

VOLUME - XX

ISSUE - CXVII

MAY/JUN 2023

The CruX

BIMONTHLY FORUM FOR THE LABORATORIANS

Editorial

CONTENTS

1 Editorial

2 Disease
Diagnosis

7 Interpretation

9 Troubleshooting

11 Bouquet

12 Tulip News

Scrub typhus or **bush typhus** is a form of typhus caused by the intracellular parasite *Orientia tsutsugamushi*, a Gram-negative α -proteobacterium of family Rickettsiaceae first isolated and identified in 1930 in Japan.

Although the disease is similar in presentation to other forms of typhus, its pathogen is no longer included in genus *Rickettsia* with the typhus bacteria proper, but in *Orientia*. The disease is thus frequently classified separately from the other typhi.

Signs and symptoms include fever, headache, muscle pain, cough, and gastrointestinal symptoms. More virulent strains of *O. tsutsugamushi* can cause hemorrhaging and intravascular coagulation. Morbilliform rash, eschar, splenomegaly, and lymphadenopathies are typical signs. Leukopenia and abnormal liver function tests are commonly seen in the early phase of the illness. Pneumonitis, encephalitis, and myocarditis occur in the late phase of illness. It has particularly been shown to be the most common cause of acute encephalitis syndrome in Bihar, India. Scrub typhus is transmitted by some species of trombiculid mites ("chiggers", particularly *Leptotrombidium deliense*), which are found in areas of heavy scrub vegetation. The mites feed on infected rodent hosts and subsequently transmit the parasite to other rodents and humans. The bite of this mite leaves a characteristic black eschar that is useful to the doctor for making the diagnosis. This issue deals with Scrub Typhus under the **DISEASE DIAGNOSIS**.

Scrub Typhus is usually a disease that is acquired after having been to a forest. What if forests disappear in near future! Are we looking at the forests throwing up more diseases from their natural habitats as reservoirs. Interpretation has given way to **UNDERSTANDING** this month. This issue is laid threadbare for you to understand.

TROUBLE SHOOTING section outlines the problems faced while employing immunochromatography rapid assay techniques and how to resolve them.

Wisdom shots, laughter vaccines and mental quiz therapy – are all there under **BOUQUET**.



PUBLISHED FOR TULIP CUSTOMERS
FOR PRIVATE CIRCULATION ONLY

DISEASE DIAGNOSIS

SCRUB TYPHUS

Background

Scrub typhus is an acute, febrile, infectious illness that was first described in China in 313 AD. It is caused by *Orientia* (formerly *Rickettsia*) *tsutsugamushi*, an obligate intracellular gram-negative bacterium, which was first isolated in Japan in 1930. Although scrub typhus was originally recognized as one of the tropical rickettsial diseases, *O. tsutsugamushi* differs from the rickettsiae with respect to cell-wall structure and genetic composition. The term scrub typhus derives from the type of vegetation (ie, terrain between woods and clearings) that harbors the vector. However, this term is not entirely accurate, in that scrub typhus can also be prevalent in areas such as sandy beaches, mountain deserts, and equatorial rain forests. US cases have been imported from regions of the “tsutsugamushi triangle,” which extends from northern Japan and eastern Russia in the north to northern Australia in the south and to Pakistan and Afghanistan in the west, where the disease is endemic. The range includes tropical and temperate regions, extending to altitudes greater than 3200 meters in the Himalayas. Scrub typhus is often acquired during occupational or agricultural exposures because active rice fields are an important reservoir for transmission. Western medicine became especially interested in scrub typhus during military campaigns fought in East Asia. During World War II, 18,000 cases were observed in Allied troops stationed in rural or jungle areas of the Pacific theatre. Scrub typhus was the second or third most common infection reported in US troops stationed in Vietnam and still infects troops in the region. The US military continues to work on vector control, more accurate diagnostic tests, better vaccines, and improved surveillance methods. Currently, it is estimated that about 1 million cases of scrub typhus occur annually and that as many as 1 billion people living in endemic areas may have been infected by *O. tsutsugamushi* at some time. Because of reports of *O. tsutsugamushi* strains with reduced susceptibility to antibiotics, as well as reports of interesting interactions between this bacterium and HIV, a renewed interest in scrub typhus has emerged.

Practice Essentials

Scrub typhus is an acute, febrile, infectious illness that is caused by *Orientia tsutsugamushi*. The name derives from the type of vegetation (ie, terrain between woods and clearings) that harbors the vector. The image below depicts a typical eschar seen in scrub typhus.



Typical eschar.

Signs and symptoms

Elements brought out in the history may include the following:

- Travel to an area where scrub typhus is endemic
- Chigger bite (often painless and unnoticed)
- Incubation period of 6-20 days (average, 10 days)
- Headaches, shaking chills, lymphadenopathy, conjunctival injection, fever, anorexia, and general apathy
- Rash; a small, painless, gradually enlarging papule, which leads to an area of central necrosis and is followed by eschar formation

Although many other conditions can present with a high fever, the presentation of the rash, a history of exposure to endemic areas, and the presentation of the sore caused by the bite can be diagnostic of scrub fever.

