



INTERNATIONAL JOURNAL OF TRENDS IN EMERGING RESEARCH AND DEVELOPMENT

Volume 4; Issue 1; 2026; Page No. 196-201

Received: 17-10-2025
Accepted: 28-12-2025
Published: 30-01-2026

Diagnostic Evaluation of Scrub Typhus: Comparative Analysis of Serological and Molecular Techniques with Emphasis on Sensitivity and Specificity

¹Bijoy Lakhi Sarmah and ²Dr. Rachana Swami

¹Research Scholar, Sunrise University, Alwar, Rajasthan, India
²Assistant Professor, Sunrise University, Alwar, Rajasthan, India

DOI: <https://doi.org/10.5281/zenodo.23320121>

Corresponding Author: Bijoy Lakhi Sarmah

Abstract

Scrub typhus is an emerging zoonotic infection caused by a small obligate intracellular bacterium, *Orientia tsutsugamushi*, which is transmitted by the bite of infected trombiculid mites. Problems with diagnosis because of the nondescript clinical characteristics of the disease and similarities with other tropical infections are considerable. This paper discusses the origin, pathogenesis, immunopathogenesis, and diagnosis of scrub typhus with emphasis on the sensitivity and specificity of serological and molecular diagnostic techniques. Various methods have been used to review the principles, effectiveness, and limitations of the immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA), conventional polymerase chain reaction, and real-time PCR. It turned out that molecular techniques are the most effective in detecting *O. tsutsugamushi* DNA in the acute phase of infection while serological techniques have more significant advantages later when immunization occurs. The accuracy of diagnosis is influenced by various factors like the time of sample collection, concentration of the pathogen in the body, the presence of antibodies, cross-reaction, and variations among strains depending on the geographical location. The paper has shown that diagnostic sensitivity and specificity vary according to the method of diagnosis chosen and the stage of the disease the patient is in.

Keywords: Scrub Typhus, *Orientia tsutsugamushi*, Molecular Diagnosis, Serological Techniques, PCR, Real-Time PCR, Sensitivity, Specificity

Introduction

Scrub typhus is a dangerous infectious disease that is transmitted to people through the bite of infected larval trombiculid mites, which are mites that have the obligate intracellular bacterium *Orientia tsutsugamushi* and can cause infection in living beings. The disease has infected areas of tropical and subtropical regions, especially in South Asia and Southeast Asia including India. There is a range of lesions caused by this disease, and they also include symptoms of mild febrile illness and severe systemic problems such as pneumonia, acute respiratory distress syndrome, hepatitis, renal problems, and multiple-organ failure. The signs of the disease include fever, headache,

myalgia, lymphadenopathy, and skin rash as well as formation of an eschar which indicates local lesions. The mechanism behind the disease development is associated with bacteria penetrating host cells and multiplying inside them, improper functioning of blood vessels, and inflammatory responses resulting in being disorder. Hence, timely diagnosis and treatment are critical for decreasing the severity of the disease.

The diagnosis of scrub typhus is quite complex because the clinical manifestations may be similar to that of a number of tropical diseases such as dengue fever, malaria, leptospirosis, and enteric fever. Although clinical symptoms and the history of potential exposition may give some

valuable first clues about the diagnosis, laboratory examination is crucial for making the right diagnosis. The most commonly used laboratory examinations are the enzyme-linked immunosorbent assay, indirect immunofluorescence assay and PCR. Although serologic tests are used for detecting the presence of specific antibodies against the bacteria, molecular techniques help to determine the bacterial material in clinical samples. It is worth noting that the molecular diagnosis is of a particular importance when the disease is in its early stages, as the concentration of antibodies may not be sufficient to make a successful diagnosis. However, the serologic methods are likely to be excellent once the immune response is developed in the patient.

The efficiency of the diagnostic methods is based on such criteria as sensitivity and specificity, which allow determining disease presence. High sensitivity ensures the low percentage of false-negative results, whereas high specificity equals the small number of false positives. However, factors that affect the result of the diagnostics include the time of collection, concentration of pathogens, presence of an immune response, cross-reactivity, and variations in strains. The knowledge of these factors is necessary for proper choice of laboratory methods. Therefore, this study evaluates the diagnostic validity of serological and molecular methods with reference to their sensitivity and specificity.

