Comparative Immunology, Microbiology and Infectious Diseases 90–91 (2022) 101888

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Revisiting scrub typhus: A neglected tropical disease

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ARTICLE INFO

Keywords:
Re-emerging disease
Coinfection
COVID-19
Dengue
Zoonotic infection

ABSTRACT

Scrub typhus is an under diagnosed re-emerging vector borne disease caused by an intracellular gram negative bacteria, *Orientia.* The disease is commonly prevalent in rural and hilly areas of Tsutsugumashi triangle. The diagnosis of the disease is very challenging due to similarity of its early symptoms with other febrile illnesses, like dengue and COVID 19, as well as non-availability of rapid, reliable and cost-effective methods. Moreover, the diverse clinical presentation in severe cases make it significant health problem. The occupational and behavioral risks responsible for the transmission lead to urgent need of vaccine development against the disease. The complete knowledge about its pathogenesis and the interaction with host's immune cells may help the scientists in developing the appropriate diagnostic methods as well as the vaccines.

1. Introduction

Scrub typhus, also called as Bush Typhus or Tsutsugumashi disease, is a re-emerging but neglected zoonotic acute febrile illness. It affects about one million people worldwide annually with a mortality rate of about 50%, if untreated [1]. About one billion people across the world are at risk of infection with *Orientae* per year [2]. This is a disease caused by the obligate intracellular, gram negative bacteria, the *Orientiae*, the members of genus *Orientia.* Two species of *Orientia* are known to cause the disease, i.e. *O. tsutsugamushi* and *O. chuto* [3,4]. The genus *Orientia* along with the other intracellular zoonotic pathogens, *Anaplasma, Coxiella, Ehrlichia* and *Rickettsia*, belong to the order rickettsiales, and has recently been separated from the genus *Rickettsia* [5]. The members of genus *Orientia* may be easily differentiated from the *Rickettsia* as they lack lipopolysaccharide, peptidoglycan, and the slime layer. Moreover, in contrast to the members of genus *Rickettsia*, the outer cell envelop of *Orientiae* is thicker than the inner one [5]. The *Orientia tsutsugamushi* is known for absence of lipopolysaccharide (LPS) and peptidoglycan layer; therefore, they are naturally resistant to all β-lactam antibiotics [6]. A recent report showed that *Orientia* has expressed peptidoglycan like structure and disulphide cross-linked outer membrane proteins here upon sensitive to cell wall targeting drugs and these components are required for bacterial growth, cell integrity and host cell invasion [7]. The pathogen is transmitted to humans through the bite of infected larval trombiculid mites of genera, *Leptotrombidium*, and *Schoengastiella* of family Trombiculidae. These larval mites, also known as chiggers, primarily feed on rodents, but occasionally on humans. The chiggers act as both vector as well as reservoir for the pathogen [8].

Scrub typhus was previously thought to be endemic only within an area of ~13,000,000 km^2, known as Tstsugumashi triangle, but later it

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https://doi.org/10.1016/j.cimid.2022.101888
Received 23 August 2022; Received in revised form 18 September 2022; Accepted 29 September 2022
Available online 1 October 2022
0147-9571/

was reported from outside the region also [1]. Currently, the disease is re-emerging and endemic in rural areas of Southeast Asia, Thailand, Korea, Australia, Russia, The Pacific Islands, and Japan [9]. The disease is prevalent in areas that favor the growth and reproduction of mites such as forests, mountains, deserts, and beaches [10]. Even after the re-emergence, the disease is still a neglected one among the public as well as the physicians. The symptoms of scrub typhus in early phase are similar with that of other infections, such as dengue, encephalitis, influenza and coronaviruses, causing acute febrile illness. Besides, one of the serious complications of scrub typhus, acute respiratory distress syndrome (ARDS) is similar with the symptom observed in SARS-CoV-2 infection. The earlier reports on co-infection of scrub typhus with dengue, chickangunya, H1N1 influenza indicate the possibility of its co-infection with the ongoing COVID 19 pandemic in the rainy and winter season. Thus, the negligence of scrub typhus poses the confusion and challenges in the diagnosis of the viral infections. Therefore, this review provides the complete picture of Scrub typhus including prevalence, clinical manifestation, pathogenesis, immunology, diagnosis, treatment and prophylaxis, as well as is an effort to aware the public and physicians both about the disease.

2. Prevalence of scrub typhus

In tropical areas, scrub typhus is prevalent throughout the year while in temperate regions seasonal pervasiveness has been observed [10]. In Asia, prevalence of disease occurs in rainy and winter season [11]. The seasonal prevalence of the disease is due to the fact that wet and cool season allows the attachment of more chiggers to the body of rodents [12]. The low temperature and high rainfall are the major weather factors responsible for development of the scrub typhus infection. The presence of scrub vegetation, wood piles, cattle and rodents around the residential places are considered as significant risk factors for scrub typhus infections. In addition, the behavioral factors like resting on muddy or grassy fields, use of unhygienic underclothes, working bare hands or in short sleeves, open defecation are also reported to help in transmission and development of infection [13,14]. The occupational risk is higher in farmers, hunters, sportsman, shepherds etc., particularly in rural highlands [14]. In the current decade, the outbreaks of the disease have been reported from across the world. The details of scrub typhus outbreak in last five years are given in Table 1 & 2.

Table 1
Scrub typhus prevalence across the India during last five years.

Places	Season	Number of scrub typhus cases	References
Assam (Dhemaji),	Autumn	3 cases	[68]
Bangalore	Late Summer, Autumn	53 cases	[69]
Rajasthan	Summer, Autumn	28 patients	[70]
Arunachal Pradesh	Autumn	30 cases: 10 male, 20 female	[71]
Chandigarh	Late summer, Autumn	228 cases; 111 males, 117 females	[72]
Chennai	Autumn, Winter	23 cases	[73]
Meghalaya	Autumn, Winter, Spring, summer	90 cases	[74]
Uttarakhand	Summer, Autumn	284 cases	[75]
Gorakhpur	Late summer, Autumn	46 cases	[76]
Mizoram	Winter, Autumn	283 cases	[77]
Parganas and Kolkata	Summer, Autumn, Winter,	5 cases	[10]
Himachal Pradesh,	Autumn, Winter	2 cases	[78]
Maharashtra	Late Autumn, Winter	48 cases; 21 males, 27 females	[79]
Odisha	summer, Autumn	201 cases	[80]
Sikkim	Manson	3 cases	[81]
Andhra Pradesh	Autumn, winter	60 cases	[82]
Telangana	Late summer	89 cases	[83]
Chandigarh	Summer, Autumn, Winter	77 cases	[84]

