

**Sero-prevalence of spotted fever, typhus and scrub typhus group
rickettsioses among patients with acute febrile illness in Kenya.**

Jacqueline Wairimu Thiga

**A thesis submitted in partial fulfillment for the degree of Master of
Science in Molecular Medicine in the Jomo Kenyatta University of
Agriculture and Technology**

2015

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature..... Date:

Jacqueline Wairimu Thiga

This thesis has been submitted for examination with our approval as University supervisors.

Signature: Date:

Dr. John N. Waitumbi
USAMRU-Kenya

Signature: Date:

Prof. Zipporah Ng'ang'a
JKUAT, Kenya

DEDICATION

I dedicate this thesis to my dear husband, Martin Kibe, son Liam Kibe, our parents, Mr. and Mrs. Thiga together with Mr. and Mrs. Ng'ang'a. And not forgetting our siblings Eli, Carol, Patrick, Eunice, Kimani and Leah. Thank you all for your support.

ACKNOWLEDGEMENT

I would like extend my sincere gratitude to Dr. John Waitumbi, Lab Director Walter Reed Project/USAMRU Kisumu for funding this project and for giving me the opportunity to work in his laboratory. His mentorship and consistent guidance throughout the whole project were invaluable. I have learnt a lot during the time I have been at the Walter Reed Project under him.

My appreciation goes to my University Supervisor, Prof. Zipporah Ng'ang'a for her guidance, mentorship, encouragement and contribution to this work. I would also like to thank Dr. Allen Richards and Ju Jiang, Naval Medical Research Center, Silver Spring MD for donating the whole cell antigens and to the entire Walter Reed Laboratory Personnel: George Awinda, Maureen Maraka, Clifford Obuya, Jackson Cheruiyot, and Joram Siangla who trained me in various laboratory techniques. My special appreciation goes to Beth Mutai and Nancy Nyakoe who supervised and assisted me in running the laboratory assays and not forgetting my fellow MSc students: Martin Wahome, Clement Masakhwe, Mishael Oswe and Josphat Nyataya.

To my good friends: Joan Mativo and Asenath for their support and valuable relationship. Special thanks go to my parents Mr. and Mrs. Thiga, husband Martin Kibe, son Liam Ng'ang'a, sisters Carol, Eunice and Leah, niece Ashley, brothers Eli and Patrick for their unflagging love and support. This thesis would have been impossible to complete without their encouragement.

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
ACKNOWLEDGEMENT.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
ABBREVIATIONS AND ACRONYMS	xi
ABSTRACT.....	xii
CHAPTER ONE: INTRODUCTION	1
1.1 Background Information.....	1
1.2 Statement of the problem.....	2
1.3 Study Justification.....	3
1.4 Null Hypothesis.....	3
1.5 Objectives.....	3
1.5.1. General Objectives.....	3
1.5.2 Specific Objectives.....	3
CHAPTER TWO: LITERATURE REVIEW	4
2.1 Rickettsiae.....	4
2.2 Taxonomy of Rickettsiae.....	4
2.2.1 Spotted Fever Group Rickettsiae(SFG)	5
2.2.2 Typhus Group Rickettsiae (TG).....	6
2.3 Transmission of Rickettsiae.....	10
2.4 Virulence of Rickettsiae.....	13
2.4.1 Adherence to Host Cell.....	13
2.4.2 Invasion and release of Rickettsiae from Host Cells	14
2.5 Clinical Presentation of Rickettsiae.....	14
2.6 Diagnosis of Rickettsiae.....	15

2.6.1. Serology	15
2.6.2 Culture of Rickettsiae.....	18
2.6.3 PCR-Based Detection of Rickettsiae	18
2.7 Treatment of Rickettsiae	19
2.8 Prevention Of Rickettsiae	20
CHAPTER THREE: MATERIALS AND METHODS	21
3.1 Study design	21
3.2 Study Population	21
3.3 Inclusion Criteria.....	23
3.4 Exclusion Criteria.....	23
3.5 Collection of blood.....	23
3.6 ELISA for the detection of rickettsiae	23
3.7 Data Management and Analysis.....	24
3.8 Ethical Clearance	24
CHAPTER FOUR: RESULTS	25
4.1 Study Participants	25
4.2 Sero-prevalence of Spotted Fever	25
4.2.1 Sero- prevalence of Scrub typhus	27
4.2.2 Mixed Rickettsiae infections	29
4.3 Risk factor indicators for Spotted Fever rickettsioses.....	29
4.3.1 Risk factor indicators for Typhus Group rickettsioses.....	31
4.3.2 Risk factor indicators for Scrub Typhus Group rickettsiose.....	31
4.4 Clinical characteristics associated with rickettsioses.....	33
CHAPTER FIVE: DISCUSSION.....	36
5.1 Sero-prevalence of Spotted fever	37
5.2. Sero-prevalence of Scrub Typhus rickettsia	37
5.3 Risk factors for Rickettsioses.....	37
5.4 Clinical characteristics associated with rickettsioses.....	38
5.5 Limitations of the study	38

5.6 Conclusions	38
5.7 Recommendations	39
REFERENCES	40
APPENDICES	51

LIST OF TABLES

Table 4. 1: Demographic characteristics of study participants	25
Table 4. 2: Risk factors for SFG among patients with acute febrile illness	30
Table 4. 3: Risk factors for TG among patients with acute febrile illness	31
Table 4. 4: Risk factors for ST among patients with acute febrile illness	32
Table 4.5: Clinical characteristics of patients who were sero-negative and seropositive for SFG rickettsioses	33
Table 4. 6: Clinical characteristics based on antibody titers for SFG	34
Table 4.7: Clinical characteristics of patients both sero-negative and seropositive for ST rickettsioses.....	34
Table 4. 8: Clinical characteristics based on antibody titers for ST.....	35

LIST OF FIGURES

Figure 2.1: Taxonomic classification of Rickettsiae	4
Figure 2.2: Map showing the distribution of Spotted fever rickettsioses	5
Figure 2.3: Transmission cycle of Rickettsiae	10
Figure 2.4: Life cycle of scrub typhus (Sharma 2010).....	12
Figure 3.1: Map of Kenya showing the general locations of surveillance hospitals..	21
Figure 4.1: Sero-prevalence of spotted fever group rickettsiae in participants recruited from the different surveillance sites.....	26
Figure 4. 2: Distribution of antibody titers to spotted fever in patients recruited from the different surveillance sites.	27
Figure 4.3: Sero-prevalence of Scrub typhus in patients recruited from the different surveillance hospitals	28
Figure 4.4: Distribution of antibody titers to scrub typhus in patients recruited from the different surveillance sites.....	29

LIST OF APPENDICES

Appendix 1: SSC Ethical approval letter. 51

ABBREVIATIONS AND ACRONYMS

AFI	Acute Febrile Illness
ALH	Alupe District Hospital
ELISA	Enzyme Linked Immunosorbent Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KSI	Kisii District Hospital
KSM	Kisumu District Hospital
MDH	Malindi District Hospital
MGT	Marigat District Hospital
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
RMSF	Rocky Mountain spotted fever
SFG	Spotted Fever Group Rickettsia
ST	Scrub Typhus
TG	Typhus Group
USAMRU	US Army Medical Research Unit.

ABSTRACT

Rickettsiosis is a pathogenic bacterial infection which is rarely identified as a cause of illness in patients presenting with common symptoms such as fever and headache. In any case, rickettsiae are difficult to diagnose especially in health facilities with limited diagnostic capability. Reported cases of rickettsioses among travelers to the Kenyan games parks have contributed to increased awareness of this disease. Unfortunately, very little is known about this disease among Kenyans. To address this knowledge gap, this study aimed at determining the sero-prevalence of the spotted fever group (SFG), typhus group (TG) and scrub typhus (ST) rickettsioses in patients presenting with acute febrile illness (AFI) at clinical sites across different regions in Kenya. The study enrolled 2225 participants ≥ 1 year who presented with fever (≥ 38 °C) without a readily diagnosable cause of infection. The study began in March 2008 to 2012 and collected blood samples for serum preparation from patients attending health facilities in different parts of Kenya: Kisii highlands (Kisii District Hospital), Kisumu (Obama Children's Hospital and Kisumu District Hospital), Busia (Alupe District Hospital), Marigat (Marigat District Hospital), Garissa (Iftin Sub-district hospital, Garissa Police Line dispensary and at the Kenyan coast (Malindi District Hospital). A total of 2225 archived sera were all were tested for antibodies to SFG (100%), 1611 (72.4%) to TG and 1401 (63.0%) to ST rickettsioses by ELISA. 212/2225 (9.5%) were sero-positive for SFG (95% C.I 8.3-10.7), 4/1611 (0.2%) for TG (95% CI, 0.0 –0.5) and 67/1401 (4.8%) for ST (95% CI, 3.7–5.9). A higher prevalence rate was seen in Garissa (57/226, 25.2%) than in Alupe (27/176, 15.3%), Marigat (37/320, 11.6%), Malindi (9/102, 8.8%), Kisii (39/656, 5.9%), or Kisumu (43/745, 5.8%). Of the total sera, (36.8%) had a titer of 1:1600 and 1:6400 (38.2%) with the highest number reported from Garissa. Antibodies to TG were only seen in Malindi (3/4, 75%) and Kisumu (1/4, 25%). Sero-prevalence of ST was highest in Marigat 28/238 (11.8%) followed by Alupe 4/68 (5.9%), Garissa 6/134 (4.5%), Kisumu 19/464 (3.5%), and Kisii 10/458 (2.2%). No cases of ST were noted in Malindi. 62.7% of individuals had a titer of 1:400, with 45.2% coming from Marigat. Significant associations were found between males and females with higher sero-prevalence among females (43/694, 6.2%) compared to males (24/707, 3.4%)

($P=0.017$) and between the different age groups for both SFG and ST ($P < 0.0001$), and between sero-positivity and having animal contact with dogs ($P=0.0296$) and camels ($P < 0.0001$) for SFG and goats for ST ($P=0.0003$) and between having symptoms (headache, joint ache and muscle ache) and ST and SFG ($P < 0.0001$) sero-positivity. In conclusion, SFG and ST rickettsioses are common among patients with acute febrile illness in Kenya. This is the first report of scrub typhus in Kenya. The findings of this study suggest that rickettsial infections should be considered in the differential diagnosis of febrile cases in Kenya and that diagnostic capacity with health facilities should be established.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Acute febrile illness (AFI) is a common presentation by patients attending health care facilities in Kenya. AFI is defined as fever of $> 38^{\circ}\text{C}$ lasting for a period of at least 24 hours, and can have other accompanying symptoms such as myalgia, malaise, headache and loss of appetite (Murdoch *et al.*, 2004b). Although AFI can have multiple causes, such as bacteria, viruses, parasites and fungi, most are empirically misdiagnosed as malaria (Dunster *et al.*, 1997). Other infrequently reported diseases, including Rickettsioses can cause AFI, but are poorly documented and or remain unknown. The majority of patients presenting with AFI are nearly always diagnosed on clinical grounds and treated for malaria, leading to inappropriate treatment and delayed diagnosis.