Scrub Typhus

Etiology of Scrub Typhus

Scrub typhus is an acute infectious disease caused by the obligate intracellular bacterium *Orientia tsutsugamushi*. The bacterium is primarily transmitted to humans through the bite of an infected larval mite, commonly known as chiggers (the larvae of trombiculid mites). These mites are usually found in rural areas, particularly in Southeast Asia, the Pacific Islands, and parts of Japan, India, and the Middle East. *Orientia tsutsugamushi* is classified within the genus *Orientia*, a group of bacteria that belongs to the family *Rickettsiaceae*. This group also includes the bacteria responsible for diseases such as Rocky Mountain spotted fever and epidemic typhus.

Orientia tsutsugamushi is a highly adaptive bacterium that resides within the cells of its vector, the mite, and can be transmitted to humans through the bite of the infected mite, which deposits the bacterium onto the skin. Once inside the human host, the bacterium enters endothelial cells, where it can multiply and spread throughout the body, causing systemic inflammation. The pathogen is genetically diverse, with multiple strains that differ in virulence, clinical outcomes, and geographical distribution.

Pathogenesis of Scrub Typhus

The pathogenesis of scrub typhus begins when a human is exposed to the bite of an infected chigger. During the bite, the mite injects saliva containing *Orientia tsutsugamushi* into the skin. The bacteria enter the body via the skin puncture and infect host cells, primarily endothelial cells that line blood vessels. This is facilitated by the bacterium's ability to bind to host cell surface receptors, which allows it to enter the cell and survive within a specialized compartment called the phagosome.

Once inside the endothelial cells, *O. tsutsugamushi* escapes from the phagosome into the host cell cytoplasm, where it replicates and forms large numbers of bacteria. This intracellular replication leads to the release of bacterial progeny that infect neighboring cells, spreading the infection. The endothelial cell infection is a hallmark of the disease, and the damage to blood vessel walls often leads to vasculitis, which is a significant feature of the disease's pathogenesis. This vasculitis can cause leakage of fluid into tissues, leading to edema, organ dysfunction, and, in severe cases, multi-organ failure.

In addition to endothelial cells, *O. tsutsugamushi* has been shown to infect various other cell types, including macrophages and dendritic cells, which play a role in immune responses. The bacterium also has mechanisms to evade host immune defenses, including inhibiting apoptosis (programmed cell death) and modulating the host's immune system to reduce the efficiency of immune clearance. This allows *O. tsutsugamushi* to persist within the host for prolonged periods, even in the face of an immune response. The clinical symptoms of scrub typhus are a result of both the direct effects of the bacteria on the cells and tissues, and the host's immune response. Early symptoms often include fever, headache, and muscle pain, which are common in many infectious diseases. As the infection progresses, a characteristic eschar (a dark, necrotic tissue area) may form at the site of the bite, which is a diagnostic feature of scrub typhus. The presence of the eschar indicates localized tissue destruction due to bacterial proliferation and immune response at the site of infection.

As the infection disseminates, systemic symptoms such as lymphadenopathy, rash, and gastrointestinal disturbances may appear. Severe cases may progress to complications such as pneumonia, acute respiratory distress syndrome (ARDS), hepatitis, renal failure, and even death, especially if left untreated. The severity of the disease depends on several factors, including the strain of *O. tsutsugamushi* involved, the immune status of the host, and the promptness of treatment.

Immunopathogenesis

The immune response to *Orientia tsutsugamushi* infection is complex. Upon initial infection, the host's innate immune system, including macrophages and neutrophils, is activated to combat the bacteria. The adaptive immune response is also triggered, with the production of antibodies, primarily IgM and IgG, which help neutralize the bacteria. However, *O. tsutsugamushi* is capable of modulating the immune system to evade complete elimination, leading to persistent infection.

One of the critical components of the immune response involves the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and interleukins, which contribute to the development of systemic inflammation and the characteristic vasculitis seen in scrub typhus. However, excessive cytokine production can also result in cytokine storms, which can exacerbate tissue damage and organ dysfunction.

Additionally, *Orientia tsutsugamushi* is known to induce specific immune responses that influence the outcome of the infection. Strain variability plays a significant role in the immune response: certain strains are more virulent and can

cause more severe disease, while others may lead to milder illness. This variability may be due to differences in the surface proteins and lipopolysaccharide components of the bacteria, which can influence the host immune recognition and response.

In conclusion, the etiology and pathogenesis of scrub typhus involve complex interactions between the pathogen, the host immune system, and environmental factors. The ability of *Orientia tsutsugamushi* to infect endothelial cells, evade immune detection, and cause systemic inflammation are key features that drive the clinical manifestations of scrub typhus. Early detection and appropriate antibiotic treatment are critical in preventing severe disease and complications.