Physical findings may include the following:

- Site of infection marked by a chigger bite
- Eschar at the inoculation site (in about 50% of patients with primary infection and 30% of those with recurrent infection)
- High fever (40-40.5°C [104-105°F]), occurring more than 98% of the time
- Tender regional or generalized lymphadenopathy, occurring in 40-97% of cases
- Less frequently, ocular pain, wet cough, malaise, and injected conjunctiva
- Centrifugal macular rash on the trunk
- Enlargement of the spleen, cough, and delirium
- Pneumonitis or encephalitis
- Central nervous system (CNS), pulmonary, or cardiac involvement
- Rarely, acute renal failure, shock, and disseminated intravascular coagulation (DIC)

See **Presentation** for more detail.

Diagnosis

Laboratory studies in patients with scrub typhus may reveal the following:

- Early lymphopenia with late lymphocytosis
- Decreased CD4:CD8 lymphocyte ratio
- Thrombocytopenia
- Hematologic manifestations may be confused with dengue infection
- Elevated transaminase levels (75-95% of patients)
- Hypoalbuminemia (50% of cases)

Laboratory studies of choice are serologic tests for antibodies, including the following:

- Indirect immunoperoxidase test
- Indirect fluorescent antibody test
- Dot immunoassay
- Rapid immunochromatographic tests for detection of IgM and IgG
- Polymerase chain reaction (PCR) assay
- Rapid diagnostic reagent for scrub typhus
- Weil-Felix OX-K strain agglutination reaction

Chest radiography may reveal pneumonitis, especially in the lower lung fields.

See **Workup** for more detail.

See **Treatment** and **Medication** for more detail.

Pathophysiology

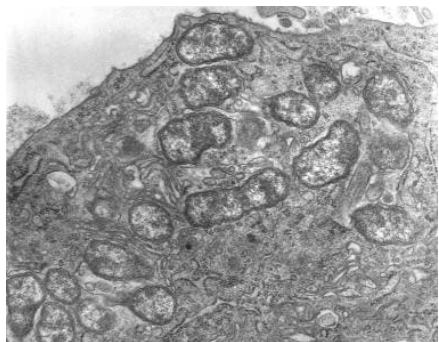
O. tsutsugamushi, the pathogen that causes scrub typhus, is transmitted to humans through the bite of an infected chigger (see the image below), the larval stage of *Leptotrombidium* mites. These 6-legged, 0.2-mm larvae are not host specific and feed for 2-10 days on the skin fluids of the host. Wild rats serve as the natural reservoir for the chiggers (and

represent a risk factor for human infection), but they are rarely infected with *O tsutsugamushi*. When the chiggers feed on humans, infection occurs.



Chigger.

Orientia is also transmitted transovarially in mites and can unbalance the sex ratio of offspring in favor of females, further propagating infection. Chigger activity and subsequent human infection rates are determined by the particular *Leptotrombidium* species present (eg, *akamushi*, *deliense*, or *pallidum*), as well as by local conditions. Not surprisingly, positive correlations have been noted between chigger population abundance and human cases of scrub typhus. In tropical regions, scrub typhus may be acquired year round. In Japan, the chigger of *Leptotrombidium akamushi* is only active between July and September, when the temperature is above 25°C (77°F). In contrast, *Leptotrombidium pallidum*, which is found over a wide range, is active at temperatures of 18–20°C (64.4–68°F), from spring into early summer and autumn). Humans acquire scrub typhus when an infected chigger bites them while feeding and inoculates *O tsutsugamushi* pathogens. The bacteria multiply at the inoculation site, and a papule forms that ulcerates and becomes necrotic, evolving into an eschar, with regional lymphadenopathy that may progress to generalized lymphadenopathy within a few days. In experimental infection, humans developed an acute febrile illness within 8–10 days of the chigger bite. Bacteremia was present 1–3 days before onset of fever. As in rickettsial diseases, perivasculitis of the small blood vessels occurs. The endothelium is involved; however, the basic histopathologic lesions suggest that macrophages might be more affected. *O tsutsugamushi* stimulates phagocytosis by the immune cells, and then escapes the phagosome. It replicates in the cytoplasm (see the image below) and then buds from the cell. The bacteria are able to harness the microtubule assembly inside the human cell for movement. Antibody-opsonized bacteria are still able to escape the phagosome but cannot effectively move on the microtubule; as a result, overall infectivity is decreased.



Transmission electron micrograph depicts peritoneal mesothelial cell of

mouse that had been experimentally infected intraperitoneally with *Orientia tsutsugamushi*. Several organisms are visible within mesothelial cell's cytoplasm.

Scrub typhus may disseminate into multiple organs through endothelial cells and macrophages, resulting in the development of fatal complications. In 2009, an apparent association was reported between high *O tsutsugamushi* blood polymerase chain reaction (PCR)-determined DNA loads and disease severity.

Etiology

Scrub typhus is caused by *O tsutsugamushi*, an obligate intracellular gram-negative bacterium that lives primarily in *L akamushi* and *L deliense* mites. This organism is found throughout the mite's body but is present in the greatest number in the salivary glands. When the mite feeds on rodents (eg, rats, moles, and field mice, which are the secondary reservoirs) or humans, the parasites are transmitted to the host. Only larval *Leptotrombidium* mites (chiggers) transmit the disease. *O tsutsugamushi* is very similar to the rickettsiae and indeed meets all of the classifications of the genus *Rickettsia*; this connection is demonstrated by the high degree of homology (90–99%) on 16S ribosomal sequencing. However, the cell walls are quite different, in that those of *O tsutsugamushi* lack peptidoglycan and lipopolysaccharide. This pathogen does not have a vacuolar membrane; thus, it freely grows in the cytoplasm of infected cells. There are numerous serotypes, of which 5—Karp, Gilliam, Kawazaki, Boryon, and Kato—are helpful in serologic diagnosis. About half of isolates are seroreactive to Karp antisera, and approximately one-quarter of isolates are seroreactive to antisera against the prototype Gilliam strain.