Rickettsiosis, a bacterial infection, has recently re-emerged as an infectious disease of concern (Richards *et al.*, 2010). *Rickettsiae* are small, gram-negative, obligate intracellular bacteria of α subdivision of Proteobacteria. *Rickettsiae* are transmitted to humans through infected arthropod vectors such as fleas, mites, ticks and lice during blood meals. They are able to invade various eukaryotic host cells such as reticulo-endothelial cells and vascular endothelial cells in vertebrates, and epithelial cells in their invertebrate arthropod vectors. The genus *Rickettsia* is divided into two main bio-groups Spotted Fever Group (SFG) and Typhus Group (TG). The Scrub Typhus Group (STG) belongs to the genus, *Orientia* (La Scola & Raoult, 1997).

The SFG rickettsiae most commonly identified in Africa are *Rickettsia conorii*, *Rickettsia africae* *Rickettsia felis* and *Rickettsia aeschlimannii*. *R. conorii*, the etiologic agent of Kenyan tick typhus, or more commonly known as Mediterranean spotted fever, is transmitted predominately through the bite of infected *Rhipicephalus* ticks. It has been reported in Central and South Africa (Raoult & Roux, 1997). *R. africae*, the causative agent of African tick bite fever, is transmitted by *Amblyomma* ticks and has also been reported in parts of Africa, including Kenya (Kelly *et al.*, 1996; Macaluso *et al.*, 2003; Mutai *et al.*, 2013).

R. aeschlimannii is the causative agent of tick-borne rickettsiosis and has been reported in South Africa (Pretorius & Birtles, 2002), Morocco (Beati *et al.*, 1997), Algeria (Mokrani *et al.*, 2008) and Kenya (Mutai *et al.*, 2013). *R. felis* is the causative agent of flea-borne spotted fever (Perez-Osorio *et al.*, 2008), has been reported in Democratic Republic of Congo, Tunisia, Algeria, Gabon, Egypt and most recently in Kenya (Richards *et al.*, 2010). There are two main TG Rickettsia: *Rickettsia typhi* and *Rickettsia prowazekii* with the latter causing epidemic or louse-borne typhus. *R. prowazekii* infects human vascular endothelial cells producing widespread vasculitis.(Svraka *et al.*, 2006). Murine typhus is caused *R. typhi* and transmitted from rats, cats and opossums to humans via a flea bite (Pether *et al.*, 1994). Scrub typhus is caused by *Orientia tsutsugamushi* and is transmitted through the bite of larval mites (chiggers) of the *Trombiculidae* family (Watt & Parola, 2003).

Rickettsioses are largely under reported and under-appreciated as major cause of illness in Sub-Saharan Africa (Rutherford *et al.*, 2004). This can be attributed to the lack of obvious clinical signs and over-reporting of most fever illness as malaria (Rutherford *et al.*, 2004). There has been an increase in the incidences of rickettsioses, reported from international travelers to Sub-Saharan Africa (Jensenius *et al.*, 2003). For example, *R. africae* was identified in 119 travelers who had recently returned from South Africa, Swaziland, Lesotho, Zimbabwe, Botswana, Gambia, Tanzania, Kenya, Gabon, and Côte d' Ivoire (Raoult *et al.*, 2001). In Kenya, incidences of rickettsioses have been reported from travelers after visiting game parks and game reserves (Yoshikawa *et al.*, 2005). Very little is known about the prevalence of this disease among resident Kenyans.

1.2 Statement of the problem

The marked increase in the incidence of rickettsioses reported in Kenya(Yoshikawa *et al.*, 2005; Richards *et al.*, 2010) has raised the profile of rickettsioses and is today ranked as one of the emerging infectious agent. A number of reports have identified widespread distribution of *Rickettsia* in ticks and domestic animals in Kenya (Mutai *et al.*, 2013). However, little is known about the prevalence and distribution of *Rickettsia* in humans in Kenyans.

1.3 Study Justification

Inadequate knowledge and clinical tools to routinely test and diagnose rickettsiae infections is a major cause of concern as majority of AFI patients who may have Rickettsioses are never identified and are probably misdiagnosed as having malaria. Reported cases of rickettsioses among travelers to the Kenyan game parks (Yoshikawa *et al.*, 2005) are a pointer to the higher prevalence of the disease, however very little is known about this disease among local Kenyans. The data obtained from this study will provide much needed information of community exposure level as well as the sero-prevalence of the disease in different geographical areas of Kenya.

1.4 Hypotheses

- Null Hypothesis
 - Rickettsioses are not common infections among patients with AFI.
- Alternate Hypothesis:
 - Rickettsiosis are common infections among patients with AFI

1.5 Objectives

1.5.1 Broad Objective

To determine the sero-prevalence of the spotted fever, typhus group and scrub typhus group rickettsioses among patients with acute febrile illness in Kenya.

1.5.2. Specific Objectives

1. To determine the sero-prevalence of the scrub typhus, typhus and spotted fever group rickettsiae in the different surveillance hospitals in Kenya.
2. To determine the risk factors associated with rickettsiosis among patients with acute febrile illness.
3. To determine the association between clinical signs and sero-positivity among patients with acute febrile illness.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rickettsiae

Rickettsiae have a typical gram negative cell wall but lack flagella. They are exclusively intracellular, requiring host cells to replicate, but are not enclosed by a vacuole (Teyssiere *et al.*, 1992; Heinzen *et al.*, 1993). SFG rickettsiae can be observed in the nuclei of host cells while TG has been observed exclusively in the cytoplasm (Teyssiere *et al.*, 1992; Heinzen *et al.*, 1993). They divide by binary fission and metabolize host derived glutamate via aerobic respiration and the citric acid cycle. Rickettsial genome sizes are small, 1 to 1.6 Megabit, and consist of a single circular chromosome (Eremeeva *et al.*, 1993).

2.2 Taxonomy of Rickettsiae

The bacterial family Rickettsiaceae of the order Rickettsiales is divided into the genus *Rickettsia*, *Orientia* and *Wolbachia*. As shown in Figure 2.1, the rickettsiae that are human pathogens are subdivided into two major groups, the spotted fever group (SFG), typhus group (TG) and another unidentified group. The scrub typhus is found in the genus *Orientia*.

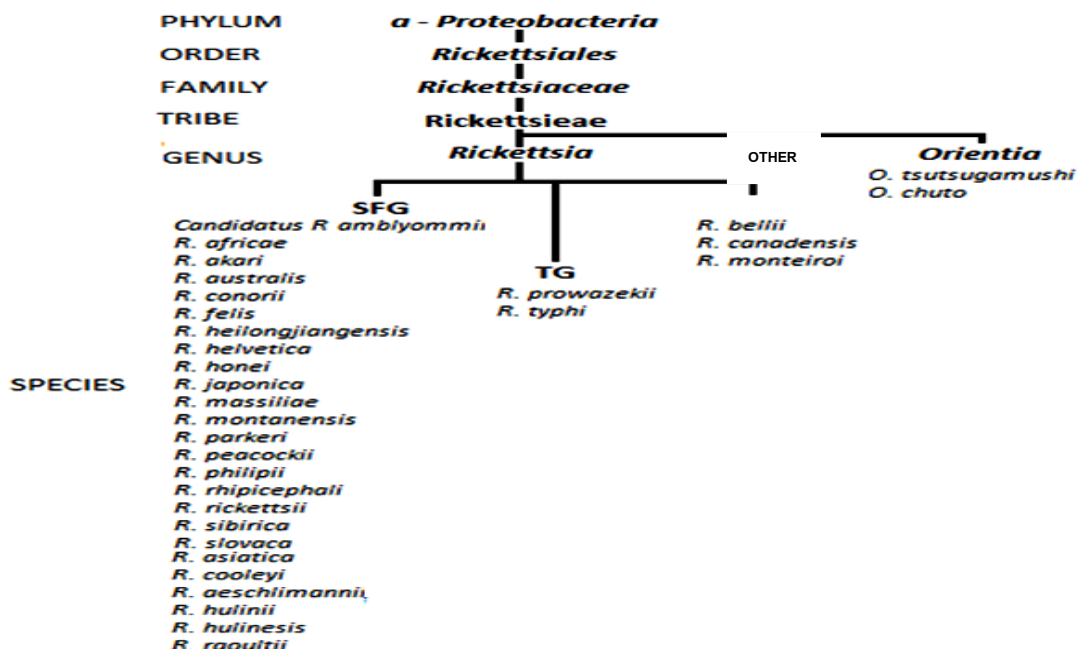


Figure 2.1: Taxonomic classification of Rickettsiae (www.textbookbacteriology.net)

2.2.1 Spotted Fever Group Rickettsiae (SFG)

SFG rickettsiae contains a number of human pathogens, most of which are transmitted by ticks. Unlike the typhus and scrub rickettsiae that have limited geographical distribution, SFG rickettsiae are cosmopolitan