Diagnosis of Scrub Typhus

Scrub Typhus is an acute febrile illness caused by the bacterium *Orientia tsutsugamushi*, transmitted by the bite of infected mites, often found in scrub or forested areas. It is prevalent in parts of Asia, including Southeast Asia, parts of the Indian subcontinent, and the Pacific Islands.

Early and accurate diagnosis of scrub typhus is essential for timely treatment, as it can be life-threatening without intervention. Several diagnostic methods can be employed:

1. **Clinical Diagnosis:** Clinical Signs: Scrub typhus should be suspected in patients presenting with fever, eschar, and a history of exposure to endemic areas (rural or scrubland areas). The presence of other nonspecific symptoms like headache, cough, muscle aches, and rash supports the clinical suspicion.
2. **Laboratory Diagnosis:** Laboratory confirmation is important because scrub typhus can mimic other febrile illnesses, including dengue, malaria, leptospirosis, and typhoid fever.

a. Serological Tests

These tests detect antibodies that the body produces in response to *Orientia tsutsugamushi* infection. They are particularly useful for confirming past or current infection.

- **Indirect Immunofluorescence Assay (IFA):** The gold standard test, detecting antibodies (IgM and IgG) against *O. tsutsugamushi*. It is highly sensitive but may take a few days for results.
- **Enzyme-Linked Immunosorbent Assay (ELISA):** Can be used for detecting IgM and IgG antibodies and is more widely available.
- **Western Blotting:** A confirmatory test that can identify specific antibodies in patient serum but is labor-intensive and less common for routine diagnosis.

b. Molecular Tests

Molecular methods directly detect the genetic material of *Orientia tsutsugamushi* and are particularly useful in the early stages of infection before the immune response is established.

- **Polymerase Chain Reaction (PCR):** PCR assays targeting *O. tsutsugamushi*'s DNA can provide a rapid and highly sensitive diagnosis. PCR can also be used to differentiate scrub typhus from other similar diseases.

c. Blood Culture

- Blood cultures for *Orientia tsutsugamushi* are difficult and are not commonly used due to the difficulty of growing the bacteria in standard lab conditions.

d. Immunohistochemical Detection

- Detection of the bacteria in tissue samples using specific antibodies may be used in some settings, especially for postmortem diagnosis.

3. Imaging and Other Tests

- **Chest X-ray:** May show signs of pneumonia in severe cases, which helps in distinguishing scrub typhus from other respiratory infections.
- **Liver Function Tests:** Can indicate liver involvement (elevated transaminases).
- **Complete Blood Count (CBC):** May show leukocytosis or leukopenia, and thrombocytopenia (low platelet count).
- **Renal Function Tests:** To monitor kidney function in severe cases (e.g., elevated serum creatinine or urea).

Differential Diagnosis: Several diseases have overlapping symptoms with scrub typhus, and differential diagnosis is critical. Some of the common conditions include:

- Dengue Fever
- Typhoid Fever
- Malaria
- Leptospirosis
- Rickettsia Infections
- Enteric Fever
- Infectious mononucleosis

Molecular techniques in scrub typhus diagnosis

Polymerase Chain Reaction (PCR) for Scrub Typhus

Polymerase Chain Reaction (PCR) is a highly sensitive molecular technique used to detect the DNA of pathogens, including *Orientia tsutsugamushi*, the causative agent of scrub typhus. PCR amplifies specific DNA sequences from the pathogen's genome, making it possible to detect even low amounts of the organism in clinical samples such as blood, tissue, or other fluids. PCR is particularly valuable in diagnosing scrub typhus during the early stages of infection, when antibodies may not yet be detectable or when the clinical symptoms are nonspecific.

Principle of PCR for Scrub Typhus Diagnosis

PCR is based on the principle of DNA amplification. The key steps include:

- Extraction of DNA from a clinical sample.
- Amplification of a specific region of the *Orientia tsutsugamushi* genome using primers.
- Detection of the amplified product, which indicates the presence of the pathogen's DNA in the sample.

There are different types of PCR, but the most commonly used for scrub typhus diagnosis is real-time PCR or quantitative PCR (qPCR), which not only amplifies but also quantifies the DNA.

