INTRODUCTION
CHAPTER I
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1. Introduction

Rickettsiosis represents the oldest recorded infectious diseases caused by a group of pleomorphic, gram negative, and obligate intracellular bacterium *rickettsiae*. Although most rickettsial organisms are non-pathogenic to humans, some of them are known to cause febrile illnesses when transmitted accidently via blood sucking arthropods such as ticks, fleas and mites. Rickettsiosis is widely distributed throughout the world. Scrub typhus was described in China in the 3rd century and it is suspected that epidemic typhus was the cause of a plague described in Athens in the 5th century (Raoult and Roux, 1997). More recently, the importance of *rickettsiae* as emerging pathogens has also been highlighted (Raoult and Roux, 1997).

The most renowned scientists who carried out research on rickettsioses are Howard Taylor Ricketts (1871-1910), attached to the University of Chicago and did extensive studies on Rocky Mountain spotted fever and Stanislaus Matthias Von Prowazek (1875-1915) a Czech protozoologist who studied the aetiology of typhus. The genus *Rickettsia* is named in the honor of Howard Taylor Ricketts, the American microbiologist who first described *rickettsiae* as causative agents of Rocky Mountain spotted fever (*Rickettsia rickettsii*) in 1906 (Ricketts, 1906). The aetiological agent of louse borne typhus was named as *Rickettsia prowazekii* in the honor of Stanislaus Matthias Von Prowazek, Elder von Lanow, cannot be isolated in artificial media and have incomplete enzyme systems. After the accessible in cell and molecular biological procedures these were classified as bacteria, together with *ehrlichiae* and *anaplasmae* (Parola et al., 2005). The precise origin of *rickettsia* remains uncertain. *Rickettsiae* and the eukaryotic cell mitochondria may have evolved from a common ancestor because many DNA sequences are shared (Weisberg et al., 1985; Emelyanov, 2003(a), 2003(b)).
Another theory suggests that *rickettsiae* may have evolved from free-living bacteria (Roux *et al*., 1997). The “original” *rickettsiae* are thought to have parasitized invertebrate host cells and when vertebrates appeared on Earth both the invertebrates and *rickettsiae* subsequently adapted to become parasites on the vertebrate newcomers. This highly successful adaptation allowed cycling of the organisms between invertebrates and vertebrates and accounts for the widespread dispersal of *rickettsiae* into many ecological systems today (Graves, 1998).

Rickettsial infection has been one of the great scourges of mankind, occurring in overwhelming epidemics during times of war and famine. Napoleon’s retreat from Moscow was forced by rickettsial disease breaking out among his troops. Lenin is said to have remarked, in reference to rickettsial disease rampant during Russian revolution that “either socialism will defeat the louse or the louse will defeat the socialism”.

Rickettsiosis are transmitted to humans by arthropod vectors namely lice, fleas, ticks and mites. *Rickettsiae* are associated with arthropods for a least a part of their life cycle and are passed to other arthropods by transovarial transmission or horizontal transmission involving vertebrates. *Rickettsiae* are divided into three groups namely, the typhus group (TG) which includes *Rickettsia prowazekii* and *R. typhi*, the causative agents of louse-borne epidemic and flea-borne murine typhus; the spotted fever group (SFG) which includes > 20 species that may cause tick-, flea-, and mite-borne rickettsioses; and the scrub typhus group (STG) which includes *R. tsutsugamushi*, the causative agent is chiggers. STG was transferred into the new separate genus *Orientia*, and renamed as *Orientia tsutsugamushi*, because it differs from *rickettsiae* in genetic composition, cell wall structure, and multiplication cycle.