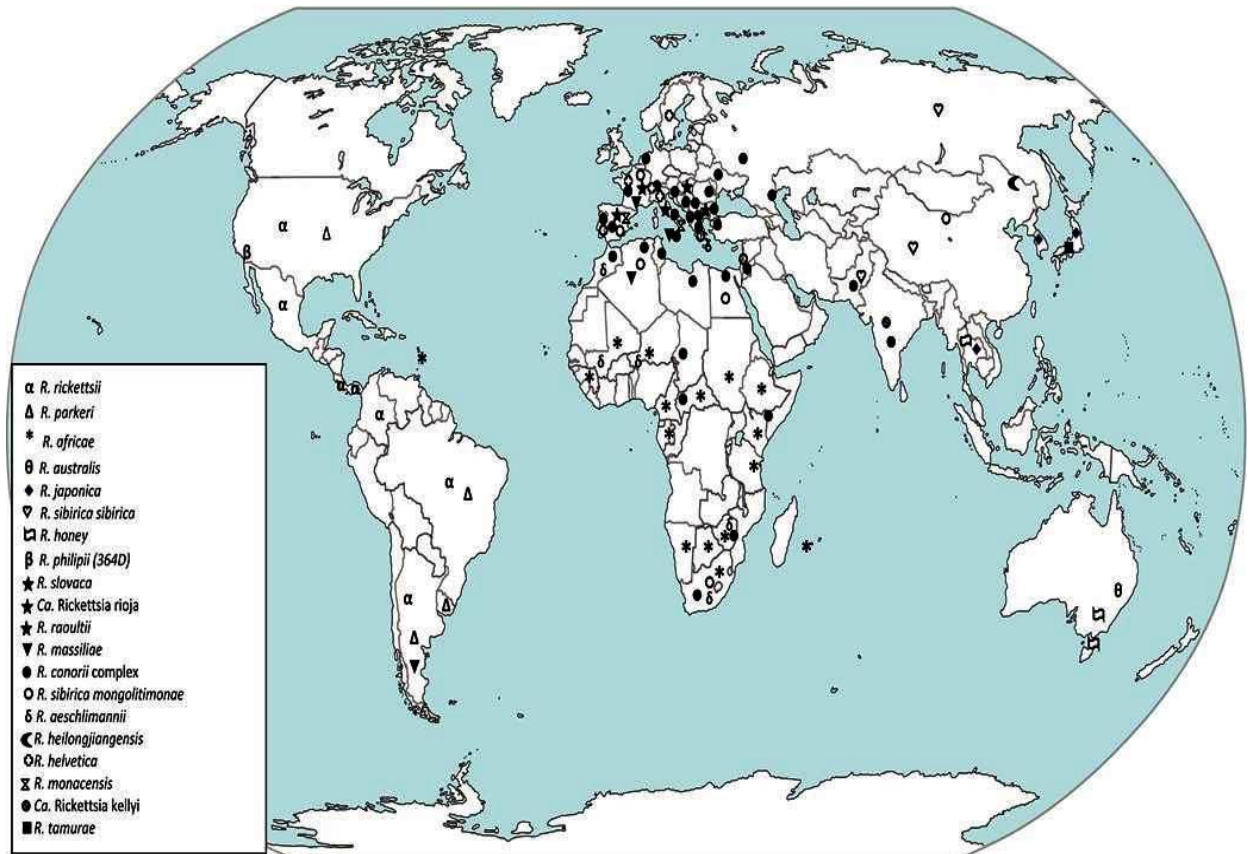


Figure 2.2: Map showing the distribution of Spotted fever rickettsioses (Faccini-martinez *et al.*, 2008)

The species of clinical importance in this group include *R. rickettsii*, the agent of Rocky Mountain spotted fever, so-called because of the area of its first discovery, but is today mainly confined to the eastern Atlantic states of USA (Sanchez *et al.*, 1992). *R. rickettsii* is transmitted to humans through three arthropod vectors, American dog tick (*Dermacentor variabilis*), the Rocky Mountain Wood Tick (*Dermacentor andersoni*), and the Brown dog tick (*Rhipicephalus sanguineus*).

The bacterium infects human vascular endothelial cells, producing spotted inflammatory responses, hence the name.

R. conorii, a common cause of human rickettsiosis is known by a variety of local names, including Boutonneuse fever, Mediterranean spotted fever, Kenya tick typhus and others. Other subspecies of *R. conorii* cause Israeli spotted fever (*R. conorii subsp. israelensis*), Astrakhan spotted fever (*R. conorii subsp. caspia*) and Indian tick typhus (*R. conorii subsp. indica*) (Parola *et al.*, 2005). The main vector for *R. conorii* is the brown dog tick *Rhipicephalus sanguineus* (Senneville *et al.*, 1991). As the local names of diseases caused by *R. conorii* indicate, the pathogen exists in most of the regions bordering the Mediterranean Sea, Black Sea, Israel, Kenya, parts of North, Central, and South Africa and India.

R. africae, the causative agent of African tick-bite fever, is transmitted by *Amblyomma hebraeum* and *A. variegatum* ticks and is found in the African savannah (Kelly *et al.*, 1994). *R. felis* is the causative agent of flea-borne spotted fever, an emerging zoonotic disease with wide cosmopolitan distribution in >20 countries and 5 continents (Perez-Osorio *et al.*, 2008). The cat flea, (*Ctenocephalides felis*), is considered the primary vector for *R. felis* infections because this arthropod can maintain stable infected progeny through transovarial and transtadial transmission. However, 10 flea species (*C. felis*, *C. canis*, *Xenopsylla cheopis*, *X. brasiliensis*, *Pulex irritans*, *Archeopsylla erinacei*, *Tunga penetrans*, *Ceratophyllus gallinae*, *Spilopsyllus cuniculi*, and *Echidnophaga gallinacean*) are vectors (Jiang *et al.*, 2006).

R. slovaca is responsible for the Tick-Borne Lymphadenitis: or TIBOLA, also called Dermacentor-borne necrosis- erythema lymphadenopathy (DEBONEL) in Spain. TIBOLA is a zoonosis transmitted to humans by Dermacentor tick-bite, notably *D. marginatus* and *D. reticulatus*, which are also the reservoirs. *R. slovaca* is transmitted trans-stadially (stage to stage) and transovarially in ticks, thus maintaining the agent in nature. *R. slovaca* has been detected in *D. marginatus* in France, Portugal, Switzerland, Yugoslavia, Slovakia, Ukraine, Armenia and Hungary (Raoult *et al.*, 1997).

R. akari causes rickettsial pox and is transmitted by the colorless mite *Liponyssoides sanguineus* (formerly *Allodermanyssus sanguineus*), which is found on mice most commonly the house mouse *Mus musculus* and other rodents (Huebner *et al.*, 1946).

It has been reported in northeastern and Midwest United States, South Africa, Costa Rica, France, Italy, Turkey, Croatia, the Ukraine, Russia, and Korea.

R. japonica first isolated in 1984 in Japan is the tick-borne agent of Japanese spotted fever and is transmitted by *Dermacentor taiwanensis* and *Haemaphysalis flava* ticks (Dong *et al.*, 2012). *R. sibirica* contains two subspecies (Fournier *et al.*, 2006); *R. sibirica* subsp. *sibirica*, the agent of North Asian tick typhus transmitted primarily by various species of *Dermacentor* and *Haemaphysalis* ticks, and *R. sibirica* subsp. *mongolotimonae*, the agent of “lymphangitis-associated rickettsiosis.” The former subspecies was first isolated in Russia but it has subsequently been found in northern China (Yu *et al.*, 1993). In contrast, *R. sibirica* subsp. *mongolotimonae* was first isolated in Inner Mongolia and then found in southern Europe and Africa (Fournier *et al.*, 2005). *R. heilongjiangensis*, first isolated from *Dermacentor silvarum* ticks in the Heilongjiang province of China (Zhang *et al.*, 2000), was subsequently demonstrated to cause human spotted fever in China and the Russian Far East (Mediannikov *et al.*, 2004).

R. hulinii was first isolated from *Haemaphysalis concinna* ticks in the Heilongjiang province of China, and its pathogenic role in humans is suspected but has not been demonstrated (Zhang *et al.*, 2000). *R. helvetica*, has been isolated from *Ixodes ricinus* ticks in many European and Asian countries, although the organism has recently been found in *Dermacentor reticulatus* ticks (Nilsson *et al.*, 2010). *R. australis* is a bacterium that causes typhus. The probable vectors are the tick species, *Ixodes holocyclus* and *Ixodes tasmani*. Small marsupials are suspected reservoirs of this bacterium which occurs in Australia (Nilsson *et al.*, 2010).

Rickettsia honei has been detected on three continents. Originally isolated in Thailand in 1962 (Robertson & Wisseman, 1973), Flinders Island (Australia) in 1993 (Stenos *et al.*, 1998) and in Texas (USA) in 1998 (Graves & Stenos, 2003). On each continent it has been associated with a different species of tick. The original isolate was from a pool of larval *Ixodes* and *Rhipicephalus* ticks. Later it was detected in *Ixodes granulatus* from *Rattus*. Its pathogenicity for humans has not yet been confirmed, but it is possibly responsible for the Spotted Fever Group human rickettsiosis in Thailand (Robertson & Wisseman, 1973). The strain from Texas (USA) was isolated from *Amblyomma cajennense* ticks taken from cattle but its

pathogenicity for humans has not yet been confirmed. The strain from Flinders Island (Australia) described as *R. honei*, has been isolated from patients with "Flinders Island Spotted Fever" and from *Aponomma hydrosauri* ticks (Graves & Stenos, 2003). *R. parkeri* rickettsiosis (sometimes known as Tidewater spotted fever or American boutonneuse fever) is a recently-recognized illness in the Americas (Romer *et al.*, 2011).

2.2.2 Typhus Group Rickettsiae (TG)

R. prowazekii, is the agent of classical epidemic typhus. It is transmitted by the human body louse, *Pediculus humanus*, but not by the head lice, from active human cases or from healthy carriers or subclinical cases (Zinsser & Castaneda, 1934). It infects the human vascular endothelial cells, producing widespread vasculitis. The infectious agent is excreted in the feces of the body louse and becomes inoculated by scratching at the site of the louse bite. (Andersson *et al.*, 1998). The other species in this group is *R. typhi*, the causal agent of murine typhus. It is transmitted by the rat flea *Xenopsylla cheopis*, and typically infects humans in markets, grain stores, breweries, and garbage depots. It causes a mild illness, but can become more aggressive in crowded human settlements (Pether *et al.*, 1994). Murine typhus is found worldwide and is endemic to areas of Texas and southern California in the United States.

2.2.3 Scrub Typhus Group Rickettsiae (STG)

Orientia (Rickettsia) tsutsugamushi is the only member in this group and causes scrub typhus. Originally called *Rickettsia tsutsugamushi*, this organism was given its own genus designation because it is phylogenetically distinct from the other rickettsiae, though closely related (Tamura *et al.*, 1995). Scrub typhus was first described by Hashimoto from Japan in 1899 (Mahajan, 2005). It is endemic to a part of the world known as the "tsutsugamushi triangle" which extends from northern Japan and far-eastern Russia in the north, to northern Australia in the south, and to Pakistan and Afghanistan in the west. Globally, over one billion people are at risk for scrub typhus and an estimated one million cases occur annually (Mahajan, 2005).

The infection is called scrub typhus because exposure generally occurs in areas with secondary (scrub) vegetation. Recent findings indicate that the disease can be prevalent in sandy beaches, mountain deserts, and equatorial rain forests (Lai *et al.*, 2009).

Scrub typhus is transmitted through the bite of larval mites or "chiggers" belonging to the family *Trombiculidae*, genus and subgenus *Leptotrombidium*. Only the larval stage takes a blood meal. Small rodents' particularly wild rats of subgenus *Rattus* are the natural hosts for scrub typhus. The seasonal occurrence of scrub typhus varies with climate in different countries. The disease occurs more frequently during the rainy season. However, outbreaks have been reported during the cooler season in southern India. Illness develops after an incubation period of 6 to 21 days and usually begins with an escher at the site of a chigger bite (Lai *et al.*, 2009).

2.3 Transmission of Rickettsiae

The *Rickettsiae* are transmitted to humans principally by infected arthropods, such as fleas, lice, mites and ticks during feeding or by scratching infectious feces into the skin. Inhaling dust contaminated with dried infected lice or flea feces may also cause infections (Oster *et al.*, 1977).