Steps in PCR for Scrub Typhus Diagnosis

Sample Collection: The first step is to collect an appropriate clinical sample, usually whole blood, serum, or tissue biopsies (e.g., from skin lesions, if present). Blood is the most common sample for PCR diagnosis.

The sample should be collected as early as possible, ideally within the first week or two of illness when the bacterial load in the blood is highest.

DNA Extraction: DNA is extracted from the collected clinical sample using specialized kits or manual methods. This involves breaking open cells (lysing) to release the DNA and purifying it to remove contaminants that could interfere with the amplification process.

PCR Amplification: Specific primers are designed to target conserved regions of the *Orientia tsutsugamushi* genome. These primers are short sequences of nucleotides that match the target DNA sequence.

The DNA extracted from the sample is mixed with the primers, nucleotides (which are the building blocks of DNA), and a DNA polymerase enzyme that is capable of synthesizing new DNA strands.

The PCR reaction occurs in a the macycler, which controls the temperature at each stage of the reaction

- **Denaturation:** The reaction mixture is heated to about 95 °C to separate the double-stranded DNA into single strands.
- **Annealing:** The temperature is lowered to around 50–60 °C, allowing the primers to bind (anneal) to their complementary sequences on the target DNA.
- **Extension:** The temperature is raised to around 72 °C, where the DNA polymerase synthesizes the new DNA strand by adding nucleotides complementary to the target DNA sequence.

Amplification Cycles: These three steps (denaturation, annealing, and extension) are repeated for 25-40 cycles, depending on the protocol. Each cycle doubles the amount of the target DNA, leading to exponential amplification of the specific region of interest.

Detection of the PCR Product: After the amplification process, the PCR product is analyzed to confirm the presence of *Orientia tsutsugamushi* DNA.

1. In real-time PCR, the amplification process is monitored in real-time using fluorescent dyes or probes that emit light when bound to the amplified DNA. The intensity of the fluorescence correlates with the amount of DNA present, which can be quantified.
2. In conventional PCR, the amplified DNA is visualized by agarose gel electrophoresis, where the PCR products are separated by size. A DNA ladder (a standard of known sizes) is used to compare the length of the amplified product to confirm its identity.

Clinical Application of PCR in Scrub Typhus Diagnosis

- **Acute Phase Diagnosis:** PCR is most useful during the first 7–10 days of infection when the pathogen is actively present in the bloodstream, and before antibodies are detectable by serological tests.

- **Differential Diagnosis:** PCR can help distinguish scrub typhus from other diseases that have similar symptoms (e.g., dengue, malaria, leptospirosis), which is crucial for proper treatment.
- **Epidemiological Surveillance:** PCR can be used in population-based studies to detect scrub typhus outbreaks and track the geographic distribution of different strains of *Orientia*.

Diagnostic Sensitivity and Specificity: When diagnosing scrub typhus, an infectious disease caused by the bacterium *Orientia tsutsugamushi*, understanding the sensitivity and specificity of diagnostic tests is crucial to ensure accurate diagnosis and effective treatment. These terms describe the performance characteristics of diagnostic tests in terms of their ability to correctly identify true positives (patients with the disease) and true negatives (patients without the disease).

Diagnostic Sensitivity: Sensitivity refers to the ability of a diagnostic test to correctly identify those with the disease (true positives). It is particularly important in situations where it is crucial not to miss any cases of infection. A highly sensitive test will have fewer false negative results.

Formula for Sensitivity

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \times 100$$

- **High Sensitivity:** If a test has high sensitivity, it is very good at detecting people who have scrub typhus and is less likely to miss cases.
- **Low Sensitivity:** If the test has low sensitivity, there may be false negatives, meaning some patients who have scrub typhus may be misclassified as not having the disease.

Impact of Sensitivity in Scrub Typhus Diagnosis

- Scrub typhus can present with nonspecific symptoms (e.g., fever, headache, muscle aches), which makes diagnosis difficult in the early stages. A high-sensitivity test is essential to identify infected patients promptly and avoid complications.
- Early detection through a sensitive test allows for appropriate antibiotic treatment, which is critical to prevent severe complications, such as organ failure, respiratory distress, or death.

Common tests for scrub typhus that have high sensitivity include

1. **Polymerase Chain Reaction (PCR):** PCR has a very high sensitivity, detecting the DNA of *Orientia tsutsugamushi* directly in the blood or tissue samples, even during the early stages of infection before antibodies are produced.
2. **Real-Time PCR:** This test can detect even small amounts of *Orientia* DNA in the blood, making it highly sensitive, particularly in the first week of infection.