3. Pathogenesis of Scrub Typhus

Orientia enters into human body through skin of the individual, who comes in contact with mite infested habitats, bush and grass, due to their frivolous or professional behaviors [15]. These organisms transmit to human through the bite or excreta of infected chiggers. The infected chiggers inoculate *Orientia tsutsugamushi* pathogens in humans during feeding. The pathogen multiplies at the entry site leading to formation of a papule that becomes necrotic and ultimately develops into an eschar [16]. After initial inoculation, *O. tsutsugamushi* invades the cells, mainly dermal dendritic cells (DCs) and activated monocytes, in the dermal layer of skin [17]. Activated DCs and monocytes may constitute a rapid, potential dissemination sites for the pathogen while circulating to lymph nodes [17]. In the lymph nodes, the pathogen causes regional lymphadenopathy that progress to generalized lymphadenopathy within a few days, followed by many clinical manifestations [18]. From the lymph nodes, the pathogen invades the endothelial cells of different organs [19]. They have been located in endothelial cells of heart, lung, brain, liver, kidney, pancreas and skin. Moreover, the post mortem studies of the infected patients have shown the presence of these pathogens in the macrophage cells of liver [17]. During systemic dissemination, involvement of macrophage and endothelial cells in infection is the hallmark of lethal illness in humans at late stage of infection [17]. Besides, other cells like fibroblasts and neutrophils, that play key roles in lung injury, may also be infected [20]. Thus if not treated instantly, the *Orientiae* can spread systemic dissemination causing interstitial pneumonia, myocardial and hepatic lesions, meningoencephalitis, acute respiratory distress syndrome (ARDS), and multi-organ failure [21]. Most life threatening disease through scrub typhus is acute respiratory distress syndrome (ARDS) [20]. In addition, renal and liver dysfunctions are common in scrub typhus which ultimately leads to death in untreated cases [22]. The infection induces both humoral and cell mediated immunity. Increased concentration of interferon alpha, IL-8 and IL-15 is observed in infected patients [23].

The mechanism of host cell invasion by *Orientiae* and its replication inside the cells is not fully understood. However, whatever is known in this regard is based on the studies involving human endothelial cells. Briefly, the pathogen attaches itself to the host cell by the interactions between its cell surface ligands and extracellular receptors of host cell. The major cell surface ligands are outer membrane protein, TSA56, and an autotransporter protein, ScaC, which bind with host's fibronectin

Table 2
Scrub typhus prevalence in rest of the world during last five years.

Places/ Country	Season	Number of scrub typhus cases	References
Anhui Province, China	Autumn	19 cases	[85]
Yamagata, Japan	Autumn	1 case	[86]
Djibouti, Africa	Autumn	3 cases	[87]
Sri Lanka	Winter, Spring, Summer	16 cases	[88]
Jeonju, South Korea	NA*	2 cases	[33]
Nepal	Summer, Autumn	358 cases	[89]
Chile, South America	Late winter, Spring	9 case; 7 males and 2 females.	[90]
Northern Vietnam	Spring, Summer, Autumn Winter	116 cases	[91]
Southern Taiwan	Summer, Autumn	265 cases	[92]
Bhutan	Autumn, Winter	470 cases	[93]
Tribhuvan (Nepal)	Spring, Summer, Autumn, Winter	84 cases; 39 boys and 45 girls	[94]
Mobarakeh, Iran	Summer, Autumn	44 cases	[95]

* NA; Not available.

glycoprotein. This interaction between the bacterial and host cell proteins, activates the integrin mediated signaling pathways involving cytoplasmic protein; focal adhesion kinase (FAK), signaling protein; Src kinases, and the cytoskeletal proteins; Talin and Paxillin [24]. The integrin mediated signaling pathway in turn activates the RhoA GTPase protein which also acts as a signaling molecule and ultimately induces the actin cytoskeleton rearrangement in the host cell. Subsequently, the pathogen is internalized in the host cell by clathrin and adaptor protein 2 mediated endocytosis [25] (Fig. 1).

During the endocytosis, the pathogen escapes the endosomal pathway by get off from late endosomes and comes in cytoplasm. In the cytoplasm, the bacterium escapes from phagocytosis with the help of cytosolic hemolysin TlyC and Phospholypase D protein [26]. Subsequent to escape from phagosome, *Orientiea* move beside dynein-dynactin motor protein complex with microtubules to microtubule organizing center near nucleus [27]. Once in the perinuclear region, the pathogen undergoes replication within a polysaccharide-enriched microcolony by binary fission [17,27]. The daughter cells exit the infected host cells through an unusual process, budding from host plasma membrane [27]. This exit process involves the various proteins like membrane cholesterol rich lipid raft and caveolin raft protein from host cell, and high-temperature requirement A (HtrA) from the pathogen.

Syndecan-4, a transmembrane heparan sulfate proteoglycans (HSPGs), which are extensively distributed in mammalian cells, are also reported to play significant role in early adhesion process of *Orientiae* to host cells [17]. However, the detailed mechanism elucidating the role of the HSPGs in the adhesion process and its ligand on the surface of *Orientiae* are still not known, and needs investigation.

Additionally, another autotransporter protein of *Orientia,* ScaA, is thought to mediate *Orientia* adhesion to nonphagocytic host cells, as suggested by its universal distribution and conserved sequences [17]. However, the host cell receptor for ScaA protein is yet not reported [17]. Further, *Orientia* ScaA proteins represent conserved targets for vaccine development [28]. This protein also provides immunity against homologous strain of *Orientia tsutsugamushi* infection or also against heterologous strain when combined with bacterial outer membrane antigen TSA56 [28]. Thus, the ScaA protein variability affects the clinical manifestations of different *Orientia* isolates [29].

4. Interaction of *Orientia tsutsugamushi* with immune cells

Orientia tsutsugamushi infection involves different cells such as endothelial cells, dendritic cells, monocytes, neutrophils, and macrophages. The interactions of the pathogen with these host cells are critical to disease progression as well as effective treatment management strategies [20]. These interactions with each type of host cell are described below.

4.1. Endothelial cell

After the entry of *Orientia*, endothelial cells (ECs) are an important target in human *Orientia* infection. Activation of EC biomarkers including intercellular adhesion molecule 1 (ICAM-1), angiopoietin 2 (Ang2), angiopoietin 1 (Ang1) levels and vascular cell adhesion molecule 1 (VCAM-1), has been observed with disease severity and clinical prognosis [17,20,30]. ECs activation can be activated directly via *Orientia* replication at entry site and the recognition of pathogen associated molecular patterns(PAMP), or indirectly through recognition of damage-associated molecular patterns (DAMP), inflammatory cytokines and chemokines [20]. ECs activation promotes leukocyte adhesion/-transmigration, antigen presentation, and cytokine production [20]. However, excessive activation of ECs leads to tissue hypoxemia, and ARDS [31]. The interaction of *Orientia* with endothelial cells leads to production of cytokines such as IL-1, IL-6, IL-8, IL-10, IL-15, IL-32, IL-33,