Risk factors

In 2009, behavioral factors were shown to be associated with scrub typhus during an autumn epidemic season in South Korea. Taking a rest directly on the grass, working in short sleeves, working with bare hands, and squatting to defecate or urinate posed the highest risks. Wearing a long-sleeved shirt while working, keeping work clothes off the grass, and always using a mat to rest outdoors showed protective associations.

Epidemiology

International statistics

Scrub typhus is endemic in regions of eastern Asia and the southwestern Pacific (Korea to Australia) and from Japan to India and Pakistan. Recently, there have been increased reports of infection in Northern India. It is generally a disease of rural villages and suburban areas and is normally not encountered in the cities. Although most cases are undiagnosed, prospective studies in endemic areas reveal an incidence of 18–23%. Community surveys in Malaysia reported an incidence of 3.2–3.5% per month and a seroprevalence exceeding 80% in those older than 44 years. Surveillance of military personnel deployed in Southeast Asia demonstrated seroconversion in 484 per 1000 population. The seasonal occurrence of scrub typhus varies with the climate in different countries because the mites are able to thrive as conditions change. The mites prefer the rainy season and certain areas (eg, forest clearings, riverbanks, and grassy regions). In the past few years, cases have been noted earlier in the season because of increased mite activity as the weather warms. Areas in which mites thrive pose a greater risk to humans. The prevalence of scrub typhus in Japan has been rising, and much of the current research is based in Japan.

Age-, sex-, and race-related demographics

People of all ages are affected equally by scrub typhus. Men and women are affected with equal frequency. No race-related differences in

incidence have been documented.

Prognosis

Prognosis varies and depends on the severity of illness, which relates to the different strains of *O tsutsugamushi*, as well as to host factors. Severe disease is uncommon with antimicrobial treatment. Prognostic indicators for severe disease have not been established. Incomplete immunity and strain heterogeneity open the door to frequent reinfections. Immunity to the same strain is believed to last 3 years, whereas immunity to other strains may last as little as 1 month; however, repeat infections may be attenuated. **In patients who are not treated, mortality ranges from 1% to 60%**, depending on the patient's age, the geographic area, and the particular strain responsible for the infection. In the preantibiotic era, mortality in Japan averaged 30%: 15% in patients aged 11-20 years, 20% in those aged 21-30 years, and 59% in those older than 60 years. In Taiwan, overall mortality was estimated at 11% but was only 5% in children and 45% in the elderly. **With appropriate antibiotic treatment, mortality from scrub typhus is quite rare**, and the recovery period is short and usually without complications. However, mortality is still approximately 15% in some areas as a consequence of missed or delayed diagnosis. If severe complications such as acute respiratory distress syndrome (ARDS) arise, mortality may still be high.

Clinical Presentation

History

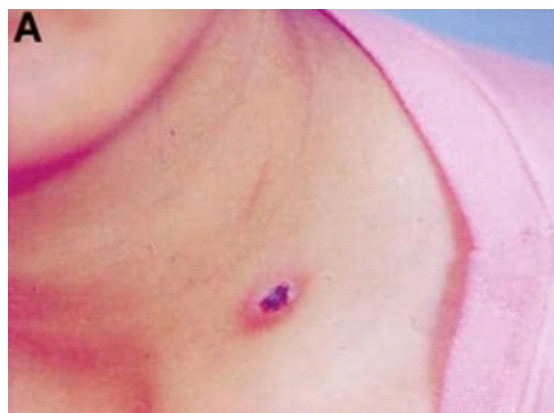
Patients with scrub typhus may present early or later in the course of their disease. In the United States, a history of travel to the endemic area must be sought, specifically probing for exposures in rural areas and contact with vegetation or the ground. **Inoculation through the chigger bite is often painless and unnoticed.** The incubation period lasts 6-20 days (average, 10 days). After incubation, persons may experience headaches, shaking chills, lymphadenopathy, conjunctival infection, fever, anorexia, and general apathy. The fever usually reaches 40-40.5°C (104-105°F). **A small painless papule initially appears at the site of infection and enlarges gradually.** An area of central necrosis develops and is followed by eschar formation. The eschar (if present) is well developed at the initiation of the fevers, which may drive the patient to seek medical attention. **Diagnosing scrub typhus early in its course can be difficult** because many conditions can present with a high fever; however, the presentation of the rash, a history of exposure to endemic areas, and the presentation of the sore caused by the bite can be diagnostic.

Physical Examination

The site of infection is marked by a chigger bite. Approximately 50% of patients with primary infection and 30% of patients with recurrent infection develop an eschar at the inoculation site (see the images below). Given the appropriate history, the eschar is often pathognomonic, but it may be missed by an inexperienced observer.



Typical eschar.



Eschar on neck.



Eschar on scrotum.