Transmission through blood transfusion is rare but has been reported during the asymptomatic incubation period of some diseases (Wells *et al.*, 1978). Figure 2.3 summarizes the different modes of transmission of SFG and TG Rickettsiae. A) Shows the transmission cycle of *R. rickettsii*. *Dermacentor* spp. ticks acquire *R. rickettsii* upon feeding, as larvae or nymphs, on rodents; they then transmit during subsequent feeding as nymphs or adults. In addition, *R. rickettsii* infection can be maintained transovarially in *Dermacentor* ticks, resulting in some larvae hatching from the egg mass already harboring an infection. B) Transmission cycle of *R. akari*. The house mouse is the reservoir host and the Mite, *Allodermanyssus sanguineus*, transmits *R. akari* to mice causing disease. C) Transmission cycle of *R. typhi* which is transmitted by the flea *Xenopsylla cheopis* to rodents or man. Humans are infected by contamination of flea bites, broken skin or conjunctiva by flea feces. Domestic animals may transport the flea vector to humans. Inhalation of contaminated dust may be a route of infection (www.phsource.com).

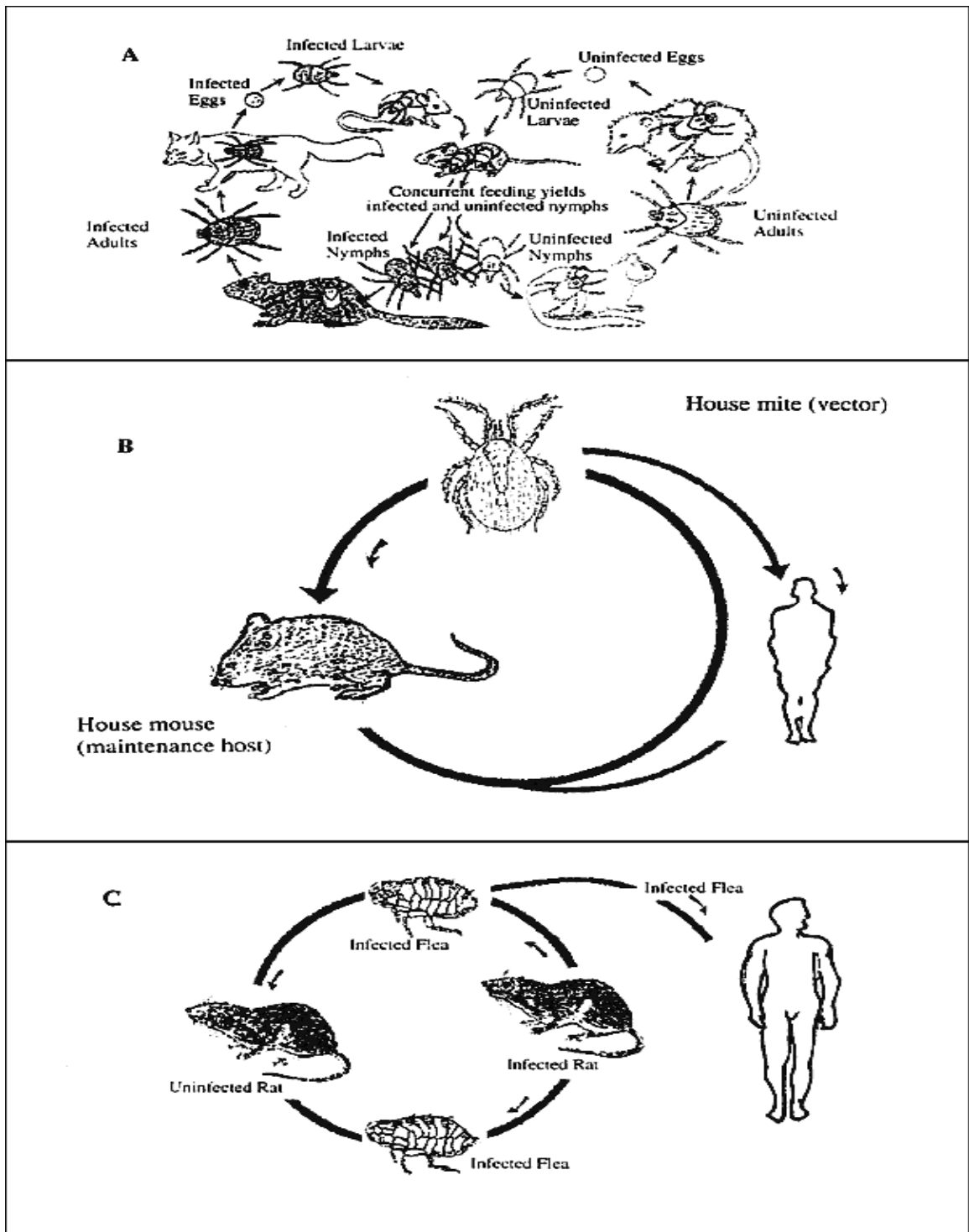


Figure 2.3: Transmission cycle of Rickettsiae. A. Life cycle of *Rickettsia rickettsii* in its tick and mammalian hosts; B. Life cycle of *Rickettsia akari*; C. Life cycle of *Rickettsia typhi*: Source (www.phsource.us)

Figure 2.4 summarizes the transmission cycle of ST. The infection is transmitted to humans and rodents by larval stages of some species of infective trombiculid mites ("chiggers", *Leptotrombidium deliense* and others) which feed on lymph and tissue fluids rather than blood. The mite is very small (0.2 - 0.4 mm) and can only be seen under a microscope or magnifying glass (Suputtamongkol *et al.*, 2009). Once the mites are infected by feeding on the body fluid of small mammals, including the rodents, they maintain the infection throughout their life stages and, as adults, pass the infection eggs (transovarial transmission). Similarly, the infection passes from the egg to the larva and adult (transtadial transmission). In this way, chigger mite populations can autonomously maintain their infectivity over long periods of time (Lai *et al.*, 2009; Suputtamongkol *et al.*, 2009). Rather than biting or piercing the skin, mite larvae prefer to insert their mouthparts down hair follicles or pores. A large number of the *Orientia tsutsugamushi* are present in the salivary glands of the larvae and are injected into its host when it feeds. The bite of the mite leaves a characteristic black eschar that is useful to the doctor for making the diagnosis (Suputtamongkol *et al.*, 2009). Human infection takes place when man accidentally picks up an infective larval mite while walking, sitting or lying on infested ground. The larva is the only stage (chigger) that can transmit the disease to humans and other vertebrates (Kang & Chang, 1999).

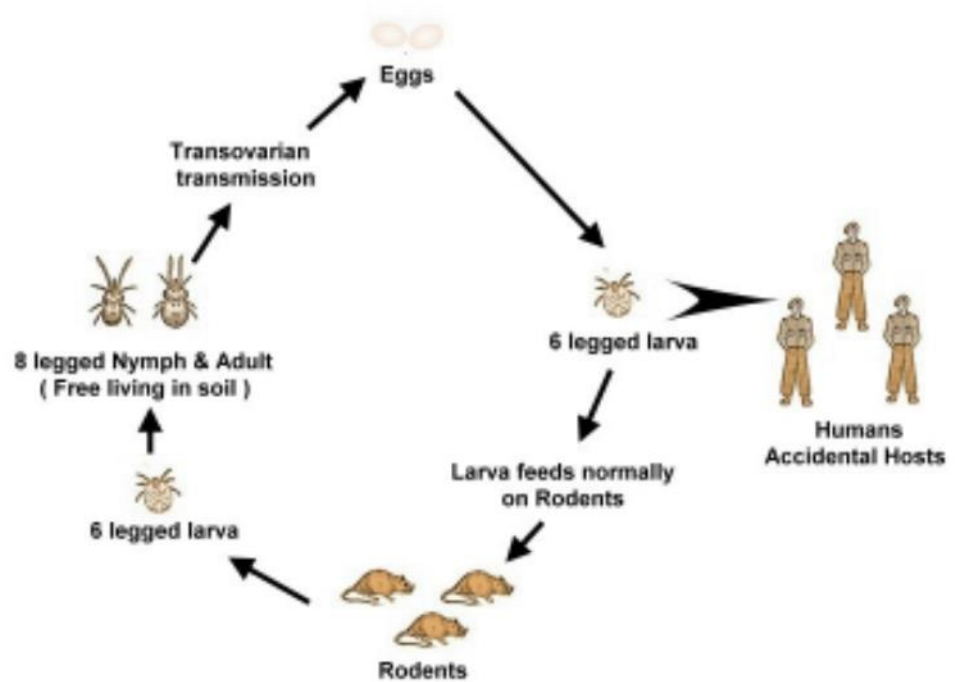


Figure 2.4: Life cycle of scrub typhus (Sharma 2010). The infection in human and rodents is by bites of larval stages of some species of infective trombiculid mites.

2.4 Virulence of Rickettsiae

2.4.1 Adherence to the Host Cell

Rickettsiae are inoculated into the dermis of the skin by a tick bite or through damaged skin from the feces of lice or fleas. From the site of inoculation, the bacteria spread through the bloodstream and infect the endothelium. Adherence to the host cell is the first step of rickettsial pathogenesis (Todar, 2009) The adhesins are presumed to be outer membrane proteins. The outer membrane protein A (OmpA) has been implicated in adherence of *R. rickettsii* because antibodies to OmpA have been shown to block adherence (Feng *et al.*, 2004). The host cell receptor for the *Rickettsia* has yet to be identified. Although the main *in vivo* target cells are the endothelial cells, *Rickettsia* can infect virtually every cell line *in vitro*. Thus, either the receptor for *Rickettsia* is ubiquitous among cells, or rickettsiae can bind to different receptors (Yun Chan *et al.*, 2010)

2.4.2 Invasion and release of Rickettsiae from Host Cells

Upon attaching to the host cell membrane, rickettsiae are phagocytosed by the host cell. Once phagocytosed, rickettsiae quickly escape from the phagosome membrane and enter the cytoplasm. The mechanism of escape from the phagosome membrane is not well understood, but it is thought to be mediated by a rickettsial enzyme, phospholipase A2. (Todar, 2009). TG rickettsiae are released from host cells by lysis. After infection with *R. prowazekii* or *R. typhi*, the rickettsiae continue to multiply until the cell is packed with organisms and then bursts. Phospholipase A2 may be involved in cell lysis. TG rickettsia-infected host cells have a normal ultra-structural appearance. SFG rickettsiae seldom accumulate in large numbers and do not lyse the host cells (Yun Chan *et al.*, 2010). They escape from the cell by stimulating polymerization of host cell derived actin tails, which propel them through the cytoplasm and into tips of membranous extrusions, from which they emerge. Infected cells exhibit signs of membrane damage associated with an influx of water, but the means by which rickettsiae damage host cell membranes is uncertain. There is evidence to suggest a role of free radicals of oxygen, phospholipase, and a protease. The protein responsible for the actin based movement in spotted fever group rickettsiae is yet to be identified, but it is apparently different from the proteins responsible for actin polymerization by *Listeria monocytogenes* and *Shigella flexneri* (Todar, 2009)

2.5 Clinical Presentation of Rickettsiae

Although the clinical presentations vary with the causative agent, some common symptoms that typically develop within 1–2 weeks of infection include fever, headache, malaise, and sometimes nausea and vomiting (Raoult & Roux, 1997). Most symptoms associated with acute rickettsial infections are nonspecific and require further tests to make an accurate diagnosis. Most tick-transmitted rickettsioses are accompanied by a maculopapular, vesicular, or petechial rash or an eschar at the site of the tick bite. While many rickettsial diseases cause mild or moderate illness, epidemic typhus and Rocky Mountain spotted fever can be severe and may be fatal in 20–60% of untreated cases (Parola *et al.*, 2005).