However, serological tests such as Indirect Immunofluorescence Assay (IFA) or Enzyme-Linked

Immunosorbent Assay (ELISA) might have lower sensitivity in the early stages of the disease, as antibodies may not have been produced yet.

Diagnostic Specificity: Specificity refers to the ability of a diagnostic test to correctly identify those without the disease (true negatives). It is the capacity of the test to exclude individuals who do not have the disease, and a test with high specificity will have fewer false positives.

Formula for Specificity

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}} \times 100$$

- **High Specificity:** A test with high specificity is very good at identifying people who do not have scrub typhus and minimizes the chances of wrongly diagnosing someone with the disease.
- **Low Specificity:** A test with low specificity may incorrectly identify people who do not have scrub typhus as being infected, leading to false positives.

Impact of Specificity in Scrub Typhus Diagnosis

- Scrub typhus shares clinical features with other tropical infectious diseases (e.g., malaria, dengue, leptospirosis), so a test with high specificity can help avoid misdiagnosing non-infected individuals as having scrub typhus.
- Misdiagnosis can lead to unnecessary treatments, such as antibiotics, which may not be appropriate and could result in adverse effects.

Tests with high specificity include

1. **Real-Time PCR:** This method specifically targets the DNA of *Orientia tsutsugamushi* and has a high specificity, as it amplifies only the pathogen’s genetic material.
2. **IFA and ELISA:** Although these serological tests are not as sensitive in the early phase of infection, they typically have high specificity once the immune system has produced antibodies, especially in later stages of infection.

Factors Affecting Sensitivity and Specificity in Scrub Typhus Diagnosis

Timing of Sample Collection

- Early in the infection, before the body has mounted an immune response, serological tests (such as ELISA or IFA) may not be reliable, resulting in lower sensitivity. In contrast, PCR can detect the pathogen's DNA in the early stages, offering higher sensitivity.
- Timing of serological tests: IgM antibodies typically appear within the first 7-10 days of infection, while IgG antibodies develop later. False negatives are more common in the early phase of infection when antibody levels are low, affecting the specificity and sensitivity of serological tests.

Cross-Reactivity

- Cross-reactivity with other rickettsial infections (e.g., typhus, murine typhus, or other species of *Orientia*) can affect the specificity of serological tests, such as IFA or ELISA. This results in false-positive results, especially in areas where multiple rickettsial infections are prevalent.
- To mitigate this issue, more specific tests such as PCR or real-time PCR are used to directly detect the pathogen.

Test Type

- PCR is highly sensitive and specific, especially in the acute phase of infection when bacterial DNA is still present in high quantities. However, PCR requires specialized equipment and expertise.
- Serological tests like ELISA and IFA are less invasive and easier to perform but tend to have lower sensitivity in the early stage of infection and are more useful in the later stages when antibodies have been produced. Despite this, they have high specificity once the immune response is established.

Geographical Variability: Sensitivity and specificity can vary based on geographic regions and the presence of different strains of *Orientia tsutsugamushi*. Different strains may have genetic variations that affect the detection sensitivity and specificity of molecular or serological tests.

Table 1: Comparison of Sensitivity and Specificity in Common Diagnostic Tests for Scrub Typhus

Test	Sensitivity	Specificity	Notes
Polymerase Chain Reaction (PCR)	Very high (early detection)	Very high (direct detection of pathogen)	Gold standard for early detection of scrub typhus.
Real-Time PCR (Quantitative PCR)	Very high (detects even small amounts of DNA)	Very high	Quantifies pathogen load, useful for monitoring treatment.
Indirect Immunofluorescence Assay (IFA)	Moderate to low (early stages)	High	High specificity for confirmed cases, but less sensitive in early infection.
Enzyme-Linked Immunosorbent Assay (ELISA)	Moderate to low (early stages)	High	Typically used for IgG/IgM antibody detection, high specificity but low sensitivity in early infection.
Culture	Low (takes time)	High (if positive)	Takes a long time (several days to weeks), not practical for early diagnosis.

Impact of Sensitivity and Specificity on Clinical Decision-Making

- High Sensitivity is essential for early detection and treatment of scrub typhus to avoid complications, especially in regions where the disease is endemic and

symptoms overlap with other tropical diseases.

- High Specificity helps to rule out other diseases, ensuring that patients are not misdiagnosed and do not receive inappropriate treatment.
- Combined Approach: In clinical practice, a

combination of diagnostic tests with varying sensitivities and specificities is often used. For example, PCR might be employed for early detection, while serological tests (like IFA or ELISA) could be used for confirmation during the later stages of infection.