Scrub typhus is an acute febrile illness widely distributed in the eastern hemisphere, especially in south-eastern Asia. It accounts for up to 23% of all febrile episodes in areas of the Asia-Pacific region where scrub typhus is endemic and has a mortality rate of up to 35% if it is left untreated. The numerous synonyms for scrub typhus include: tsutsugamushi (disease mite) disease; Kedani (hairy mite) fever; akamushi (red mite) fever; flood fever; Japanese river fever; tropical typhus; rural typhus; mite-borne typhus and chigger-borne disease (Blake *et al*., 19459(a)).
The name “scrub typhus”, which was coined by Fletcher in 1927 to stress the association of the disease with wasteland, was easy to pronounce and carried some meaning in English (Savoor, 1951). The name gained widespread usage during World War II when military forces operating in the Asiatic/Pacific region encountered outbreaks of the disease which they associated with exposure to a “scrub” environment. The name “typhus” is derived from the Greek word typhos which means stupor. Savoor and Audy in their introduction to the Chapter on Typhus in the Jubilee volume, The Institute for Medical Research 1900-1950, stated, “this is a doubly appropriate name, for not only is stupor a striking and characteristic feature of the intoxication of both typhus and the typhoid or enteric fevers, but the Greek word typhos also means smoke or haze – and until fairly recent times the complex of diseases related to typhus was but darkly seen through the smoky clouds of our ignorance” (Savoor et al., 1951). An estimated one million cases occur annually and as many as one billion people living in endemic areas may have been infected at some time.

The causative agent of scrub typhus Orientia tsutsugamushi is transmitted to humans by the bite of the larval stage of trombiculid mites, acarians belonging to the genus Leptotrombidium. Proliferation of the bacterium occurs at the site, forming a characteristic skin lesion known as an eschar. After incubation for 10-12 days, the affected persons experience headaches, fever, anorexia, and general lymphadenopathy. Additional symptoms include enlargement of the spleen, nervous disturbance, delirium, and prostration. Mortality ranges from 6 to 10%. Death occurs as a direct result of the disease or from secondary effects such as pneumonia, encephalitis, and circulatory failure. Failure to suppress the rickettsial infection is manifested as a symptomatic infection or rickettsemia. Disease severity and manifestations vary widely from asymptomatic to fatal and show marked geographical differences. The general course of the disease and the prognosis vary considerably depending on the character of the endemic strain. Primarily, the antigenic diversity of the three prototype strains, Gilliam, Karp and Kato were illustrated from New Guinea, Japan and Burma respectively (Shishido, 1962). Later on, more than thirty antigenically distinct serotypes are present in the endemic areas of the ‘tsutsugamushi triangle’ (Ohashi et al., 1996).
In the wild, trombiculid mite larva can be found at any location that is suitable for rodent populations and has ground moisture sufficient to nourish the mite vectors (Kawamura et al., 1995). Presently, people living in urban areas are increasingly at risk of acquiring an *O. tsutsugamushi* infection that is antigenically similar to those that cause scrub typhus in rural populations (Richards et al., 1997). The reason for change in the incidence of new cases is not clear. Scrub typhus is usually successfully treated with doxycycline, tetracycline or chloramphenicol (Twartz et al., 1982). Currently, no effective and acceptable vaccine for human use against the rickettsial disease is available.

Scrub typhus often presents as fever and cannot be distinguished clinically from co-endemic diseases such as malaria, typhoid, leptospirosis and dengue. The presence of eschar supports the diagnosis but this is often missed. Diagnosis, therefore, depends on clinical suspicion, promoting the clinician to request to an appropriate laboratory investigation, and failure to diagnose the disease will likely result in treatment with ineffective β-lactam drugs.

### 1.1. Burden of Rickettsiosis

Rickettsial infections are major cause of febrile illness throughout Asia-Pacific region (Rapmund, 1984; WHO, 1993). In India Rickettsiosis have been reported since 1930s with reports of scrub typhus from Kumaon region (Blewitt, 1938). During second world in Assam among the soliders (Mackie, 1946; Sayen et al., 1946). Scrub and murine typhus was reported from Jabalpur area in Madhya Pradesh (Kalra, 1949) where as murine typhus was also reported from Kashmir (Kalra and Rao, 1951).

Rickettsial infections have also been documented from many states in India namely, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Assam, West Bengal, Maharashtra, Kerala, Tamil Nadu, and Delhi. (Mathai et al., 2001; Sundhindra et al., 2004; Mahajan et al, 2006; Batra, 2007; Mittal et al., 2012).
1.2. Global burden of Scrub typhus

Scrub typhus is one of the common forms of Rickettsial infection. During the last two decades outbreak of scrub typhus have been reported from various regions. It was reported from Camp Fuji in 2000 and 2001; (Jiang et al., 2003), from Palau in 2001–2003; (Durand et al., 2004) from Maldives in 2002; (Lewis et al., 2003) and from southern India, Sri Lanka, and the Maldives in 2003 (Mathai et al., 2003; Kularatne, 2003).