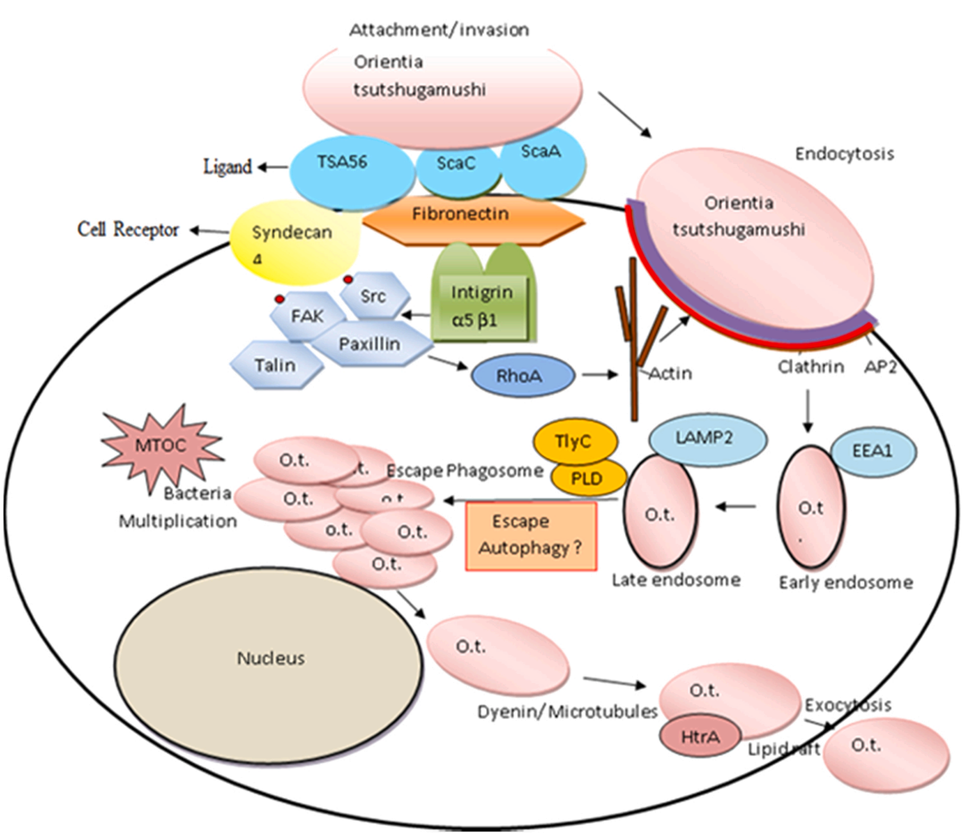

Fig. 1. Attachment and invasion of *Orientia tsutsugamushi* to host cell surface, Ot: *Orientia tsutsugamushi*; TSA56: type surface antigen 56; ScaC: surface cell antigen C; ScaA: surface cell antigen A; AP2: Adaptor protein2; FAK: focal adhesion kinase; Src: tyrosine-protein kinase; MTOC: Microtubule organizing centre.

IL-1β and tumor necrosis factor (TNF) [17].

4.2. Dentritic cell and Monocytes

Orientia infection of dendritic cells is more potent at the dermal–epidermal layer and Langerhans cells [32]. Upregulation of secretion of costimulatory molecules such as CD40, CD80, CD86 (65), CD83 and major histocompatibility complex (MHC) class II molecules provide evidence for activation of dendritic cells [17]. Other proinflammatory molecules including tumor necrosis factor alpha (TNF-α), IL-6, IL-8, IL-12p70, and the chemoattractant molecules CCL3 and CCL5 are specific features of O*rientiae* infected dendritic cells activation [17]. After activation of DC, *Orientia* disseminate in the direction of lymph nodes, where it develops adaptive immunity through secretion of T cells specially CD4 cells.

The monocytes are primary inflammatory response cells for *Orientia* at the entry site of the dermal tissue and circulating system also. Monocytes cells are main target of *Orientia* where it can replicate [33]. *O. tsutsugamushi*-infected monocytes exhibit a type I interferon (IFN) response.

4.3. Neutrophils

Neutrophils are the most abundant polymorphonuclear leukocytes in circulation; its recruitment at site of *Orientia* infection plays an important role in early detection and control of human pathogens [34]. Earlier the presence of *O. tsutsugamushi* in phagosomes within neutrophils and in the extracellular space has been studied [35]. Some studies have noted neutrophils at the site of the mite (chigger) bites in humans [32]. The increased level of neutrophil chemoattractant, CXCL 8, during *Orientia tsutsugamushi* infection shows the severity of the disease [36]. Biomarkers of neutrophil activation and the neutrophil extracellular traps (NETs) formation are also remarkably upregulated in severe scrub typhus patients as compared with less severe cases [20].

5. Clinical manifestation

Clinical manifestation of scrub typhus occurs in early stage of infection. Early symptoms also include acute onset of fever with headache and myalgia. Thus, these symptoms are similar to that of dengue fever, chikungunya, paratyphoid, and pyrexia of unknown origin (PUO) [21,37]. Sometimes, it appears as only flu- like symptoms, and creates confusion with other infections causing acute febrile illness such as influenza and coronavirus infections. The disease usually leads to gastrointestinal disturbances like abdominal pain, vomiting, rash, diarrhea. The maculopapular rash appears on about 5th to 6th day in about 50% patients and appears first on the trunk later extend to the extremities of arms and the legs but sparing the face, palms and soles [10]. An eschar at the wound site is the most characteristic feature of scrub typhus, but may not be present in all patients. Eschar is a black necrotic lesion resembling a cigarette burn usually found in areas where skin is thin, moist or wrinkled and where clothing is tight [38]. However, the presence of eschar is rare in Indian and other Asian population [9]. Hepatosplenomegaly and splenomegaly has also been reported in most of the cases. The disease is still regarded as a life threatening disease. Serious complications of this disease include pneumonia, acute respiratory distress syndrome (ARDS), myocarditis, meningoencephalitis, confusion and deafness, cardiac failure, acute renal failure, gastrointestinal bleeding, septic shock, lymphadenopathy and leukocytosis [39]. The disease is usually under diagnosed in India due to the non-specific clinical manifestations, lack of access to specific and sensitive diagnostic tests in most places and low index of suspicion among the clinicians [40].

Co-infections are common in tropical countries and can be present in scrub typhus infection with the overlapping clinical features [41]. If not treated well-timed, co-infections may lead to life-threatening consequences. The co-infection of scrub typhus with various diseases like dengue, chikungunya, H1N1 influenza, has already been reported [42,43]. Keeping in view the co-infection of scrub typhus with different viral infections, the possibility of its co-infection with COVID 19 pandemic in the rainy and winter season cannot be ruled out. The co-infections pose a diagnostic challenge to the treating physician, which are the most important causes of morbidity and mortality.

6. Diagnosis

Scrub typhus is one of the under-diagnosed illnesses. The symptoms similar to other diseases and low positivity of the eschar, a characteristic feature of the scrub typhus in the Asian population left the physicians to rely on the laboratory tests only. In laboratories, the disease can be diagnosed by serological, molecular and microbiological methods (Fig. 2). However, there is urgent need for discovery of new simple, rapid, reliable, accessible and cost effective diagnostic methods [44].