2.6 Diagnosis of Rickettsiae

2.6.1 Serology

Serological assays are the simplest diagnostic tests to perform, since serum can readily be sent to a reference laboratory. For a test to be useful in the diagnosis of an acute rickettsial infection, the most important criteria are sensitivity and the length of delay between the onset and appearance of detectable antibody titers. On the other hand, when the test is to be used for sero epidemiologic studies, it should be highly specific to prevent false-positive results due to cross-reacting antibodies. Other criteria which need to be considered include the amount of antigens needed, their costs and the minimal material required (La Scola & Raoult, 1997). A number of tests exist for serological diagnosis of rickettsiae infections.

The Weil-Felix test is based on the detection of antibodies to alkali based carbohydrate antigen which are shared by some rickettsiae and certain strains of *Proteus* species bacteria: *P. vulgaris* OX2, *P. vulgaris* OX19, and *P. mirabilis* OXK. (Raoult & Dasch, 1995). OX-2 reacts strongly with sera from persons infected with SFG rickettsiae with the exception of those of Rocky Mountain Spotted Fever (RMSF) while the whole cells of *P. vulgaris* OX-19 react with sera from persons infected with TG rickettsiae as well as with RMSF. The OX-K strain of *Proteus mirabilis* was demonstrated to agglutinate with sera from scrub typhus patients and was further used in the diagnosis of *O. tsutsugamushi*- related infections (La Scola & Raoult, 1997). By the Weil-Felix test, agglutinating antibodies are detectable after 5 to 10 days following the onset of symptoms, with the antibodies detected being mainly of the immunoglobulin M (IgM) type (Amano *et al.*, 1992a; Amano *et al.*, 1992b). However, the test was said to lack sensitivity and specificity (Kaplan & Schonberger, 1986).

The complement fixation test (CF) is utilized in the detection of antibodies specific to *Rickettsiae*. The rickettsial antigens used are species-specific for the SFG and TG. However, cross-reacting antibodies among groups are observed (Shepard *et al.*, 1976). The CF test is strain specific for *O. tsutsugamushi*. Antibody titer obtained by the CF test correlate better with IgG titers than with IgM titers in

immunofluorescence assay (IFA). A limitation of this test is the variation in test results which is attributed to the method of antigen production and the amount of antigen used in the assay (Hersey *et al.*, 1957).

Microagglutination is a test based on the detection of interactions between antibodies and whole rickettsial cells. It has not been widely used because of the need for large amounts of purified rickettsial antigens and these antigens are not available commercially (Fiset *et al.*, 1969).

Indirect Hemagglutination Test detects antibodies to an antigenic erythrocyte-sensitizing substance (ESS) used to coat human or sheep erythrocytes that are either fresh or fixed in glutaraldehyde. The ESS is rickettsial group specific with cross-reactivity among RMSF and rickettsialpox. This test detects both IgG and IgM antibodies, but agglutination is more efficient with IgM antibodies. (Anacker *et al.*, 1979)

In the latex agglutination test, ESS is used to coat latex beads. The reactivity is not exactly the same as that of the indirect hemagglutination test, because the ESS on latex beads probably contains more antigenic fractions than the ESS adsorbed onto erythrocytes (Hechemy *et al.*, 1983). This test is rapid (15 min) and does not require elaborate instrumentation. Latex agglutination test is reactive with both IgG and IgM antibodies. The agglutination efficiency in this test is increased when the anti-rickettsial IgM/IgG ratio is greater than 1. This test can detect antibodies 1 week after onset of illness (Hechemy *et al.*, 1980).

Enzyme-linked Immunosorbent Assay (ELISA) was majorly introduced for detection of antibodies against *R. typhi* and *R. prowazekii* in 1977 (Halle *et al.*, 1977). This technique is highly sensitive and reproducible, allowing differentiation of IgG and IgM antibodies. This technique was later adapted to the diagnosis of RMSF (Clements *et al.*, 1983) and ST (Dasch *et al.*, 1979). A "paper ELISA" was proposed for the detection of anti-*O. tsutsugamushi* antibodies. The initial steps of the assay are similar to those used for the IFA, but an anti-human IgG peroxidase conjugate and substrate-saturated filter paper, on which the reaction is visualized, are used (Crum *et al.*, 1980). ELISA has been performed for the detection of SFG and TG rickettsia using *R. typhi* and *R. conorii* whole cell antigens (Graf *et al.*, 2008).A

commercial enzyme-linked immunosorbent assay (ELISA) using a recombinant 56-kDa protein (r56) to detect specific immunoglobulin M (IgM) produced in ST infections has been developed and is available (Land *et al.*, 2000).

Indirect Immunofluorescence Antibody (IFA) is the gold standard and is used as a reference technique in most laboratories. For ST, the sensitivity of IFA is low if high specificity is required. Detection of rickettsiae by using immunofluorescence allows the confirmation of infection in patients prior to their seroconversion. (La Scola & Raoult, 1997) Samples can be tested fresh or after formalin fixation or on paraffin embedment histological tissues. Biopsy specimens of the skin with a rash around the lesion, preferably petechial lesions are the most common samples used. In animals or patients with fatal cases of infection, bacteria are detectable at autopsy in the tissues of numerous organs such as liver, spleen, kidney, heart, meningeal membranes, or skin (Dumler *et al.*, 1991). IFA assays are time consuming, requiring specialized equipment and trained personnel.

Indirect Immunoperoxidase (IIP) has been developed as an alternative to IFA for the diagnosis of scrub typhus and was later evaluated for use in the diagnosis of infections due to *Rickettsia conorii* (Raoult *et al.*, 1985; Sarov *et al.*, 1992) and *R. typhi* (Kelly *et al.*, 1988). The procedure is the same as IFA, but fluorescein is replaced by peroxidase. The advantage of the immunoperoxidase assay is that the results can be read with an ordinary light microscope. In addition, it provides a permanent slide record (Kelly *et al.*, 1988). IIP assays are time consuming, requiring specialized equipment and trained personnel.

The micro-IFA has the advantage of simultaneously detecting antibodies to a number of rickettsial antigens (up to nine antigens) with the same drop of serum in a single well containing multiple rickettsial antigen dots. IFA allows the detection of IgG and IgM antibodies or both. The identification by IFA of specific IgM antibodies to the various species of rickettsiae provides strong evidence of recent active infection, although the diagnosis may be obscured by a prozone phenomenon where the antibodies exist in excess of antigens (Philip *et al.*, 1976). Unfortunately, this technique is affected by rheumatoid factors, thus requiring the use of a rheumatoid factor absorbent before IgM determination.

Western immunoblot assay (Raoult & Dasch, 1989) with sodium dodecyl sulfate-gel electrophoresed and electroblotted antigens is a powerful serodiagnostic tool for seroepidemiology and confirmation of serologic diagnoses obtained by other tests. The test detects two types of antigens, lipopolysaccharide and two high molecular-weight proteins (rOmpA and rOmpB). It is especially useful in differentiating true-positive from false-positive results created by cross-reacting antibodies. These cross-reacting antibodies, observed both between biogroups (SFG and typhus group) and between species, appear to be directed against lipopolysaccharide (LPS) and to be of the IgM class, although IgG antibodies directed against both LPS and protein antigens have also been observed (Raoult & Dasch, 1989; 1995). Although this technique is accurate, it is also very expensive and time-consuming, since a large number of rickettsiae are required for each absorption.

2.6.2 Culture of Rickettsiae

Cultures are used to isolate *Rickettsia* from clinical samples. Ticks, amphibians and mammalian cell lines such as L-929, Vero, BGM, HeL, XTC, human embryonic lung cells and primary chick embryo cells may be used for growing *Rickettsia* (Simser *et al.*, 2001). *Rickettsia* cannot grow in the presence of most antibiotics, thus strict attention must be paid to aseptic techniques to avoid contamination. *Rickettsiae* have a relatively slow growth rate with doubling time of 10 hours (Raoult & Roux, 1997). The disadvantages of culture is that it is limited to laboratories with bio hazard facilities, vials need to be inoculated the day of sampling, negative for patients with prior antibiotic therapy.

2.6.3 PCR-Based Detection of Rickettsiae

Rickettsiae may be detected by PCR amplification from an array of samples that include blood, skin biopsy and arthropod tissues including ticks (Gage *et al.*, 1992; Gage *et al.*, 1994), fleas, and lice (Higgins & Azad, 1995). Detection strategies are based on recognition of sequences within the 16S rRNA gene, and those encoding a 17-kDa protein, citrate synthase, the rOmpB and rOmpA, for SFG rickettsiae (Raoult *et al.*, 1996; Roux *et al.*, 1996).

The 16S rRNA gene is useful for identification of rickettsia species, and provides accurate identification (Phan et al., 2011). The gene encoding the 17-kDa protein has not yet been studied enough to become an identification tool, although nucleotide sequence comparison revealed homologies of 99.8, 88.1, and 88.7% between *R. rickettsii* and *R. conorii*, *R. typhi*, and *R. prowazekii*, respectively (Anderson et al., 1987), indicating its potential as a diagnostic test. The citrate synthase gene (*gltA*) of all rickettsiae with the exception of *O. tsutsugamushi* has now been sequenced. Species-specific sequences can be recognized in a 1,234-bp fragment of this gene, which is bordered by conserved regions which act as suitable hybridization sites for consensus primers. Nevertheless, this gene is not divergent enough to allow one to distinguish among all rickettsial species (Roux et al., 1997).

The *ompA* gene is specific for the SFG rickettsiae and exhibits enough heterogeneity to ensure accurate identification of bacteria from this group by comparison of a 632-bp region at the 5' end of the gene. Indeed, the gene is polymorphic enough in this region to allow the differentiation of some strains of *R. conorii* (Roux et al., 1996). Unsurprisingly, this differentiation is in accordance with the previously described antigenic diversity among strains of this species (Walker et al., 1992). However, this approach does not allow the identification of *Rickettsia bellii*, *R. akari*, *Rickettsia helvetica*, *R. australis*, *Rickettsia canada*, *R. typhi*, *R. prowazekii*, or *O. tsutsugamushi*, either because of an absence of this gene or because the primers used do not hybridize (especially to *R. canada*). In the absence of amplifiable fragments of the *gltA* and *ompA* genes, the molecular identification of *O. tsutsugamushi* has been achieved by a nested PCR which allows the differentiation of strains to the serotype level (Furuya et al., 1993; Murai et al., 1995). The first primer pair allows the amplification of a fragment of the gene that encodes a 56-kDa protein, which is responsible for typing strain antigenic specificity (Ohashi et al., 1990; Stover et al., 1990) and the second primer pair allows the determination of the serotype strain.