In prospective studies, trained investigators were able to locate an eschar on 68-87% of patients. In adults, the eschar is often truncal, whereas children may have lesions in the perineum. The incidence of an eschar on head, face or neck is estimated to be approximately 5%. Multiple eschars may be present. The eschar may also abrade, leaving an ulcer reminiscent of primary syphilis. **The presence or absence of eschar was thoroughly examined** in a study of 176 Korean patients with scrub typhus confirmed by immunofluorescent assay. In this study, 162 (92%) cases had eschar, with 128 (79.5%) on the front of the body. In men, eschars were detected within 30 cm below the umbilicus (19 patients; 35.8%), on the lower extremities (12 patients; 22.6%) and on the chest above the umbilicus (11 patients; 20.8%). In women, the most prevalent area was the chest above the umbilicus (44 patients; 40.7%). **Patients experience abrupt onset of high fever** (40-40.5°C [104-105°F]), headache, malaise, and myalgia approximately 10 days after infection. At that time, the eschar (if present) is well formed. Fever is the most commonly reported complaint, occurring more than 98% of the time. Tender regional or generalized lymphadenopathy may provide a clue to diagnosis and is reported in 40-97% of cases. Less frequently, ocular pain, wet cough, malaise, and injected conjunctiva are present. **Toward the end of the first week, approximately 35% (reported range, 15-93%) of patients** develop a centrifugal macular rash on the trunk. The rash may progress to become papular (see the image below). It may be transient and easily missed. Additional symptoms at this time may include enlargement of the spleen, cough, and delirium. Pneumonitis or encephalitis may develop during the second week.



Maculopapular rash.

Some patients may have central nervous system (CNS) involvement with tremors, nervousness, slurred speech, nuchal rigidity, or deafness during the second week of the disease. However, results from cerebrospinal fluid (CSF) analysis are either normal or indicate a low number of monocytes. Severe CNS involvement (eg, seizure or coma) is rare. If acute hearing loss is present (as may be the case in as many as one third of patients, according to some reports), it strongly points toward scrub typhus. [Some evidence of pulmonary involvement \(eg, cough, tachypnea, or pulmonary infiltrates\) is often present.](#) Respiratory compromise may progress to acute respiratory distress syndrome (ARDS), especially in the elderly. Cardiac involvement is often minor and rare; however, cases of fatal myocarditis have been reported. Infection with *O tsutsugamushi* may cause a relative bradycardia, which, when combined with rash, may raise concern for typhoid fever. [Scrub typhus may rarely cause acute renal failure,](#) shock, and disseminated intravascular coagulation (DIC). If the patient does not receive treatment, symptoms may last for more than 2 weeks; with treatment, the patient recovers within 36 hours.

Complications

Scrub typhus patients who are not treated may develop serious complications and may even die. Mortality ranges from 1% to 60%, depending on the geographic area and the pathogenic strain. Death can occur either from the primary infection or from secondary complications (eg, pneumonitis, encephalitis, or circulatory failure). Most fatalities occur by the end of the second week of infection. [Scrub typhus has an increased potential for complications when patients are older than 60 years,](#) present without eschar, or have white blood cell (WBC) counts higher than 10,000/ μ L. This condition represents an important cause of fever associated with poor pregnancy outcomes in refugee camps on the Thai-Burmese border. Another study reported that more than a third of pregnant women with murine typhus or scrub typhus infection have poor neonatal outcomes.

Differential Diagnoses

Diagnostic Considerations

Scrub typhus may rarely be first seen with fever and a tender neck swelling, mimicking a deep neck infection. [Diagnosing scrub typhus in international travelers can be challenging.](#) Such travelers may become sick before or within a few days of return from an endemic region. An illness that begins more than 18 days after return is unlikely to be scrub typhus. If empiric therapy does not result in defervescence within 48 hours, an alternative diagnosis should be strongly considered. Travelers to endemic areas should be educated regarding the importance of being aware of bites and seeking treatment immediately if they are affected.

- Hemorrhagic Fever with Renal Failure Syndrome
- Leptospirosis
- Rickettsial infections
- Severe fever with thrombocytopenia syndrome - See the report "S. Korea says death toll from tick-borne virus rises to 8"

Differential Diagnoses

- Anthrax
- Dengue
- Malaria
- Severe fever with thrombocytopenia syndrome
- Tularemia
- Typhoid Fever
- Typhus

Workup

Approach Considerations

Routine laboratory studies in patients with scrub typhus reveal early lymphopenia with late lymphocytosis. A decrease in the CD4:CD8 lymphocyte ratio may also be noted. Thrombocytopenia is also seen. The hematologic manifestations may raise the suspicion of dengue infection. [Elevated transaminase levels may be present in 75-95% of patients.](#) Hypoalbuminemia occurs in about 50% of cases, whereas hyperbilirubinemia is rare. These findings may be especially prevalent in children. In adults, elevated transaminase levels relate to severity of disease. Transaminitis combined with other symptoms and exposure history may suggest possible leptospirosis. Coinfection with leptospirosis has been reported. [Laboratory studies of choice are serologic tests for antibodies.](#) The main confirmatory tests are the indirect immunoperoxidase test and the immunofluorescent assay. An infection is confirmed by a 4-fold increase in antibody titers between acute and convalescent serum specimens. A single high titer with classic clinical features is considered a probable case. Serology for all suspected subtypes should be requested. [The indirect fluorescent antibody test is sensitive and provides results in a couple of hours.](#) It uses fluorescent antihuman antibody to detect specific antibody from patient serum bound to a smear of scrub-typhus antigen. A dot immunoassay has also been used in the serodiagnosis of scrub typhus. [A study of 2 rapid immunochromatographic tests for detection of IgM and IgG against *O tsutsugamushi* determined that both assays were more sensitive and specific than the standard immune immunofluorescence assay for the early diagnosis of scrub typhus.](#) [A study by Varghese et al that included 203 patients](#) previously confirmed to have scrub typhus reported that IgM levels gradually declined but remained elevated above the diagnostic cutoff up to 12 months post-infection. [O tsutsugamushi has been identified by means of the polymerase chain reaction \(PCR\) technique in clinical specimens.](#) Performing nested PCR on the eschar might be a rapid diagnostic test for scrub typhus in the early, acute stage. In 2007, Cao et al reported on the development of a rapid diagnostic