Serological methods include the detection of IgM antibodies in first week of infection and IgG antibody in second week of infection [45]. The oldest known serological method is Weil-Felix test which is based on agglutination of IgM antibodies with an antigen commonly shared by the *Orientia tsutsugamushi* and strains of *Proteus.* These antibodies can be detected in patient's blood after 5–10 days of symptoms onset. The test detects the titer of the agglutinins in the patient's serum against the *Proteus* strains OX-19, OX-2 and OX-K [46]. This is the cheapest and easily available method with 99.85% specificity However, the method has very low sensitivity (69.76%) sensitivity with 96.77% positive predictive value at a titer of $\geq$1: 160 [47]. The test exhibits cross-reactivity with epitopes of antigens from species of the genus *Rickettsia*, and can detect all kind of rickettsial infection except *Rickettsia akari*.

Another serological test, immunofluorescence assay (IFA), is a gold standard technique for diagnosis of scrub typhus. The test detects IgM antibodies in first week of infection using fluorescent anti-human antibody [48]. This method has 84.00% sensitivity and 94.82% specificity [49]. The test is more sensitive, and requires less time as compared to Weil-Felix test. However, it is costly and requires expertise and specific instruments like Fluorescence microscope. The test uses the antigens from four different strains of *O. tsutsugamushi* namely, Karp, Kato, Gilliam and Boryong [50]. The antigenic pool may contain the antigens from different strains according to country/locality for efficient diagnosis. Indirect immunoperoxidase (IIP) test is a modification of the

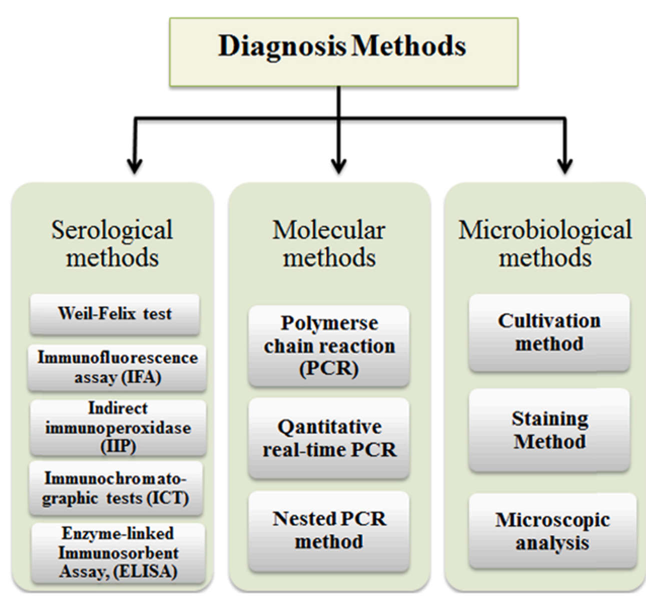

Fig. 2. Flow Diagram of diagnosis methods.

standard IFA method [51]. The method overcomes the cost of fluorescent microscope by replacement of fluorescein to peroxidase. It can be performed with a light microscope. The test has 88% sensitivity while specificity of 87% [52]. However, the test required paired samples and provides retrospective diagnosis.

Immunochromatographic tests (ICT) are simple and rapid serological test which detects total IgM, IgG or IgA antibodies in human serum against *O. tsutsugamushi,* and does not require any specialized facility and expertise [53]. These tests have been applied in the countries like India, China and Thailand. The comparison of results obtained through ICT with standard IFA test revealed that the ICTs have greater specificity and sensitivity for diagnostic of scrub typhus. However, these rapid tests need further assessment for confirmatory diagnosis and hence are being discouraged by ICMR [44].

ELISA (enzyme-linked immunosorbent assay) is automated, rapid, and cheapest test and can be done at room temperature [54]. It detects IgM antibodies against *Orientia tsutsugamushi* and uses recombinant antigens, 56-, 47-, 22-kDa proteins and cell surface proteins (ScaA and ScaC), derived from Karp, Kato, Gilliam and TA716 strains of *O. tsutsugamushi* [50]. The method provides digitized result that can be read by the naked eye and are more objective. Commercial IgM ELISA kit has greater sensitivity (98.5%) and specificity (96.3%) as comparable to IFA [54,55]. IgM ELISA test is useful for measuring the cross-sectional seroprevalence and best for large samples analysis [56]. Thus, ELISA tests are potential method for diagnosis of scrub typhus and are easily available up to secondary level of health care settings (district hospitals) in India and other endemic countries [49]. However, the test does not detect antigenically variable strains and the samples need to be pooled for performing ELISA which may cause delay in the diagnosis and influence the overall conclusion about the disease.

The molecular detection by PCR (polymerase chain reaction) based nucleic acid amplification tests (NAAT), quantitative real-time PCR assay, nested PCR method, are easy to perform, rapid methods and offer potential alternative for scrub typhus diagnosis with good sensitivity (86%) and specificity (100%) [57,58]. These tests can be performed with skin rash biopsies, lymph node biopsies and blood [59]. The assays target the 47-kDa, 56 kDa, 16S rRNA, and groEL gene of the *Orientia tsutsugamushi* IFN-β gene as control [57,60]. However, these methods have limitations due to diverse genetic makeup of *Orientia* strains [9]. Besides, the method is expensive, requires trained personnel and hence is not available beyond the secondary level of health care settings in India [44].

Microbiological methods include the isolation and identification of the causal organism by microscopy. *Orientia tsutsugamushi* organism can be isolated from blood samples using animal inoculation followed by cultivation in Vero cell or mouse fibroblast cell culture and can be identified by IFA method. Alternatively, *Orientia* can be directly identified by direct microscopy after staining with Giemsa or Gimenez stains. These stains provide dark-purple or pale-greenish blue color, respectively. The organism appears as bright red cell in oval shape measuring 05–08 μ wide and 12–30 μ long. However, the method is time consuming and provides retrospective results.

7. Treatment and prophylaxis

Scrub typhus infected patient can be treated easily with antibiotic, doxycycline. The drug is a preferred choice as it is highly efficient and show quick action. The recommended dosage is 45 mg/day in two divided doses for patients below 45 kg and 200 mg/day in two divided doses for in adult individuals above 45 kg for duration of seven days [61]. The patients allergic to doxycycline may be given the 50–100 mg/kg/day chloramphenicol divided every 6 hourly, maximum 3 g/day. Although doxycline and chloramphenicol are standard therapy but cause adverse effect in pregnant women, so single dose of azithromycin 500 mg for five days was successfully used in pregnant females without relapse and with favorable pregnancy outcomes [62]. In adults, azithromycin 500 mg in a single dose for five days and in children 10 mg/kg body weight for five days is recommended [61]. Over the time, few studies have reported the failure of treatment with doxycycline and chloramphenicol due to development of drug resistance in south East Asia, but these strains are sensitive to azithromycin [61]. However, the later evidences suggest that doxycycline resistance was a misconception and there are several other determinants which can explain the treatment failure [63].