Scrub typhus was proven or suspected to be endemic in countries like India, Indonesia, Pakistan, Maldives and Sri Lanka but limited data have been or only recently being gathered (Kelly et al., 2009). Pakistan lies at or near the western fringe of the area of endemcity where Shirai and Wisseman tested 79 Orientia isolates recovered from patients, rodents, and mites in multiple locations throughout Pakistan (Shirai and Wisseman, 1975). Serological evidence of scrub typhus has been reported from Kunduz and Badakhshan provinces of Afghanistan, but no clinical cases have been identified (Arsen’eva, 1982). The scrub typhus is well described in West Pakistan in ecozones similar to those of Afghanistan (Traub et al., 1967; Wisseman et al., 1967; Traub and Wisseman, 1968).

1.3. Scrub typhus in India

Scrub typhus is prevalent in parts of India but the reported number of cases and specific laboratory tests for diagnosis are not available. In India, scrub typhus was recognized as a typhus-like fever in 1917 (Tattersall, 1945). During World War II scrub typhus was the major cause of fever among the troops deployed along the Assam-India-Burma (Myanmar) border with a mortality rate of 5 % (Tattersall, 1945; Sayen et al., 1946). In 1970s a seroepidemological survey was conducted in different ecologically different areas in the states of Rajasthan and Jammu Kashmir by using Complement Fixation test (Menon et al., 1978). There was an appearance of the disease in 1990 in a unit of an army deployed at the Pakistan border of India that resulted in fatality of several people (Singh, 2004).
Mathai et al., reported an outbreak of scrub typhus in southern India during the cooler months from October 2001 to February 2002 (Mathai et al., 2003). An outbreak of scrub typhus was reported during autumn of 2003 in Himachal Pradesh. (Sharma et al., 2005). Epidemics of scrub typhus have been reported from (1) Tamil Nadu during 2002-2003; (Varghese et al., 2006) (2) Himachal Pradesh during 2003 and 2004; (Mahajan et al., 2006) (3) Tamil Nadu in 2007; (Abrahamsen et al., 2013); (4) Pondicherry during 2006-2008; (Vivekananda et al., 2010) (5) Goa during 2009 -2010; (Narvencar et al., 2012) (5) Assam and Nagaland during 2010-2011 (Khan et al., 2012); (6) Rajasthan during 2012 (Bithu et al., 2014); (7) Jaipur during 2012 (Sinha et al., 2014); (8) Uttrakhand during 2012 to 2013 (Singh et al., 2014). A study was conducted in 2011 on adult patients from the rural Telangana region (Subbalaxmi et al., 2012), but there are no documented studies related to the prevalence of scrub typhus from Andhra Pradesh.

1.4. Need for the study
Although the Scrub typhus disease is endemic in our country, it is grossly underdiagnosed. The reasons for this are non-specific clinical presentation of the disease, lack of access to the specific diagnostic facilities in most areas, and low index of suspicion by the clinicians.

In this background this cross-sectional prospective study was undertaken. The study was aimed at determining the prevalence of sero-positivity for scrub typhus in Southern districts of Andhra Pradesh, comparing the diagnostic utility of Weil-Felix test, ELISA and Nested PCR tests and determining the serotype of *O. tsutsugamushi* that is clinically relevant here.
1.5. Study Objective

1.5.1. General Objective

The objective of this cross-sectional prospective study was to identify the most common cause of acute undifferentiated febrile illness in health settings with primary objective to develop evidence to guide management of acute Scrub typhus fever. Secondary objective were to determine available diagnostic tools in field conditions and compare the three diagnostic modalities namely Weil-Felix test, ELISA and Nested PCR for detection in blood. Finally, it was intended to show that scrub typhus is endemic in this region and to enhance further research and to build capacity for laboratory testing and research within the country.

1.5.2. Specific Objectives

i. Seroprevalence of Scrub typhus among febrile ill patients by Weil-Felix test – preliminary study.

ii. Serological and molecular diagnosis of scrub typhus by three diagnostic modalities – Weil-Felix test, ELISA and Nested PCR for detection in blood.

iii. Comparison of clinically suspected scrub typhus patients with the three diagnostic modalities - Weil-Felix test, ELISA and Nested PCR for detection in blood.

iv. Molecular detection of clinically relevant *O. tsutsugamushi* serotypes from Southern Andhra Pradesh.