

**UNIVERSITI TEKNOLOGI MARA**

**MOLECULAR  
CHARACTERISATION OF  
RICKETTSIAL DISEASES IN  
ADULTS PRESENTING TO TELUK  
INTAN HOSPITAL**

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Thesis submitted in fulfillment  
of the requirements for the degree of  
**Master in Science**  
**(Medical Microbiology & Parasitology)**

**Faculty of Medicine**

**February 2023**

## ABSTRACT

Rickettsioses are emerging infectious diseases caused by Rickettsia species, a genus of obligate intracellular gram-negative bacteria found in various arthropods vectors such as ticks, lice, fleas, and chiggers. The three main diseases caused by Rickettsia species are scrub typhus (e.g. *Orientia tsutsugamushi*, *Orientia chuto*), murine typhus (e.g. *Rickettsia typhi*), and spotted fever (e.g. *Rickettsia conorii*, *Rickettsia helvetica*). Rickettsioses is one of the most common agriculture and animal farming-associated zoonotic diseases, and it is potentially fatal if left unrecognised and untreated. Infections in humans are manifested as acute undifferentiated febrile illnesses, headaches and rashes following the bites from various arthropods vectors. The disease has often been overlooked due to its similar symptoms to other vector-borne diseases such as dengue fever, leptospirosis, and malaria. Misdiagnosis and improper antibiotic therapy may result in severe complications and even death if not addressed promptly. The exact burden of rickettsial diseases in Malaysia is limited due to the disease being underdiagnosed. Serology methods such as indirect immunoperoxidase and indirect hemagglutination tests are commonly used in the diagnosis of the infection. However, these techniques are laborious and require reference serum and antigens. Moreover, it can only detect a limited number of rickettsia species and strains. Hence, the molecular technique is the method of choice for studying rickettsia's genetic diversity as it can detect the causative agents at the genomic level. Therefore, this study aimed to identify and characterise the rickettsia species among adults presented to Teluk Intan Hospital by polymerase chain reaction and DNA sequencing. Briefly synthetic DNA of the 56-kDa type-specific antigen (TSA), the 17-kDa, and the OmpB, were utilised as templates and positive controls in the validation of PCR for scrub typhus, murine typhus, and spotted fever, respectively, using published primers. The PCR product was visualised on a 2% agarose gel. A total of 141 blood samples that fulfilled the inclusion and exclusion criteria were collected from adults presented to Teluk Intan Hospital, Perak, Malaysia, from November 2019 to November 2020. Only one sample was detected positive for scrub typhus and sequence-verified to be *Orientia tsutsugamushi* strain UT302, which was initially isolated from Thailand. The experimental findings have demonstrated that the optimised PCR methods are rapid, straightforward, precise, and reproducible. The number of positive cases was lower than anticipated; this was most likely due to the hospital being gazetted as a COVID-19 hospital during the global COVID-19 pandemic in 2020. This has greatly affected patient recruitment as the number of patients visiting the hospital has been reduced. In conclusion, from this study, we have detected and identified an *Orientia tsutsugamushi* strain TA 763 from a hospitalised patient at Teluk Intan Hospital using the molecular technique. This finding is important as the identified strain has not been previously discovered in Malaysia, hence it is not part of the antigen control used in the serology test performed in Malaysian government hospitals. This could be one of the reasons that cause the underdiagnosis of rickettsia in the country. The findings of this study will be useful for future rickettsia epidemiological studies and improvement of the diagnostic methods.

## ACKNOWLEDGEMENT

Firstly, I wish to thank God for allowing me to embark on my MSc and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor, Associate Professor Dr Wang Seok Mui, and co-supervisors Dr Siti Farah Alwani Mohd Nawi and Dr Muhamad Yazli Yuhana.

My appreciation goes to all my postgraduate friends especially Aishah Syakirah, Norhidayah Rosman, Noor Hanisa Harun, Mohd Radzi, Zulaika Roslan and all staff at the Institute for Medical Molecular Biotechnology (IMMB), Faculty of Medicine, for their constant support, assistance, and encouragement. I am grateful to the staff at Teluk Intan Hospital, Dr Akmal Binti Mokhtar, Dr Izian Munirah Binti Ahmad Khusairi, Dr Norlisa Khalid, and Ms Haryati Binti Ishak, who provided the facilities and assistance during the sample recruitment. I would also like to thank the Ministry of Health for providing me with full paid leave and the Ministry of Higher Education for the FRGS-RACER grants for my study. My special thanks to Dr Fairuz Amran from Institute for Medical Research (IMR) and also Dr Siti Norbahiyah Awad for assisting me whenever I need them.

Finally, this thesis is dedicated to my husband, and both of my kids for their patience and sacrifice in completing this master's studies. This victory would not be complete without the blessing of my beloved mother and family. This achievement is for all of you. Alhamdulillah!

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Research Background

Rickettsioses are emerging infectious diseases caused by rickettsiae (rickettsia species), a group of obligatory small intracellular gram-negative bacteria consisting of scrub typhus, murine typhus and spotted fever Rickettsia typhus (Faccini-Martinez *et al.*, 2014).

Infections in humans are manifested as acute undifferentiated febrile illnesses. Unspecific symptoms such as fever, headache and rash occur following the bites from various arthropods vectors such as chigger mites in scrub typhus, rat fleas in murine typhus and ticks in spotted fever rickettsial typhus. Howard Taylor Ricketts (1871-1910), a United States pathologist and microbiologist, was the first person who discovered and isolated Rickettsia bacteria that caused the Rocky Mountain Spotted Fever in the Bitterroot Valley (Gross & Schafer, 2011) in 1906. Unfortunately, he died at the age of 39 in 1910 after being affected by epidemic typhus. Both a taxonomy family (Rickettsiaceae) and an order (Rickettsiales) were named after the scientist (Gross & Schafer, 2011).

The species of rickettsia were classified into three major groups based on serology. They were scrub typhus, murine typhus, and spotted rickettsia typhus fever. Humans typically become rickettsia's accidental host. A person who is afflicted with a febrile illness, such as rickettsiosis, dengue, or leptospirosis, would most likely develop a fever and a rash. These are the classic signs of a febrile sickness. Due to similar symptoms of illnesses, the clinical diagnosis of rickettsia becomes complicated (Blacksell *et al.*, 2015).

*Orientia tsutsugamushi* is an etiological agent of scrub typhus, which caused tsutsugamushi fever and was first reported in 1920. Scrub typhus is distributed in most countries in the Asia Pacific region. More than 30 antigenically distinct serotypes of *O. tsutsugamushi* has been reported from Japan, Sumatra, Taiwan, Philippines, India, Malaysia, Thailand, Australia, and many other countries. It is also known as chigger borne rickettsioses as the chiggers, the larval stage of mites are the vectors of the disease