseases while cough and abnormal chest radiograph suggests pneumonia or bronchitis.
- Severe illness including seizures, coma, renal insufficiency and respiratory failure are seen in approximately 10% of cases, only 1% of cases are fatal.

R.typhi and *R.prowazekii* are closely similar but may be differentiated by biological and immunological tests. When male guinea pigs are inoculated intraperitoneally with blood from



Eschar of tick bite over the left side of the abdomen

a case of endemic typhus or with a culture of *R.typhi*, they develop fever and a characteristic scrotal inflammation. The scrotum becomes enlarged and the testes cannot be pushed back into the abdomen because of inflammatory adhesions between the layers of the tunica vaginalis. This is known as the Neil-Mooser or the tunica reaction. The Neil-Mooser reaction is negative with *R. prowazekii*.

SPOTTED FEVER GROUP

They are all transmitted by ticks, except *R.akari*, which is mite borne. Rickettsiae of this group possess a common soluble antigen and multiply in the nucleus as well as in the cytoplasm of host cells. Many species have been recognized in this group.

Organism	Disease
<i>R.rickettsii</i>	Rocky Mountain spotted fever
<i>R.siberica</i>	Siberian tick typhus
<i>R.conori</i>	Indian, Mediterranean, Kenyan and South African tick typhus
<i>R.australis</i>	Queensland tick typhus
<i>R.japonica</i>	Oriental spotted fever

The rickettsiae are transmitted transovarially in ticks, which therefore act as both vectors and reservoirs. The infection may be transmitted to vertebrate hosts by any of the larval stages or by adult ticks. Ticks are not harmed by the rickettsiae and remain infected for life. The rickettsiae are shed in tick faeces but transmission to human beings is primarily by bite, as the rickettsiae also invade the salivary glands of the ticks. All rickettsiae of this group pass through natural cycles in domestic and wild animals or birds.

ROCKY MOUNTAIN SPOTTED FEVER

Rocky Mountain Spotted Fever (RMSF) is the most serious type of spotted fever and is the first to have been described. It is prevalent in many parts of North and South America and is transmitted by *Dermacentor andersoni* and related species of ticks.

TICK TYPHUS (INDIAN TICK TYPHUS)

Tick typhus, in several parts of Europe, Africa and Asia is caused by *R. conori*, strains of which isolated from the Mediterranean region, Kenya, South Africa and India are indistinguishable. The species is named after Conor, who provided the first description of the Mediterranean disease. Tick typhus was first observed in India in the foothills of the Himalayas. Subsequently, the disease was reported from many parts of the

country namely Allahabad, Narsapatnam, Ratlam, Secunderabad, Trichinapally, Bangalore, Jhansi, Darjeeling, Pune and Lucknow. The tick sp. *Rhipicephalus sanguineus* is the most important vector and is generally found infesting dogs all over. Some species of *Haemaphysalis* and *Hyalomma* ticks may also transmit the infection.

The incubation period ranges from 2 to 7 days. In >50% of the patients, a primary lesion with central necrosis (eschar) appears at the site of the tick bite. The lesion is covered with a brownish black scab (tache noire) and may ulcerate. Recall of a tick bite cannot always be elicited from the patient. Regional lymphadenitis is common. The fever lasts for 1 to 2 weeks and is accompanied by headache, arthralgias, myalgias and a generalized maculopapular rash which develops between the third and fifth days of illness or which may not appear. It disappears at the time of defervescence. Alterations in cytokine profiles, hypercoagulability and deep venous thrombosis may occur. In severe cases – particularly in elderly patients and those with diabetes mellitus, alcoholism or heart failure – meningoencephalitis with coma and seizures and/or disseminated vasculitis of internal organs (e.g. in the heart, lungs, kidneys, liver and pancreas) are observed. The mortality rate is 1 to 5% but is higher among patients with severe cases.

...about CD Alert

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Acknowledgement: Financial assistance by WHO/USAID is duly acknowledged.