2.7 Treatment of Rickettsiae

The standard treatment regimen for *Rickettsiae* is 2.2 mg/kg body weight of doxycycline administered twice a day (orally or intravenously) for children weighing <45.4 kg and 100 mg/daily in adults and pregnant women (Holman *et al.*, 2001). Doxycycline has a high degree of efficacy and low toxicity in treating rickettsial infections, even in children and pregnant women. Depending on the specific pathogen, chloramphenicol, azithromycin, fluoroquinolones, and rifampin may also be considered, but these are not universally effective for all rickettsial agents, nor have they been evaluated in controlled clinical trials (Parola *et al.*, 2005).

Treatment in low resource countries has challenges in that, rickettsioses are rarely considered when evaluating patients with acute, undifferentiated febrile illnesses. This situation can be attributed in part to unavailability of specific laboratory tests, equipment, and expertise, and also the limited economic resources in many countries of the region (Ndip *et al.*, 2004). In most third world countries, laboratory diagnosis is limited to simple methods such as microscopy and rapid diagnostic tests for diseases such as malaria and typhoid. Patients receive inappropriate treatment.

2.8 Prevention of Rickettsiae

The best prevention measure to minimize exposure to infectious arthropods particularly lice, fleas, ticks and mites is the proper use of insect repellents, self-examination after visits to vector-infested areas and wearing protective clothing (Parola *et al.*, 2005).

Rickettsioses commonly present as nonspecific febrile illnesses that are difficult to diagnose clinically. This situation can be attributed in part to unavailability of specific laboratory tests, equipment, and expertise, and also the limited economic resources in many African countries. (Ndip *et al.*, 2004). Although studies identifying etiologies of fever are limited in sub-Saharan Africa, human seroprevalence studies have reported presence of SFG (Ndip *et al.*, 2004) and TG (Kaabia *et al.*, 2006) . In the study reported here, we sought to determine the prevalence of rickettsiae infections among patients with acute febrile illness in Kenya and the risk factors that predispose to rickettsioses.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was a laboratory based study where archived serum samples were used and was an arm of a larger parent surveillance study to determine the etiologies of acute febrile illness in Kenya. The parent study began in March 2008 and blood samples for serum preparation were collected from participants attending the different surveillance hospitals shown in Figure 3.1 that had been recruited under a protocol entitled “Acute Febrile Illness” that was approved by the KEMRI Ethical Review Committee (KEMRI SSC # 1282). Samples had been coded to maintain anonymity and data collected through questionnaires stored in records.

The Names and location of the surveillance hospitals were:

Western Kenya: Kisumu District Hospital, New Nyanza Provincial Hospital-Kisumu, Kisii District Hospital, and Alupe Sub-district Hospital-Busia

Northeastern Kenya: Iftin Sub-district Hospital-Garissa and Garissa Police line clinic.

Coastal Kenya: Malindi District Hospital

Rift Valley: Marigat District Hospital

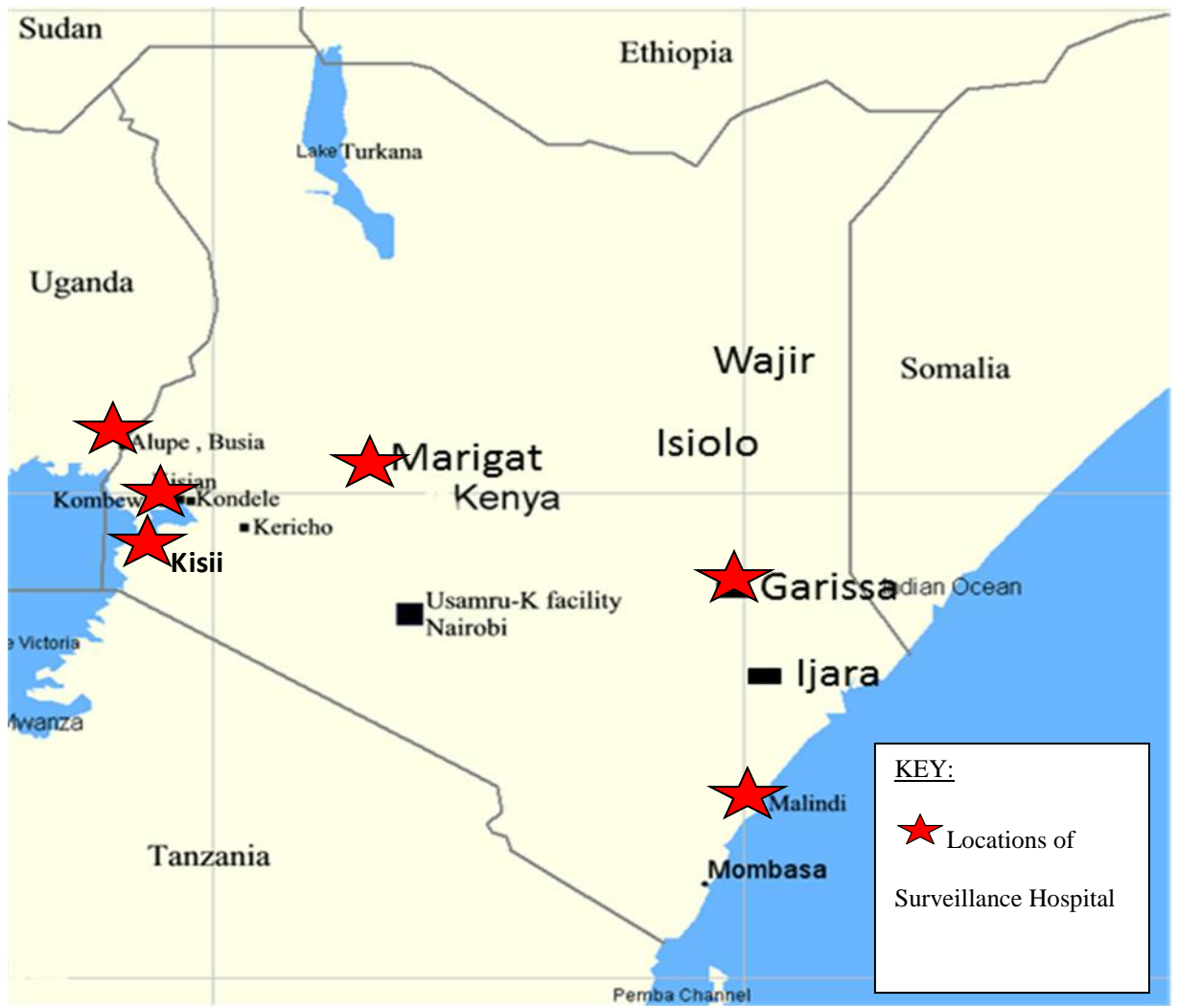


Figure 3.1: Map of Kenya showing the general locations of surveillance hospitals.

Locations selected are malaria endemic zones Obama Children Hospital in Kisumu, Kisumu District Hospital, Kisumu, Alupe District Hospital in Busia, Malindi District Hospital, Kisii District Hospital, Ifin Sub-district Hospital and Garissa Police line clinic in Garissa.

3.2 Study Population

This comprised of patients presenting at the outpatient departments of the surveillance hospitals with fever (temperature $\geq 38^{\circ}\text{C}$).

3.3 Inclusion Criteria

Adult or child one year of age or older, presenting with fever (temperature $\geq 38^{\circ}\text{C}$) that consented to participate in the study were recruited. Parents or guardian consented for the minors.

3.4 Exclusion Criteria

Children under one year of age, volunteers unwilling to complete the questionnaire or to participate and unwilling to consent or assent to having their blood drawn or used for research purposes.

3.5 Collection of blood

Blood samples were collected by field officers. Blood was first drawn by finger prick and used to test for malaria by rapid diagnostic technique (RDT). Patients who tested negative for malaria by RDT and fulfilled inclusion criteria had 5 mL of venous whole blood collected in a vacutainer tube and allowed to clot. The clotted blood was then centrifuged at 3000rpm for 5 minutes and serum collected, aliquoted into multiple aliquots and kept in dry liquid nitrogen shippers and transported to the KEMRI/Walter Reed Project, Kisumu for storage at -80 °C until use.

3.6 ELISA for detection of rickettsiae

ELISA was conducted as described by (Graf *et al.*,2008) with modifications on the type of whole cell antigens used and their final dilutions. For assessment of SFG, *R. cornorii* whole-cell antigen was used. For TG, *R. typhi* whole-cell antigen was used and for *O. tsutsugumishi*, Karp and Gilliam (Jiang *et al.*,2003) whole-cell antigen strains were used. For all the three rickettsiae targets, the assay procedure was the same. One half of a 96 well micro titer plate was pre-coated with 100 µL/well of rickettsial antigen diluted in 1X PBS (NaCl 0.138 M; KCl - 0.0027 M). The SFG antigen was diluted at 1:1500, 1:300 for the TG antigen and 1:1000 for Gilliam mixed with 1:2000 Karp for the scrub typhus antigen (0.1µg/well). The other half of the plate was coated with 100 µL/well of PBS only. The coated plates were then covered with a plastic wrap and incubated for 48 hours at 4 °C to allow maximum adsorption of the antigens to the plate. The pre-coated plates were then washed three times in wash buffer (0.1% Tween 20 in 1X PBS) using an ELISA plate washer (Skan washer 400, Molecular Devices, California USA), then blocked with 200 µL/well of blocking buffer (5% Difco skim milk Becton, Dickson and Company, Sparks Maryland USA in wash buffer) for 1 hr, and rinsed with wash buffer three times. The sera was diluted 1:100 in blocking buffer, and 100 µL/well added to the pre-coated plates and incubated for 1 hr at 22 °C in an incubator to allow

antigen/antibody interaction. The plates were then washed three times with wash buffer, and 100 μ L/well of peroxidase conjugated to goat anti-human IgG (Kirkegaard & Perry Laboratories, Gaithersburg Maryland USA) at a 1:2000 dilution added and then incubated for 1 hr at room temperature. The plates were finally washed three times in wash buffer and a two component mixture of horse radish peroxidase substrate, 2,2'-azino-di-[ethylbenzthiazoline sulfonate] (Kirkegaard and Perry Laboratories), was added., mixed at 1:1 ratio before use, added to the plates at 100 μ L/well, then incubated for 15 minutes at room temperature in the dark. The plates were then read at 405 nm using an ELISA plate reader (Molecular devices Vmax kinetic microplate reader, California USA) and the optical densities (OD) from the wells without antigen used to subtract background absorbance. A serum sample was considered reactive when ≥ 0.5 OD. Serum samples that were reactive at 1:100 dilution (net OD greater than 0.5), were further titrated using fourfold dilutions of the serum (1:100 to 1:6400) and tested again as described above. ELISA-positive titers were determined to be the inverse of the highest dilution in which a net optical density (absorbance with antigen minus absorbance without antigen) of >0.2 was obtained. For each target antigen, QC samples included three negative and one positive control serum samples that were evaluated with each run.