Apart from using antibiotics, there is another way to protect the disease by herbal medicine. In herbal medicine system, different parts of plants are used either in raw or extracted form to treat diseases and improve overall wellbeing. Like many other diseases, scrub typhus can also be treated using plant extracts with antibacterial properties against *Orientia*. Ayurveda recommends to use lightly boiled cow milk with broth of *Phaseolus roxburghii* for treatment of typhus fever. In addition, *Echinacea* alone or in combination with other antiseptic herbs such as *Allium sativum* may also be used to cure scrub typhus infection. During the world war I, Dr Albert Schweitzer used *Allium sativum* as treatment for soldiers suffering from scrub typhus, and other diseases. Of late, the herbal medicine, Cap Bunga SIANTAN Demam T, is used to heal a damaged digestive tract as a result of typhus infection. However, further experiemtal studies are needed to confirm the role of these herbal medicines in treating scrub typhus before using them as regular alternative medicine [96].

The initial efforts for vaccine development were focused on preparation of killed vaccines, inactivated vaccines, attenuated vaccines and subunit vaccines [64]. Recent advances in molecular biology and immunology have clarified the antigenic organization of *Orienta tsutsugamushi* including 70, 58, 56, 47,110 and 22KD proteins on outer surface [64,65]. The proteins with masses of 47 KD and 56KD are the crucial surface antigens and are called as [Sta 47, Sta 56] scrub typhus antigens. Thereafter, the efforts were made to develop recombinant fusion proteins to provide a more efficient cellular and humoral immunity against scrub typhus. Chakraborty and Sharma [10] investigated a major outer membrane protein (OMP), the 56-kDa type-specific antigen (TSA56), as a vaccine candidate. They have also compared the efficiency of vaccines containing recombinant TSA56 (rec56) protein and tsa56-expressing DNA (p56), and observed that both vaccines induced a cell-mediated immune response to TSA56 in mice model. The study has suggested that both recombinant TSA56 protein and tsa56-expressing DNA may be used for the development of an effective vaccine and provided the background for advance studies to optimize the vaccine performance using different adjuvant like liposome, FIA and titermax. Recently, another outer membrane protein, 47-kDa antigenic surface protein was used for development of a subunit vaccine containing recombinant 47-kDa protein (rec47) and a corresponding DNA vaccine (p47). The comparison of these two vaccines in mice model has revealed that the subunit vaccine alone or in combination with adjuvant exhibited higher amount of antibodies and stronger cellular immunity induction in mice model as compared to the DNA vaccine. The study suggested the use of 47-kDa OMP for development of effective vaccine against scrub typhus. However, a multisubunit vaccine combined with an effective adjuvant need to be developed to increase the efficacy of this vaccine [66]. Till date, no any commercial vaccine is available for prophylaxis against scrub typhus [67].

Although no vaccine is available for scrub typhus, other preventive measures at both personal as well as community level may be taken to prevent the infection. Community level prevention may be achieved using the insect repellents such as DEET, dusting sulphur, dimethyl phthalate or benzyl benzoate in and around the residential areas, parks, and other open fields to refrain the growth of infected chiggers, the vector [10]. Moreover, the herbal repellents can also be used to retain the infected chiggers away from human colonization. Eamsobhana et al., [97] studied the effects of various essential oils on *Leptotrombidium imphalum* chiggers, and reported that the oils extracted from *Zingiber cassamunar*, *Eucalyptus globules*, exhibited 100% repelency against mites.

Nonetheless, the essential oils from *Syzygium aromaticum* and *Melaleucea alternifolia* also exhibited 100% repellency, when mixed with absolute ethanol in ratio of 19:1 and 3:2, respectively. Further, the use of insecticides and clear vegetation can impede the propagation of chiggers and the transmission cycle of vector. Personal prophylaxis may be achieved by applying permethrin on clothes, and diethyltoluamide on socks to prevent chigger bites. Despite of this, disease can also be prevented by dodging the use of dirty and wet undergarments, covering the whole body with clothes whenever entering in an exposed area, and avoiding barefoot work culture in risky occupations.

8. Conclusion

Scrub typhus is most common re-emerging rickettsial disease in Asia-Pacific region, particularly in developing countries. Due to non- availability of cost-effective, rapid, specific and sensitive diagnostic methods, the disease remains under diagnosed and under reported. Further, low index of suspicion among the clinicians and limited awareness about the diseases makes it more difficult to diagnose. The symptoms of the disease similar to other febrile illnesses highlighted the significance of its differential diagnosis. The knowledge about the pathogenesis of the disease will help the scientists to design and develop the cost-effective rapid and reliable methods for diagnosis of scrub typhus. Moreover, increased awareness to clinicians as well as public about the unusual manifestations of scrub typhus such as ARDS and multiorgan dysfunction, and its timely treatment may greatly reduce the mortality and morbidity of the disease. Owing to frequent outbreaks of scrub typhus infection and its risk for occupational transmission, the efficient efforts for vaccine development are also required.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors are thankful to their respective employers for providing necessary facilities.