reagent for scrub typhus. The Weil-Felix OX-K strain agglutination reaction can be used to aid in diagnosis of scrub typhus. It may be the only serologic test available in less developed countries; unfortunately, it is not a very sensitive assay. Chest radiography may reveal pneumonitis, especially in the lower lung fields. Histologically, the basic pathologic change is focal or disseminated vasculitis caused by the destruction of endothelial cells and the perivascular infiltration of leukocytes.

Management

Current treatment for scrub typhus is based on antibiotic therapy. Relapses may occur if the antibiotics are not taken for long enough. Agents that have been used include the following:

- Tetracycline derivatives (standard; especially doxycycline)
- Macrolides (eg, azithromycin, roxithromycin, and telithromycin)
- Fluoroquinolones (not currently recommended; results have been mixed)

Diet and activity are as tolerated. Inpatient care may be necessary for patients with severe scrub typhus. In such cases, meticulous supportive management is necessary to abort progression to DIC or circulatory collapse.

Preventive measures in endemic areas include the following:

- Protective clothing
- Insect repellents
- Short-term vector reduction using environmental insecticides and vegetation control

Chemoprophylaxis regimens have included the following:

- A single dose of doxycycline given weekly, started before exposure and continued for 6 weeks after exposure
- A single oral dose of chloramphenicol (typically not used in the United States) or tetracycline given every 5 days for a total of 35 days, with 5-day nontreatment intervals
- No vaccine is available.

INTERPRETATION

UNDERSTANDING

How Forest Loss Is Leading To a Rise in Human Disease

A growing body of scientific evidence shows that the felling of tropical forests creates optimal conditions for the spread of mosquito-borne scourges, including malaria and dengue. Primates and other animals are also spreading disease from cleared forests to people.

In Borneo, an island shared by Indonesia and Malaysia, some of the world's oldest tropical forests are being cut down and replaced with oil palm plantations at a breakneck pace. Wiping forests high in biodiversity off the land for monoculture plantations causes numerous environmental problems, from the destruction of wildlife habitat to the rapid release of stored carbon, which contributes to global warming. **But deforestation is having another worrisome effect:** an increase in the spread of life-threatening diseases such as malaria and dengue fever. For a host of ecological reasons, the loss of forest can act as an incubator for insect-borne and other infectious diseases that afflict humans. The most recent example came to light this month in the *Journal of Emerging Infectious Diseases*, with researchers documenting a steep rise in human malaria cases in a region of Malaysian Borneo undergoing rapid deforestation. **This form of the disease was once found mainly in primates called macaques**, and scientists from the London School of Tropical Medicine and Hygiene wondered why there was a sudden spike in human cases. Studying satellite maps of where forest was being cut down and where it was left standing, the researchers compared the patchwork to the locations of recent malaria outbreaks. They realized the primates were concentrating in the remaining fragments of forest habitat, possibly increasing disease transmission among their own populations. Then, as humans worked on the new palm plantations, near the recently created forest edges, mosquitoes that thrived in this new habitat carried the disease from macaques to people. Such phenomena are not uncommon. "In years when there is a lot of land clearance you get a spike in leptospirosis [a potentially fatal bacterial disease] cases, and in malaria and dengue," says Peter Daszak, the president of Ecohealth Alliance, which is part of a global effort to understand and ameliorate these dynamics. "Deforestation creates ideal habitat for some diseases." **The Borneo malaria study is the latest piece of a growing body** of scientific evidence showing how cutting down large swaths of forests is a major factor in a serious human health problem — the outbreak of some of the world's most serious infectious diseases that emerge from wildlife and insects in forests. Some 60 percent of the diseases that affect people spend part of their life cycle in wild and domestic animals. **The research work is urgent** — land development is rapidly taking place across regions with high biodiversity, and the greater the number of species, the greater the number of diseases, scientists say. They are deeply concerned that the next global pandemic could come out of the forest and spread quickly around the world, as was the case with SARS and Ebola, which both emerged from wild animals. **Mosquitoes are not the only carriers of pathogens from the wild to humans.** Bats, primates, and even snails can carry disease, and transmission dynamics change for all of these species following forest clearing, often creating a much greater threat to people.

The risk of disease outbreaks can be greatly magnified after forests are cleared for agriculture and roads.