3.7 Data Management and Analysis

The data obtained from records were stored in a Microsoft excel sheet, and computational analysis performed with either Graph pad prism 5 (Graph Pad Software Inc., San Diego, CA) and SPSS version 20. The sero-prevalence was calculated as an overall percentage of sero-reactive patients and by surveillance sites. Fisher's exact test was used to determine significant differences in prevalence's between groups. Associations between subject demographic characteristics and serological results were determined using Chi-square test. Significance levels were set at 0.05 alpha with confidence levels held at 95%.

3.8 Ethical clearance

Scientific and ethical clearance was sought from the Ethical Review Committee (ERC), Scientific Steering Committee (SSC#1282) of KEMRI, and the Walter Reed Army Research Institute of Human Use Research Committee, (WRAIR), USA (Appendix 1).

CHAPTER FOUR

RESULTS

4.1 Study Participants

A total of 2,225 participants were enrolled into the study. Of these, all were tested for SFG (100%), 1611 (72%) for typhus group rickettsia and 1401 (63%) for scrub typhus due to reagent limitations. The demographic characteristics of the study participants as obtained from records are as shown in Table 4.1. Their ages ranged from 1 to 72 years with a mean age of 5 years with majority (77.8%) being children (up to 12 years). They had a male to female ratio of 1:1 across all groups.

Table 4. 1: Demographic characteristics of study participants

Characteristic	SFG (%) (n =2225)	TG (%) (n=1611)	ST (%) (n=1401)
Gender			
Male	1131 (50.8)	784 (48.7)	707 (50.5)
Female	1094 (49.2)	827 (51.3)	694 (49.5)
Age			
<5 yrs.	1107 (49.8)	804 (49.9)	687 (49.0)
5- 12 yrs.	622 (28.0)	469 (29.1)	423 (30.2)
13 - 26 yrs.	290 (13.0)	196 (12.2)	166 (11.8)
>26 yrs.	206 (9.3)	142 (8.8)	125 (8.9)

Spotted fever group (SFG), Scrub Typhus (ST) and Typhus group (TG).

4.2 Sero-prevalence of Spotted Fever

Overall, 212 out of 2225 (9.5%) AFI participants were sero-prevalent for SFG rickettsiae (95% CI 8.3-10.7). Figure 4.1 shows the regional prevalence with their respective 95% confidence intervals. Significantly higher prevalence rate was seen in Garissa, 57/226 (25.2%) (Combined sero-prevalence at Iftin Sub-district Hospital

and Garissa Police Line as this is one region) compared to 27/176 (15.3%) for Alupe, 37/320 (11.6%) for Marigat, 9/102 (8.8%) for Malindi, 39/656 (5.9%) for Kisii and 43/745 (5.8%) Kisumu ($P < 0.05$).

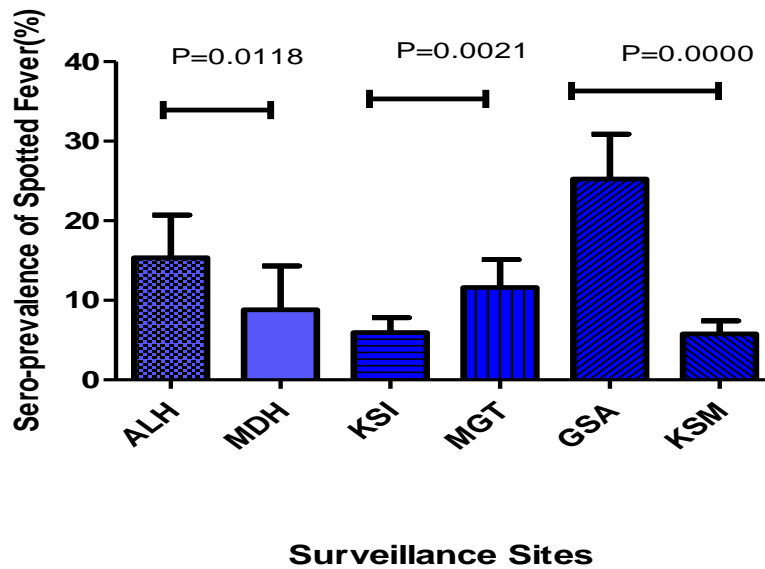


Figure 4.1: Sero-prevalence of spotted fever group rickettsiae in participants recruited from the different surveillance sites. GSA – Garissa District Hospital, MDH – Malindi District Hospital, ALH –Alupe District Hospital , MGT–Marigat District Hospital, KSI – Kisii District Hospital, KSM – Kisumu District Hospital.

As shown in Figure 4.2, majority of the seropositive patients had a titer of 1:1600 (36.8%) and 1:6400 (38.2%) with the highest numbers coming from Garissa (29%, $n=23$), followed by Kisumu (17.9%, $n=16$). Malindi had the lowest percentage of (4.9%, $n=4$) seropositive patients.

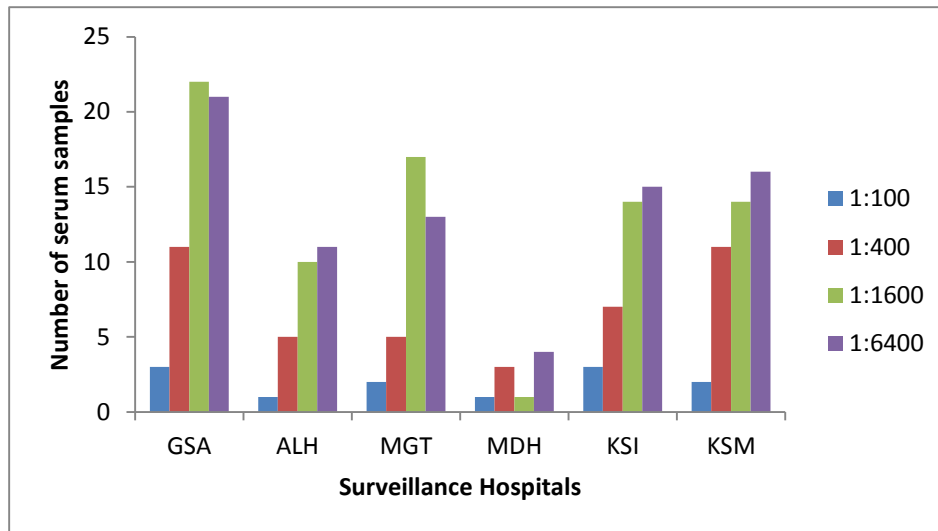


Figure 4. 2: Distribution of antibody titers to spotted fever in patients recruited from the different surveillance sites. GSA – Garissa District Hospital, MDH – Malindi District Hospital, ALH –Alupe District Hospital , MGT–Marigat District Hospital, KSI –Kisii District Hospital, KSM – Kisumu District Hospital. Titers: 1:100, 1:400, 1:1600 and 1:16400.

Sero-prevalence for TG was 0.2% (4/1611) and only occurred in Malindi (3 patients) and Kisumu (one patient).

4.2.1 Sero- prevalence of Scrub typhus

Antibodies against *ST rickettsiae* were detected in 67/1401 (4.8%) patients (95% CI, 3.7%–5.9%). The highest sero-prevalence (28/238) was seen in Marigat District Hospital (11.8%) followed by Alupe Sub District Hospital 4/68 (5.9%), Garrisa 6/134 (4.5%) for patients recruited from Ifitin Sub-district Hospital and Garissa Police Line and 19/464 (3.5%) for Kisumu for patients recruited from New Nyanza Provincial Hospital and Kisumu District Hospital. ST sero-prevalence was lowest 10/458 (2.2%) in Kisii as shown in Figure 4.3, with their respective 95% confidence intervals.

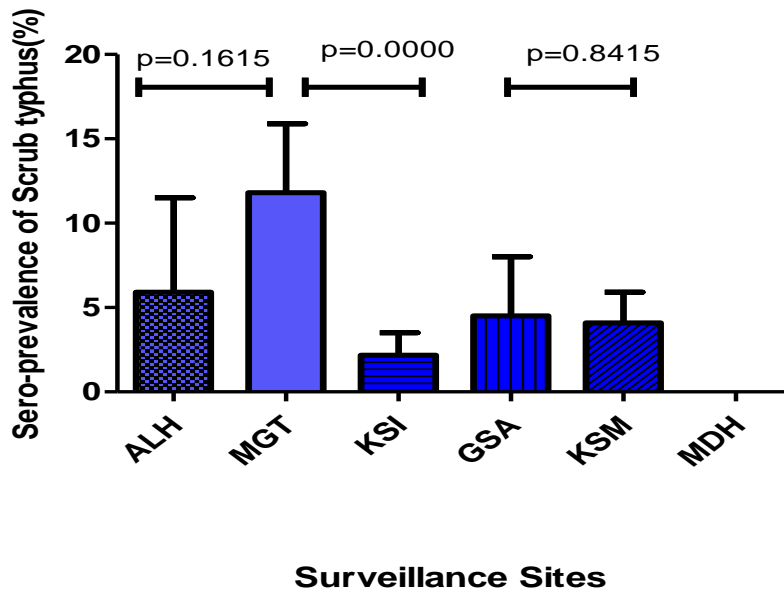


Figure 4.3: Sero-prevalence of Scrub typhus in patients recruited from the different surveillance hospitals. GSA – Garissa District Hospital, MDH – Malindi District Hospital, ALH –Alupe District Hospital , MGT–Marigat District Hospital, KSI –Kisii District Hospital, KSM – Kisumu District Hospital.

Most patients had a titer of 1:400 (62.7%) with the highest coming from Marigat (45.2%) and Kisumu (31.0%) (Figure 4.4.)