References

[1] J. Jiang, A.L. Richards, Scrub Typhus: no longer restricted to the tsutsugamushi triangle, Trop. Med Infect. Dis. 3 (2018) 11.
[2] A. Luce-Fedrow, M. Lehman, D. Kelly, K. Mullins, A. Maina, R. Stewart, et al., A review of scrub typhus (Orientia tsutsugamushi and related organisms): then, now, and tomorrow, Trop. Med Infect. Dis. 3 (2018) 8.
[3] L. Izzard, A. Fuller, S.D. Blacksell, D.H. Paris, A.L. Richards, N. Aukkanit, et al., Isolation of a novel orientia species (O. chuto sp. nov.) from a patient infected in Dubai, J. Clin. Microbiol 48 (2010) 4404–4409.
[4] L. Clark, V.F. Williams, Historical review: Rickettsial diseases and their impact on U.S. military forces, Med Surveill. Mon. Rep. 26 (2019) 28–33.
[5] A. Tamura, N. Ohashi, H. Urakami, S. Miyamura, Classification of Rickettsia tsutsugamushi in a New Genus, Orientia gen. nov., as Orientia tsutsugamushi comb. nov, Int J. Syst. Bacteriol. 45 (1995) 589–591.
[6] C. Otten, M. Brilli, W. Vollmer, P.H. Viollier, J. Salje, Peptidoglycan in obligate intracellular bacteria, Mol. Microbiol 107 (2018) 142–163.
[7] S. Atwal, S. Giengkam, S. Chaemchuen, J. Dorling, N. Kosaisawe, M. VanNieuwenhze, et al., Evidence for a peptidoglycan-like structure in Orientia tsutsugamushi, Mol. Microbiol 105 (2017) 440–452.
[8] C. Masakhwe, P. Linsuwanon, G. Kimita, B. Mutai, S. Leepitakrat, S. Yalwala, et al., Identification and characterization of orientia chuto in trombiculid chigger mites collected from wild rodents in Kenya, J. Clin. Microbiol 56 (2018).
[9] D. Kala, S. Gupta, R. Nagraik, V. Verma, A. Thakur, A. Kaushal, Diagnosis of scrub typhus: recent advancements and challenges, 3 Biotech 10 (2020) 396.
[10] S. Chakraborty, N. Sarma, Scrub typhus: an emerging threat, Indian J. Dermatol. 62 (2017) 478–485.
[11] T. Wangrangsimakul, I. Elliott, S. Nedsuwan, R. Kumlert, S. Hinjoy, et al., The estimated burden of scrub typhus in Thailand from national surveillance data (2003-2018), PLoS Negl. Trop. Dis. 14 (2020) e0008233-4.
[12] I. Elliott, I. Pearson, P. Dahal, N.V. Thomas, T. Roberts, P.N. Newton, Scrub typhus ecology: a systematic review of Orientia in vectors and hosts, Parasit. Vectors 12 (2019) 513.
[13] T. George, S. Rajan, J. Peter, S. Hansdak, J.J. Prakash, R. Iyyadurai, et al., Risk factors for acquiring scrub typhus among the adults, J. Glob. Infect. Dis. 10 (2018) 147.
[14] J.A.J. Prakash, Scrub typhus: risks, diagnostic issues, and management challenges, Res Rep. Trop. Med Volume 8 (2017) 73–83.
[15] A. Durey, H.Y. Kwon, Y.K. Park, J. Baek, S.B. Han, J.-S. Kang, et al., A Case of Scrub Typhus complicated with a Splenic Infarction, Infect. Chemother. 50 (2018) 55.
[16] K.P. Abhilash, K. Gunasekaran, Eschar: A vital clue for diagnosis of scrub typhus. Curr Med, Issues 17 (2019) 134.
[17] F.E. Díaz, K. Abarca, A.M. Kalergis, An update on host-pathogen interplay and modulation of immune responses during orientia tsutsugamushi infection, Clin. Microbiol Rev. 31 (2018) 1–30.
[18] S. Dogra, Recent advances in understanding pathophysiology of scrub typhus, JK Sci. 12 (2010) 70–71.
[19] M. Hussain, Scrub Typhus Meningoencephalitis. Meningoencephalitis: disease which requires optimal approach in emergency manner, InTech,, 2017, p. 61.
[20] B. Trent, J. Fisher, L. Soong, Scrub Typhus pathogenesis: innate immune response and lung injury during orientia tsutsugamushi infection, Front Microbiol (2019) 10.
[21] J.V. Peter, T.I. Sudarsan, J.A.J. Prakash, G.M. Varghese, Severe scrub typhus infection: clinical features, diagnostic challenges and management, World J. Crit. Care Med 4 (2015) 244.
[22] S. Gaba, N. Gaba, M. Gupta, S. Sharma, Hepatic and renal profile of scrub typhus patients at a tertiary care center in India, Cureus 12 (2020).
[23] P. Eisermann, J. Rauch, S. Reuter, L. Eberwein, U. Mehlhoop, P. Allartz, et al., Complex cytokine responses in imported scrub typhus cases, Germany, 2010-2018, Am. J. Trop. Med Hyg. 102 (2020) 63–68.
[24] K. Katoh, FAK-dependent cell motility and cell elongation, Cells 9 (2020) 192.
[25] B.-A. Cho, N.-H. Cho, S.-Y. Seong, M.-S. Choi, I.-S. Kim, Intracellular invasion by orientia tsutsugamushi is mediated by integrin signaling and actin cytoskeleton rearrangements, Infect. Immun. 78 (2010) 1915–1923.
[26] W. Tantibhedhyabgkul, J. Mege, Pathogenesis of Orientia tsutsugamushi Infection in Humans. Hum. Emerg. Re-emerging Infect, vol. II, Wiley,, 2015, pp. 877–885.
[27] J. Salje, Orientia tsutsugamushi: A neglected but fascinating obligate intracellular bacterial pathogen, PLOS Pathog. (2017) e1006657-14.
[28] N.-Y. Ha, P. Sharma, G. Kim, Y. Kim, C.-K. Min, M.-S. Choi, et al., Immunization with an autotransporter protein of orientia tsutsugamushi provides protective immunity against Scrub Typhus, PLoS Negl. Trop. Dis. 9 (2015), e0003585.
[29] M.C. Koralur, A. Ramaiah, G.A. Dasch, Detection and distribution of Sca autotransporter protein antigens in diverse isolates of Orientia tsutsugamushi, PLoS Negl. Trop. Dis. 12 (2018), e0006784.
[30] S.M. Parikh, The angiopoietin-Tie2 signaling axis in systemic inflammation, J. Am. Soc. Nephrol. 28 (2017) 1973–1982.
[31] M.D. Moussa, C. Santonocito, D. Fagnoul, K. Donadello, O. Pradier, P. Gaussem, et al., Evaluation of endothelial damage in sepsis-related ARDS using circulating endothelial cells, Intensive Care Med 41 (2015) 231–238.
[32] D.H. Paris, R. Phetsouvanh, A. Tanganuchitcharnchai, M. Jones, K. Jenjaroen, M. Vongsouvath, et al., Orientia tsutsugamushi in human scrub typhus eschars shows tropism for dendritic cells and monocytes rather than endothelium, PLoS Negl. Trop. Dis. 6 (2012), e1466.
[33] H.-J. Ro, H. Lee, E.C. Park, C.-S. Lee, S. Il Kim, S. Jun, Ultrastructural visualization of Orientia tsutsugamushi in biopsied eschars and monocytes from scrub typhus patients in South Korea, Sci. Rep. 8 (2018) 17373.
[34] A.R. Witter, B.M. Okunnu, R.E. Berg, The essential role of neutrophils during Infection with the Intracellular Bacterial Pathogen Listeria monocytogenes, J. Immunol. 197 (2016) 1557–1565.
[35] Y. Rikihisa, S. Ito, Entry of Rickettsia tsutsugamushi into polymorphonuclear leukocytes, Infect. Immun. 38 (1982) 343–350.
[36] E. Astrup, J. Janardhanan, K. Otterdal, T. Ueland, J.A.J. Prakash, T. Lekva, et al., Cytokine network in scrub typhus: high levels of interleukin-8 are associated with disease severity and mortality, PLoS Negl. Trop. Dis. 8 (2014), e2648.
[37] S. Sapkota, S. Bhandari, S. Sapkota, R. Hamal, Dengue and Scrub Typhus Coinfection in a Patient Presenting with Febrile Illness, Case Rep. Infect. Dis. 2017 (2017) 1–3.
[38] R.P. Takhar, M.L. Bunkar, S. Arya, N. Mirdha, A. Mohd, Scrub typhus: a prospective, observational study during an outbreak in Rajasthan, India, Natl. Med J. India 30 (2017) 69–72.
[39] V. Atam, A. Majumdar, D. Himanshu, V. Kumar, I. Atam, Massive splenomegaly in scrub typhus: a rare presentation, IDCases 19 (2020), e00680.
[40] K.S. Roopa, Serodiagnosis of scrub typhus at a tertiary care hospital from Southern India, J. Clin. DIAGNOSTIC Res. 9 (2015). DC05–7.
[41] K. Jagadishkumar, N. Nataraj, U.R. Dasappa, Varicella and dengue co-infection in a child: Case report, J. Compr. Pedia (2020).
[42] P.N. Rao, A.M. van Eijk, S. Choubey, S.Z. Ali, A. Dash, P. Barla, et al., Dengue, chikungunya, and scrub typhus are important etiologies of non-malarial febrile illness in Rourkela, Odisha, India, BMC Infect. Dis. 19 (2019) 572.