Throughout human history pathogens have emerged from forests. The

Zika virus, for example, which is believed to be causing microcephaly, or smaller than normal heads, in newborns in Latin America, emerged from the Zika forest of Uganda in the 1940s. Dengue, Chikungunya, yellow fever, and some other mosquito-borne pathogens likely also came out of the forests of Africa. **Forests contain numerous pathogens that have been passed** back and forth between mosquitoes and mammals for ages. Because they evolved together, these viruses often cause few or no symptoms in their hosts, providing "a protective effect from a homegrown infection," says Richard Pollack of the T.H. Chan School Public Health at Harvard. But humans often have no such protection. **What research is demonstrating is that because of a complex chain of ecological changes**, the risk of disease outbreaks, especially those carried by some mosquitoes, can be greatly magnified after forests are cleared for agriculture and roads. **A flood of sunlight pouring onto the once-shady forest floor**, for example, increases water temperatures, which can aid mosquito breeding, explained Amy Vittor, an assistant professor of medicine at the University of Florida. She is an expert in the ecology of deforestation and malaria, which is where this dynamic is best understood. **Deforestation creates other conditions conducive to mosquito breeding.** Leaves that once made streams and ponds high in tannins disappear, which lowers the acidity and makes the water more turbid, both of which favor the breeding of some species of mosquito over others. Flowing water is dammed up, deliberately and inadvertently, and pools. Because it is no longer taken up and transpired by trees, the water table rises closer to the forest floor, which can create more swampy areas. **As agriculture replaces forest, "re-growth of low lying vegetation** provides a much more suitable environment" for the mosquitoes that carry the malaria parasite, Vittor says.



A man sleeps inside a mosquito net in his home in West Papua, Indonesia.

The link between deforestation and increases in malaria has been known for some time, but research in the last two decades has filled in many of the details. Much of the work has been done in Peru, where in one region in the 1990s cases of malaria went from 600 per year to 120,000, just after a road was built into virgin forest and people began clearing land for farms. **The cascade of human-induced ecological changes dramatically reduces mosquito diversity.** "The species that survive and become dominant, for reasons that are not well understood, almost always transmit malaria better than the species that had been most abundant in the intact forests," write Eric Chivian and Aaron Bernstein, public health experts at Harvard Medical School, in their book *How Our Health Depends on Biodiversity*. "This has been observed

essentially everywhere malaria occurs.” Mosquitoes can adapt fairly quickly to environmental change. In response to a push to use bed nets to prevent nighttime bites in malaria-prone regions of the world, for example, researchers are seeing a change in the time of day mosquitoes bite — many now target their human quarry in the hours before bed. A study by Vittor and others found that one malaria-carrying mosquito species, *Anopheles darlingi*, in a deforested area in Peru was radically different than its cousins in intact forests; the *Anopheles darlingi* in deforested areas bit 278 times more frequently than in an intact forest, according to a study published in the *American Journal of Tropical Medicine and Hygiene* in 2006. “In the forest, we found almost no breeding whatsoever, and no biting by the adult mosquitoes,” Vittor said. That’s probably because the ecology of the deforested landscape — short vegetation and deep water — favored their breeding, and they need human blood to grow their eggs. The types of mosquitoes that do well in this radically altered ecosystem are more “vector competent,” which means their systems are particularly good at manufacturing a lot of the pathogen that causes malaria. A study in Brazil, published in the *Journal of Emerging Infectious Diseases* in 2010, found that clearing four percent of the forest resulted in a nearly 50-percent increase in human malaria cases. The ecology of the viruses in deforested areas is different. As forests are cut down, numerous new boundaries, or edges, are created between deforested areas and forest. A mosquito called *Aedes africanus*, a host of the yellow fever and Chikungunya viruses, often lives in this edge habitat and bites people working or living nearby. Other primates, which are also reservoirs for the pathogens, gather in the borders of these different ecosystems, providing an ongoing source of virus for the insects. Insects are not the only way that deforestation can exacerbate infectious diseases. For some unknown reason, the species of snails that can better adapt to warm open areas that occur after a forest is cut down are better hosts for parasites called flatworms, some of which cause schistosomiasis, a disease which damages human organs. Scientists are concerned that these outbreaks exacerbated by human alteration of landscapes could cause the next pandemic. The Roman Empire once stretched from Scotland to Africa and lasted for more than 400 years. No one knows exactly why the empire collapsed, but one contributing factor may have been malaria. A mass grave of babies from that era, excavated in the 1990s, found, through DNA analysis, that many of them had died from malaria, according to a study published in 2001 in the journal *Ancient Biomolecules*. Some researchers speculate that the malaria outbreak may have been exacerbated by deforestation in Rome’s surrounding Tiber River Valley to supply timber to the growing city.

One piece of the puzzle is to know what pathogens might come out of the forest in the future.