Conclusion

The current work emphasizes the importance of timely and accurate diagnosis for effective identification and treatment of scrub typhus. The comparative study of serological and molecular approaches to diagnosis shows that their effectiveness highly depends on disease progression, specimen characteristics and principles of diagnosis. Molecular techniques, especially conventional PCR and real-time PCR, have substantial benefits in terms of identification of *Orientia tsutsugamushi* DNA during the early stages of infection, when specific antibodies are not likely to be present. On the other hand, serological methods, such as indirect immunofluorescence assay and ELISA, are valuable later in the course of the disease, when measurable immune response occurs. In addition, the results prove that sensitivity and specificity of methods are important parameters for evaluating diagnostic performance, while specimen collection timing, pathogen concentration, cross-reactivity and genetic variation are factors affecting sensitivity and specificity of testing methods. The data underline the fact that the choice of diagnostic methods must be based on the clinical symptoms, the stage of the disease and laboratory capabilities.

This research provides more insight that the incorporation of molecular and serological testing techniques may enhance diagnostics reliability, help in minimizing the false-negative and false-positive diagnostic results and assist in appropriate clinical management. The application of two methods can also improve clinical decision-making process and help in effective disease monitoring in endemic zones. Further research should target the development of standardized diagnostic management techniques, improving access to molecular diagnostics and analysing effectiveness of diagnostics at different stages of the disease. Moreover, further research should involve bigger clinical population, geographical strains variations as well as adequate reference standards.

References

1. Bakshi D, Singhal P, Mahajan SK, Subramaniam P, Tuteja U, Batra HV. Development of a real-time PCR assay for the diagnosis of scrub typhus cases in India and evidence of the prevalence of new genotype of *Orientia tsutsugamushi*. *Acta Tropica*. 2007;104:63-71.
2. Chakraborty S, Sarma N. Scrub typhus: an emerging threat. *Indian Journal of Dermatology*. 2017;62:478-485.
3. Chogle A. Diagnosis and treatment of scrub typhus—the Indian scenario. *Journal of the Association of Physicians of India*. 2010;58:11-12.
4. Isaac R, Varghese GM, Mathai E, Manjula J, Joseph I. Scrub typhus: prevalence and diagnostic issues in rural southern India. *Clinical Infectious Diseases*. 2004;39(9):1395-1396.
5. Jain H, Das A, Dixit S, Kaur H, Ranjit M, Dutta A, et

al. Development and implementation of a strategy for early diagnosis and management of scrub typhus: an emerging public health threat. *Frontiers in Public Health*. 2024;12:1347183. doi:10.3389/fpubh.2024.1347183.

6. Jeong YJ, Kim S, Wook YD, Lee JW, Kim KI, Lee SH. Scrub typhus: clinical, pathologic, and imaging findings. *RadioGraphics*. 2007;27(1):161-172.
7. Kala D, Gupta S, Nagraik R, Verma V, Thakur A, Kaushal A. Diagnosis of scrub typhus: recent advancements and challenges. *3 Biotech*. 2020;10:396. doi:10.1007/s13205-020-02389-w.
8. Kim DM, Kim HL, Park CY, Yang TY, Lee JH, Yang JT, et al. Clinical usefulness of eschar polymerase chain reaction for the diagnosis of scrub typhus: a prospective study. *Clinical Infectious Diseases*. 2006;43(10):1296-1300.
9. Koh GCKW, Maude RJ, Paris DH, Newton PN, Blacksell SD. Diagnosis of scrub typhus. *The American Journal of Tropical Medicine and Hygiene*. 2010;82(3):368-370.
10. Sethi S, Prasad A, Biswal M, Hallur VK, Mewara A, Gupta N, et al. Outbreak of scrub typhus in North India: a reemerging epidemic. *Tropical Doctor*. 2014;44(3):156-159.
11. Usha K, Kumar E, Kalawat U, Kumar BS, Chaudhury A. Molecular detection of scrub typhus in Tirupati, Andhra Pradesh, India. *Journal of Vector Borne Diseases*. 2015;52:171-174.
12. Viswanathan S, Muthu V, Iqbal N, Remalayam B, George T. Scrub typhus meningitis in South India—a retrospective study. *PLoS One*. 2013;8(6):e66595.

Creative Commons (CC) License

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.