[43] L. Jhuria, V. Muthu, S. Gupta, M.P. Singh, M. Biswal, K. Goyal, et al., Coinfection of H1N1 Influenza and Scrub Typhus-A Review. QJM An, Int J. Med 113 (2020) 465–468.
[44] S. Shivalli, Diagnostic evaluation of rapid tests for scrub typhus in the Indian population is needed, Infect. Dis. Poverty 5 (2016) 40.
[45] G.M. Varghese, V.M. Rajagopal, P. Trowbridge, D. Purushothaman, S.J. Martin, Kinetics of IgM and IgG antibodies after scrub typhus infection and the clinical implications, Int J. Infect. Dis. 71 (2018) 53–55.
[46] M. Koraluru, I. Bairy, M. Varma, S. Vidyasagar, Diagnostic validation of selected serological tests for detecting scrub typhus, Microbiol Immunol. 59 (2015) 371–374.
[47] M. Srinivasagam, V. Arumugam, R. Jayakumar, Comparison of IgM enzyme linked immuno sorbent assay with Weil-felix agglutination test for diagnosis of Scrub typhus in South India, Indian J. Microbiol Res 5 (2018) 92–95.
[48] F.M. Bozeman, B.L. Elisberg, Serological Diagnosis of Scrub Typhus by Indirect Immunofluorescence, Exp. Biol. Med 112 (1963) 568–573.
[49] R. Gautam, K. Parajuli, T. Tshokey, J. Stenos, J.B. Sherchand, Diagnostic evaluation of IgM ELISA and IgM Immunofluorescence assay for the diagnosis of Acute Scrub Typhus in central Nepal, BMC Infect. Dis. 20 (2020) 138.
[50] K. Pote, R. Narang, P. Deshmukh, Diagnostic performance of serological tests to detect antibodies against acute scrub typhus infection in central India, Indian J. Med Microbiol 36 (2018) 108.
[51] D.J. Kelly, P.W. Wong, E. Gan, G.E. Lewis, Comparative evaluation of the indirect immunoperoxidase test for the serodiagnosis of Rickettsial disease, Am. J. Trop. Med Hyg. (1988).
[52] M.V. Land, W.-M. Ching, G.A. Dasch, Z. Zhang, D.J. Kelly, S.R. Graves, et al., Evaluation of a commercially available recombinant-protein enzyme-linked immunosorbent assay for detection of antibodies produced in Scrub Typhus Rickettsial Infections, J. Clin. Microbiol 38 (2000) 2701–2705.
[53] K. Saraswati, N.P.J. Day, M. Mukaka, S.D. Blacksell, Scrub typhus point-of-care testing: a systematic review and meta-analysis, PLoS Negl. Trop. Dis. 12 (2018), e0006330.
[54] W. Rodkvamtook, Z. Zhang, C.C. Chao, E. Huber, D. Bodhidatta, J. Gaywee, et al., Dot-ELISA rapid test using recombinant 56-kda protein antigens for serodiagnosis of scrub typhus, Am. J. Trop. Med Hyg. (2015).
[55] N. Gupta, R. Chaudhry, S.K. Kabra, R. Lodha, B.R. Mirdha, B.K. Das, et al., Comparative evaluation of serological and molecular methods for the diagnosis of scrub typhus in indian settings, Jpn J. Infect. Dis. 70 (2017) 221–222.
[56] S.D. Blacksell, A. Tanganuchitcharnchai, P. Nawtaisong, P. Kantipong, A. Laongnualpanich, N.P.J. Day, et al., Diagnostic accuracy of the inbios scrub typhus detect enzyme-linked immunoassay for the detection of igm antibodies in Northern Thailand, Clin. Vaccin. Immunol. (2016).
[57] W. Tantibhedhyangkul, E. Wongsawat, S. Silpasakorn, D. Waywa, N. Saenyasiri, J. Suesuay, et al., Use of multiplex real-time PCR To Diagnose Scrub Typhus, J. Clin. Microbiol 55 (2017) 1377–1387.
[58] N. Saravanan, P. Rajendiran, S. Sankar, M. Ramamurthy, A. Sasimohan, V. Vineeta, et al., Detection of scrub typhus by real-time polymerase chain reaction and immunoglobulin M ELISA among patients with acute febrile illness, J. Nat. Sci. Biol. Med (2020).
[59] V. Kamath, S. Ganguly, J. Bhatia, R. Himabindu, Rickettsial infections: past and present perspectives, APIK J. Intern Med 8 (2020) 4.
[60] K.A. Patricia, Improving the diagnosis of scrub typhus by combining groel based polymerase chain reaction and IgM ELISA, J. Clin. DIAGNOSTIC Res (2017) 27–31.
[61] M. Rahi, M.D. Gupte, A. Bhargava, G.M. Varghese, R. Arora, DHR-ICMR guidelines for diagnosis and management of rickettsial diseases in India, Ricketts. Biol. Mol. Biol. Epidemiol. Vaccin. Dev. (2016).
[62] Y.-S. Kim, H.-J. Yun, S.K. Shim, S.H. Koo, S.Y. Kim, S. Kim, A comparative trial of a single dose of azithromycin versus doxycycline for the treatment of mild scrub typhus, Clin. Infect. Dis. 39 (2004) 1329–1335.
[63] T. Wangrangsimakul, W. Phuklia, P.N. Newton, A.L. Richards, N.P.J. Day, Scrub Typhus and the Misconception of Doxycycline Resistance, Clin. Infect. Dis. 70 (2020) 2444–2449.
[64] S.B. Hussain, B. Mushtaq, M.N. Shamas, Scrub typhus vaccine: past, present and future, JK Sci. 12 (2010) 102–103.
[65] S. Chattopadhyay, A.L. Richards, Scrub Typhus vaccines: past history and recent developments, Hum. Vaccin 3 (2007) 73–80.
[66] S. Choi, H.J. Jeong, K.-J. Hwang, B. Gill, Y.R. Ju, Y.S. Lee, et al., A recombinant 47-kDa outer membrane protein induces an immune response against orientia tsutsugamushi strain boryong, Am. J. Trop. Med Hyg. 97 (2017) 30–37.
[67] H. Cho, W.-H. Lee, Y.-K. Kim, K. Kim, Extracellular vesicle-associated antigens as a new vaccine platform against scrub typhus, Biochem Biophys. Res Commun. 523 (2020) 602–607.
[68] M.K. Baruah, J. Sharma, First report of scrub typhus cases in Dhemaji, Assam, India, South Indian J. Biol. Sci. 1 (2015) 156–157.
[69] B. Kalal, P. Puranik, S. Nagaraj, S. Rego, A. Shet, Scrub typhus and spotted fever among hospitalised children in South India: Clinical profile and serological epidemiology, Indian J. Med Microbiol 34 (2016) 293.
[70] R. Masand, R. Yadav, A. Purohit, B.S. Tomar, Scrub typhus in rural Rajasthan and a review of other Indian studies. Paediatr. Int Child Health 36 (2016) 148–153.