Once a disease has left a forested region, it can travel in human beings, crossing the world in a matter of hours by airplane before the person even shows symptoms. How well it does in its new homes depends on several factors. Once Zika traveled to Brazil from Africa, for example, it flourished because *Aedes aegypti* mosquitoes hang out around people and love to lay their eggs in small containers of water. Many people in Brazil’s large slums store water in buckets, and standing water also collects in tarps, old tires, and trash. A key question about the Zika virus is whether it will enter the primate populations in South America, which means it might become a permanent resident and an ongoing source of infection. “Is it going to set up shop there?” asks Vittor. “We don’t know.” Mosquitoes aren’t the only creatures that bring fever out of the forest. Angolan free-tailed bats were believed to harbor the Ebola virus that broke out and killed more than 11,000 people last year. And AIDS, which has killed more than 25 million people worldwide, came from people eating bush meat, likely chimpanzees. A wild card in this disease scenario is the rapidly changing climate. If spring comes early, mosquitoes hatch earlier and summer populations are larger. In Southeast Asia, the spike in temperatures during El Niño weather cycles correlates with dengue fever outbreaks, because the warmer weather allows mosquitoes to breed faster and expand the population, which spreads the virus further, according to a study last year in the *Proceedings of the National Academy of Sciences*. Part of the solution is to recognize and understand these connections and teach people that keeping nature intact has protective effects. And where people do cut down forests or build roads, numerous steps can be taken to lessen the chance of mosquito-borne disease outbreaks — education campaigns, more clinics, health training, and medical monitoring. Another piece of the puzzle is to know what pathogens the world might be up against in the future as they come out of the forest. Ecohealth Alliance is cataloging wildlife-borne viruses in wild places where there is new encroachment into undisturbed nature and health care is poor or non-existent. The goal is to better understand how these viruses might spread and to potentially develop vaccines. “If we could deal with the trade in wildlife and deforestation we wouldn’t need to stop an outbreak,” like Zika or Ebola, said Daszak, the organization’s president. “We would have already dealt with it.”

TROUBLESHOOTING

Immunochromatography Assay (ICA):

- It is also called as lateral flow dipstick immunoassay or simply strip tests, commonly called as lateral flow test.
- Immunochromatography is union of chromatography and immunoassay.
- Immunochromatography is a simple device proposed to determine the presence or absence of target analyte.

Principle:

- It is based on the same principle as that of ELISA sandwich technique, differing on that the immunological reaction is carried out on the chromatographic paper by capillary action.
- For ICA, two varieties of specific antibodies are used against the antigen. One of the antibodies is immobilized on the chromatographic paper, and the other is labelled with colloidal gold and infiltrated into sample pad.
- An immunographic unit is accomplished by affixing the sample pad at the end of the membrane.
- When the liquid sample is dropped on the sample pad, the antigen in the sample forms an immunocomplex (Ag-Ab complex) with the antibody labelled with colloidal gold.
- Ag-Ab complex migrates along with the liquid sample, and comes in contact with the antibody immobilized on the membrane, followed by the formation of an immunocomplex with the immobilized antibody. As more and more Ag-Ab complexes are halted at the test line, it yields a colored red purple line.
- Appearance of red purple line on the membrane is the suggestive of the presence of antigen of interest in the sample. Since the liquid of the sample travels through the membrane very fast, it makes it possible to determine the presence or absence of antigen within 15 mins.

Basic components of a typical immunochromatographic strip:

1. Sample application pad
2. Conjugate pad
3. Nitrocellulose membrane
4. Adsorbent pad

Sample application pad:

- It is composed of cellulose and/or glass fibers.
- The commencement of assay takes place when sample is applied to this pad.
- This pad functions to migrate the sample to other components.
- It should be able to transport sample in a smooth, steady, and homogenous way.
- The pretreatment may consist of segregation of sample components, elimination of interferences, pH adjustment etc.

Conjugate pad:

- Labelled antibodies, usually nano colloid gold particle is dispensed in this pad.
- As soon as the material of the conjugate pad comes in contact with the moving liquid sample, it should release labelled conjugate.
- Labeled conjugate should stay stable throughout the life span of the lateral flow strip.

- Any alterations in distributing, drying or release of conjugate can alter the results of assay significantly.
- Sensitivity of the assay might be largely affected by the poor preparation of labeled conjugate.
- Conjugate pads are synthesized by using materials such as glass fiber, cellulose, polyesters etc.

Nitrocellulose membrane:

- It plays crucial role in the sensitivity of ICA.
- Test and control lines are marked over this piece of membrane.
- An ideal membrane should aid and provide good binding to capture probes (antibodies etc.).
- A good membrane should have lesser non-specific adsorption in the regions of test and control lines for better result.
- For the better sensitivity of the assay, proper dispensing of bio-reagents, drying and blocking plays a major role.

Adsorbent pad:

- It functions as a sink at the end of the strip.
- It also aids in regulating flow rate of liquid over the membrane and prevents back flow of the sample.

All these components are attached over a backing card. Materials for backing card are greatly flexible as they provide platform for preparing assembly of all the components and have no concern with ICA except this. Thus, backing card aids making the handling of strip easier.

Steps involved in ICA

step I: Placing of sample

- For the initiation of the test, a sample is placed on the sample pad at one end of the strip.
- The sample may be used alone as in case with urine or serum or blood tests, or along with buffer which is particular to the test.

step II: Solubilization of molecules

- As soon as the addition of the sample takes place, the detector molecules are solubilized.
- After solubilization, the detector molecules mix up and bind to the analyte in the sample (if analyte present)

step III: Capillary action

- The fluid mixture is drawn up the sample pad and into the membrane by the capillary action.
- The mixture of sample/detector molecule continues to move up the membrane until it reaches the analyte capture molecule.
- A second antibody or antigen immobilized as a thin strip in the nitrocellulose will then capture the Ag-Ab complex if it is positive for the target analyte.
- The control line should always show visible line, or else the test is considered invalid.
- If the test is positive, a colored (generally pink or purple) line develops along with the control line.

step IV: Absorption of excess buffer

- Excess buffer along with any reagents not halted at the test of control line will then travel to the absorbent wicking pad.