[71] B. Borkakoty, A. Jakharia, D. Biswas, J. Mahanta, Co-infection of scrub typhus and leptospirosis in patients with pyrexia of unknown origin in Longding district of Arunachal Pradesh in 2013, Indian J. Med Microbiol 34 (2016) 88.
[72] N. Sharma, M. Biswal, A. Kumar, K. Zaman, S. Jain, A. Bhalla, Scrub typhus in a tertiary care hospital in North India, Am. J. Trop. Med Hyg. (2016).
[73] S.M. Jacob, S. Geethalakshmi, S.J.A. Mary, K. Sivasangeetha, G. Pachiyappan, G. Thatchinamoorthy, et al., Scrub Typhus: A Cause for Pyrexia of Unknown Origin-Scenario in Chennai City, South India, Open Forum Infect. Dis. (2016) 3.
[74] S. Sivarajan, S. Shivalli, D. Bhuyan, M. Mawlong, R. Barman, Clinical and paraclinical profile, and predictors of outcome in 90 cases of scrub typhus, Meghalaya, India, Infect. Dis. Poverty 5 (2016) 91.
[75] A. Bhargava, R. Kaushik, R. Kaushik, A. Sharma, S. Ahmad, M. Dhar, et al., Scrub typhus in Uttarakhand & adjoining Uttar Pradesh: seasonality, clinical presentations & predictors of mortality, Indian J. Med Res 144 (2016) 901.
[76] M. Mittal, J.W.V. Thangaraj, W. Rose, V.P. Verghese, C.P.G. Kumar, M. Mittal, et al., Scrub typhus as a cause of acute encephalitis syndrome, Gorakhpur, Uttar Pradesh, India, Emerg. Infect. Dis. 23 (2017) 1414–1416.
[77] H. Lalrinkima, R. Lalremruata, C. Lalchhandama, L. Khiangte, F.H. Siamthara, C. Lalnunpuia, et al., Scrub typhus in Mizoram, India, J. Vector Borne Dis. 54 (2017) 369–371.
[78] V.S. Guleria, C. Sharda, A.K. Sood, V. Kumar, Scrub typhus: Atypical presentation in subhimalayan region, Med J. Armed Forces India 74 (2018) 180–182.
[79] Abhaykumar Ambilkar1, Amit Jogdande2, Subhash Thakre3, S.D. Nimgade4, V. M. Chauragade5, BP. Determinants of Scrub Typhus: A Case Control Study in Tribal Region of Eastern Maharashtra, J. Dent. Educ. 76 (2012) 1532–1539.
[80] M. Bal, M.P. Mohanta, S. Sahu, B. Dwibedi, S. Pati, M. Ranjit, Profile of pediatric scrub typhus in Odisha, India, Indian Pedia 56 (2019) 304–306.
[81] A. Mohanty, A. Kabi, P. Gupta, M. Jha, Rekha Us, Raj A. Scrub typhus – a case series from the state of Sikkim, India, Int J. Crit. Illn. Inj. Sci. 9 (2019) 194.
[82] N. Peesapati, R. Lakkapragada, S. Sunitha, P. Sivaram, Clinical manifestations and complications of scrub typhus: a hospital-based study from North Andhra, Astrocyte 2 (2015) 116.
[83] R.M. Lakshmi, T. Vijay Dharma, S. Sudhaharan, S. Malladi Venkata Surya, R. Emmadi, S. Raju Yadati, et al., Prevalence of scrub typhus in a tertiary care centre in Telangana, south India, Iran. J. Microbiol 12 (2020) 204–208.
[84] N. Singla, V. Mahajan, J. Chander, V. Guglani, Clinicoepidemiological and genotyping correlation of pediatric scrub typhus from Chandigarh, India, Indian Pedia 57 (2020) 314–316.
[85] M. Cao, L. Che, J. Zhang, J. Hu, S. Srinivas, R. Xu, H. Guo, Y. Zhang, C. Wang, Y. Feng, Determination of scrub typhus suggests a new epidemic focus in the Anhui Province of China, Sci. Rep. 6 (1) (2016) 1–7.
[86] Y. Suzuki, M. Shimanuki, J. Seto, K. Yahagi, K. Mizuta, A Case of Scrub Typhus Imported from South Korea to Yamagata, Japan, Jpn J. Infect. Dis. 69 (2016) 454–456.
[87] K.C. Horton, J. Jiang, A. Maina, E. Dueger, A. Zayed, A.A. Ahmed, et al., Evidence of rickettsia and orientia infections among abattoir workers in Djibouti, Am. J. Trop. Med Hyg. (2016).
[88] R. Premaratna, L.S. Blanton, D.N. Samaraweera, G.N.N. de Silva, Chandrasena NTGA, Walker DH, et al. Genotypic characterization of Orientia tsutsugamushi from patients in two geographical locations in Sri Lanka, BMC Infect. Dis. 17 (2017) 67.
[89] R. Gautam, K. Parajuli, J.B. Sherchand, Epidemiology, risk factors and seasonal variation of scrub typhus fever in Central Nepal, Trop. Med Infect. Dis. 4 (2019) 27.
[90] T. Weitzel, C. Martínez-Valdebenito, G. Acosta-Jamett, J. Jiang, A.L. Richards, K. Abarca, Scrub typhus in continental Chile, 2016–20181, Emerg. Infect. Dis. 25 (2019) 1214–1217.
[91] N.V. Trung, L.T. Hoi, D.D. Cuong, D.T. Ha, T.M. Hoa, V.N. Lien, et al., Analysis of the 56-kDa type specific antigen gene of Orientia tsutsugamushi from northern Vietnam, PLoS One 14 (2019), e0221588.
[92] Y.-C. Chang, K.-C. Kuo, W. Sun, J.-N. Lin, C.-H. Lai, C.-H. Lee, Clinicoepidemiologic characteristics of scrub typhus and murine typhus: A multi-center study in southern Taiwan, J. Microbiol Immunol. Infect. 52 (2019) 769–778.
[93] K. Dorji, Y. Phuentshok, T. Zangpo, S. Dorjee, C. Dorjee, P. Jolly, et al., Clinical and epidemiological patterns of scrub typhus, an emerging disease in Bhutan, Trop. Med Infect. Dis. 4 (2019) 56.
[94] L. Bajracharya, Scrub typhus in children at tribhuvan university teaching hospital in Nepal, Pedia Heal Med Ther. Volume 11 (2020) 193–202.
[95] H. Kassiri, B. Fathi, R. Dehghani, S. Dehghani, K. Doostifar, First report of human infestation dermatitis due to chigger mite (Acari: Trombiculidae) in Iran, J. Entomol. Res 44 (2020) 157.
[96] N.K. Patel, Alternative medicine for scrub typhus, JK Science 12 (2) (2010) 106.
[97] P. Eamsobhana, A. Yoolek, W. Kongkaew, K. Lerdthusnee, N. Khlaimanee, et al., Laboratory evaluation of aromatic essential oils from thirteen plant species as candidate repellents against *Leptothrombidium* chiggers (Acari: Trombiculidae), the vector of scrub typhus, Experimental and Applied Acarology 47 (3) (2009) 257–262.