Benefits of Immunochromatographic assay:

- Simple and easy to perform
- Rapid test, thus gives quicker results
- Durability over a wide range of climates
- Economical
- Single use

- Commercially accessible
- Small sample volume required

Limitations:

- Mostly qualitative results only
- Less sensitive or less accurate in comparison to other tests, i.e. can detect more than one or two analytes simultaneously.

Applications of immunochromatographic assay:

- Largely used in clinical diagnosis as a screening tests for variety of clinical markers.

- Many markers of clinical values for liver disease, sexually transmitted diseases, cardiac markers as well as markers for men's and women's reproductive health can be rapidly detected by immunochromatographic assay
- Detection of human chorionic gonadotropin (HCG), i.e. test of pregnancy.
- Diagnosis of various bacterial and viral infections. Example: rapid immunochromatographic test for syphilis, dengue etc
- Detection of parasitic infections. Eg. Rapid immunochromatographic test for malaria, filariasis etcUsed in early detection of cancer biomarker.

BOUQUET

In Lighter Vein

PEOPLE SAY EVERYTHING
HAPPENS FOR
A REASON
SO WHEN
I PUNCH
YOU IN THE
FACE,
REMEMBER
I HAVE A REASON



Don't mind me,
I'm just returning your
nose. I found it in my
business again.



Work,
Really?
Again?
Didn't I just
Do that
Yesterday?



Wisdom Whispers

YOUR BELIEFS
DON'T MAKE
YOU A
BETTER
PERSON, BUT
YOUR
BEHAVIOR
DOES.

TWO THINGS
DEFINE YOU.
YOUR PATIENCE WHEN
YOU HAVE NOTHING,
AND YOUR ATTITUDE
WHEN YOU HAVE
EVERYTHING.

A bad attitude is
like a flat tire, you'll
never go anywhere
until you change
it.

Anonymous
14 Dec 2012 10:00 AM

DON'T LOWER YOUR
STANDARDS FOR ANYONE
OR ANYTHING. SELF
RESPECT IS EVERYTHING.

Brain Teasers

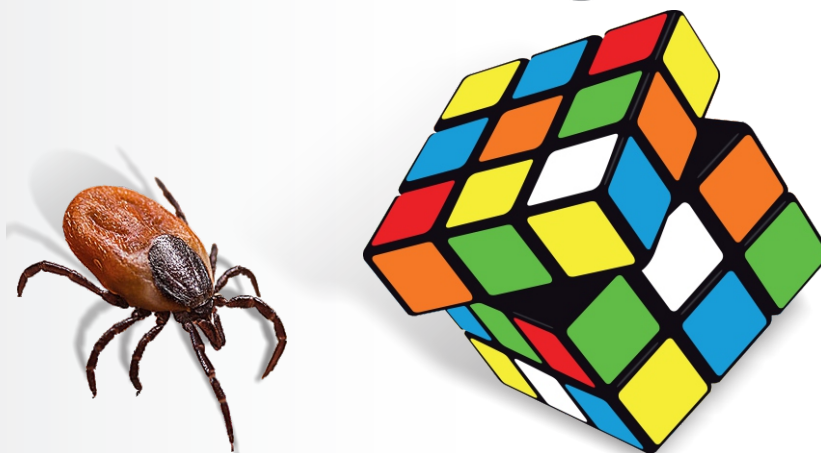
- Scrub typhus, also known as _____
 - Bush typhus
 - Mite typhus
 - Jungle typhus
 - All of the above
- Scrub typhus is caused by a bacteria _____
 - Orientia tsutsugamushi*
 - Leptospira interrogans*
 - Mycobacterium tuberculosis*
 - Bacillus anthracis*
- The site of scrub Typhus infection is marked by a _____
 - Chigger bite
 - Mosquito bite
 - Ant bite
 - None of above
- Current treatment for scrub typhus is based on antibiotics such as _____
 - Tetracycline derivatives
 - Azithromycin, roxithromycin
 - Macrolides
 - All of the above

ANSWER: 1: 4, 2: 1, 3: 1, 4: 4

Scrub Typhus Diagnosis



No more an **Enigma...**



Scrubcheck™

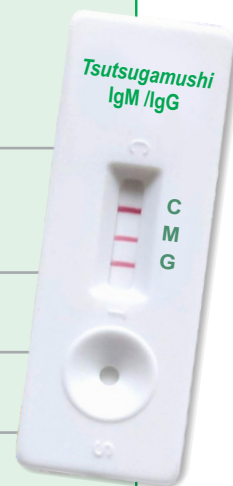
Uses *Orientia tsutsugamushi* specific recombinant antigen –
Specific detection of scrub typhus specific IgM and IgG antibodies.

Differential detection of IgM and IgG class of antibodies to scrub typhus – Precise detection of current infection and past exposure.

Reliable test result – Sensitivity 98% and specificity 100%

Storage at 4 - 30°C – Suitable for using in most climatic conditions.

10 tests pack – Suitable for all class of laboratories.



Introducing

Scrubcheck™

Reliable, quick diagnosis of Scrub Typhus infection

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
E-mail: sales@tulipgroup.com Website: www.tulipgroup.com

orchid*



Microexpress®

Coral Clinical Systems

BioShields®



Viola