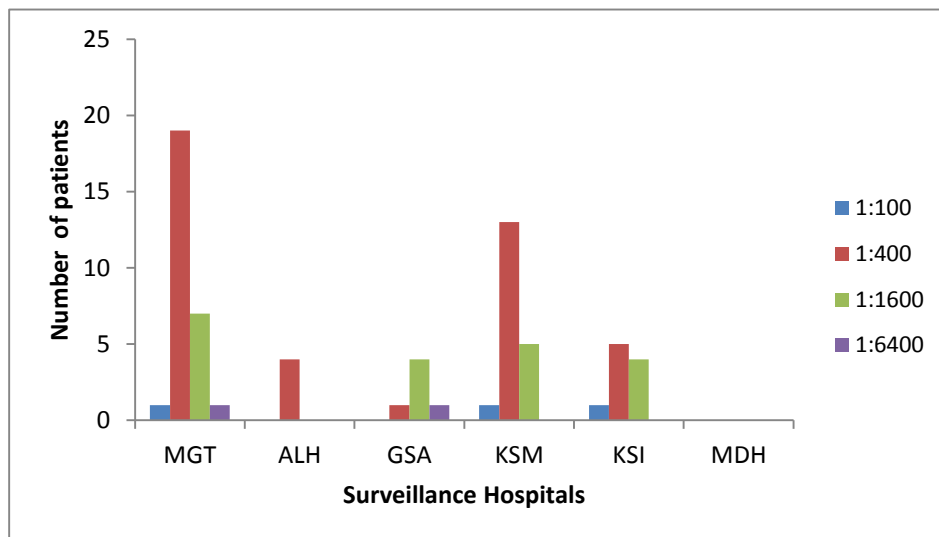


Figure 4.4: Distribution of antibody titers to scrub typhus in patients recruited from the different surveillance sites. GSA – Garissa District Hospital, MDH – Malindi District Hospital, ALH –Alupe District Hospital , MGT–Marigat District Hospital, KSI –Kisii District Hospital, KSM – Kisumu District Hospital. Titers: 1:100, 1:400, 1:1600 and 1:16400.

4.2.2 Mixed Rickettsiae infections

A total of 9/67 (13.4%) patients that were sero-positive to ST were also seropositive for SFG. Of these, 6 (66.6%) were from Marigat, 2 (22.2%) from Kisumu and 1 (11.1%) from Garissa.

4.3 Risk factor indicators for Spotted Fever rickettsioses

Table 4.2 shows the prevalence of SFG antibodies by age, gender, and animal contact. There were no significant differences in antibody prevalence rates between males (10.3%) and females (8.3%) ($P=0.2482$). Sero-prevalence of SFG increased with age: <5years having (3.7%), 5-12 years (10.0%), 13-26 years (21.6%) and >26years (22.3%) and the difference among the different age groups were significant ($P < 0.0001$). Owning camels had significantly higher odds of sero-positivity ($P < 0.0001$, OR 4.5) and dogs had a significantly lower odds of sero-positivity ($P = 0.0296$, OR 0.2). Contact with donkeys had a higher odds of sero-positivity.

Table 4. 2: Risk factors for SFG among patients with acute febrile illness

Characteristic	No. of participants	No. (%) +ve	OR (95% CI)	Fisher's P-value
Gender: Female	1094	96 (8.8)	1.0	0.2482
Male	1131	116 (10.3)	1.2 (0.9-1.6)	
Age <5yrs	1107	41 (3.7)	1.0	
5- 12yrs	622	62 (10.0)	2.9 (1.9 – 4.4)	<0.0001
13 - 26 yrs.	290	63 (21.7)	7.2 (4.7- 11.2)	<0.0001
>26yrs	206	46 (22.3)	7.5 (4.6 – 12.1)	<0.0001
Goats				
No contact	2188	205 (9.4)	1.0	0.0798
Contact	37	7 (18.9)	2.3 (0.8 – 5.3)	
Cows				
No contact	2187	207 (9.5)	1.0	0.4021
Contact	38	5 (13.1)	1.4 (0.4 – 3.8)	
Donkeys				
No contact	2223	211 (9.5)	1.0	0.1815
Contact	2	1 (50.0)	9.5 (0.1 – 748.8)	
Cats				
No contact	2106	203 (9.6%)	1.0	0.5241
Contact	119	9 (7.6%)	0.8 (0.3 – 1.5)	
Sheep				
No contact	2218	212 (9.6%)	1.0	1.0000
Contact	7	0 (0%)	0 (0 – 5.2)	
Dogs				
No contact	2146	210 (9.8%)	1.0	0.0296
Contact	79	2 (2.5%)	0.2 (0.03 – 0.9)	
Camels				
No contact	2173	196 (9.0%)	1.0	<0.0001
Contact	52	16 (30.8%)	4.5 (2.3 – 8.5)	

OR=odds ratio CI= Confidence Intervals

4.3.1 Risk factor indicators for Typhus Group rickettsioses

Unlike the Spotted Fever and scrub typhus, only 4 patients had antibodies against TG rickettsia. Three of these were from Marigat and were all females. The other was a male from Kisumu. None of the patients reported contact with animals (Table 4.3)

Table 4. 3: Risk factors for TG among patients with acute febrile illness

Sample	Hospital	Gender	Age	Animal Contact
1	MGT	Female	3	NC
2	MGT	Female	3	NC
3	MGT	Female	38	NC
4	KSM	Male	2	NC

MGT= Marigat District Hospital, KSM = Kisumu District Hospital, NC = No Contact

4.3.2 Risk factor indicators for Scrub typhus rickettsioses

Table 4.4 shows the demographic characteristics of patients that were sero-positive for ST. Females (6.2%) had significantly higher odds of ST exposure than males (3.4%) ($P=0.0169$, OR1.0). Like SFG, the sero-prevalence of ST increased with age: <5years having 2.5%, 5-12 years 5.9% ($P =0.0005$), 13-26 years 6.0% ($P =0.0262$) and >26years 8.0 % ($P =0.0016$) and this difference were statistically significant. Significant associations were found between ST sero-positivity and having contact with goats ($P =0.0003$). Contact with cows, donkeys, cats, dogs, sheep and camels had no significant association to ST sero-positivity.

Table 4. 4: Risk factors for ST among patients with acute febrile illness

Characteristic	No. of participants	No. (%) +ve	OR (95% CI)	Fisher's P-value
Gender: Female	694	43 (6.2)	1.0	0.0169
Male	707	24 (3.4)	0.5(0.3-0.9)	
Age				
<5yrs	687	17 (2.5)	1.0	
5- 12yrs	423	29 (5.9)	2.9(1.5 – 5.7)	0.0005
13 - 26 yrs.	166	10 (6.0)	2.5(1.0 - 6.0)	0.0262
>26yrs	125	11 (8.8)	3.8(1.6 – 8.8)	0.0016
Goats				
No contact	1372	60 (4.4)	1.0	0.0003
Contact	29	7 (24)	7.0 (2.4 – 17.7)	
Cows				
No contact	1377	65 (4.7)	1.0	0.3200
Contact	24	2 (8.3)	1.8 (0.2 – 7.7)	
Donkeys				
No contact	1399	67 (5.1)	1.0	1.0000
Contact	2	0 (0)	0 (0- 38.7)	
Cats				
No contact	1315	66 (5.0)	1.0	0.1203
Contact	86	1 (1.2)	0.2 (0.05 – 1.3)	
Dogs				
No contact	1387	67 (4.8)	1.0	1.0000
Contact	14	0 (0)	0.0 (0 – 5.5)	
Sheep				
No contact	1398	67 (4.8)	1.0	1.0000
Contact	3	0 (0)	0.0 (0 – 11)	
Camels				
No contact	1365	67 (4.9)	1.0	0.4129
Contact	36	0	0.0 (0.0- 2.1)	

OR=odds ratio; CI= Confidence Intervals

4.4 Clinical characteristics associated with rickettsioses

Table 4.5 shows the clinical characteristics of patients' sero-positive for SFG. In addition to fever, patients sero-positive for SFG were more likely to have headache (79.7% compared to 52.7% without headache, $P < 0.05$), muscle aches (38.7% vs. 19.6%, $P < 0.05$) and joint pains (58.0% vs. 31.0%, $P < 0.05$).

Table 4.5: Clinical characteristics of patients who were sero-negative and sero-positive for SFG rickettsioses.

Clinical Characteristic	% Sero-negative (n=2013)	% Sero-positive (n=212)	OR (95% CI)	Fisher's exact P-value
Headache	1153 (52.7)	169 (79.7)	2.9 (2.1 - 4.2)	<0.0001
Joint ache	624 (31.0)	123 (58.0)	3.1 (2.3 - 4.2)	<0.0001
Muscle ache	395 (19.6)	82 (38.7)	2.6 (1.9 - 3.5)	<0.0001
Rashes	104 (5.2)	10 (4.7)	0.9 (0.4 - 1.8)	1.0000
Tick bites	33 (1.6)	4 (1.9)	1.2 (0.3 - 3.3)	0.7752

OR=odds ratio; CI= Confidence Intervals.

As shown in table 4.6, majority of patients with high titers of 1:1600 and 1:6400 had symptoms of headache, joint and muscle ache. This was a confirmation that persons with high titers which could indicate ongoing infection had higher odds of having symptoms of headache, joint ache and muscle ache.

Table 4. 6: Clinical characteristics based on antibody titers for SFG

Clinical Characteristic	Titer			
	1:100	1:400	1:1600	1:6400
Headache	10	34	66	59
Joint ache	6	21	48	48
Muscle ache	3	16	33	30
Rashes	0	2	1	7
Tick bites	0	0	3	1

NOTE: Data are number of participants.

Table 4.7 shows clinical characteristics for patient's sero-positive for ST. For those patients seropositive for ST, 74.6% had headaches, 40.3% had muscle aches, and (50.7%) had joint pains. Statistically significant associations were found between having headache, joint ache and muscle ache ($P < 0.05$).

Table 4.7: Clinical characteristics of patients both sero-negative and sero-positive for ST rickettsioses

Clinical Characteristic	% sero- negative (n= 1334)	% sero- positive (n=67)	OR (95% CI)	Fisher's exact P-value
Headache	791(59.3)	50(74.6)	2.0(1.1 - 3.8)	0.0146
Joint ache	415(31.1)	34(50.7)	2.2(1.4 – 3.9)	0.0012
Muscle ache	270(20.2)	27(40.3)	2.7 (1.5 – 4.5)	0.0003
Rashes	69(5.2)	6(9.0)	1.8(0.6 – 4.4)	0.1665
Tick bites	23(1.7)	1(1.5)	0.9(0.02 – 5.5)	1.0000

OR=odds ratio; CI= Confidence Intervals.

Majority of patients with titers of 1:400 had symptoms of headache, rashes, joint and muscle ache followed by those with titers of 1:1600 (Table 4.8)

Table 4. 8: Clinical characteristics based on antibody titers for ST.

Clinical Characteristic	Titer			
	1:100	1:400	1:1600	1:6400
Headache	3	29	16	2
Joint ache	2	22	8	2
Muscle ache	1	17	8	1
Rashes	0	4	2	0
Tick bites	0	1	0	0

NOTE: Data are number of participants.

CHAPTER FIVE

DISCUSSION

The study reported here confirms findings of SFG and TG in Kenya and reports for the first time in Africa, presence of ST. The study population was largely pediatric (77.8%) with more than 50% being less than 5 years and not all samples were tested for ST and TG. This may have impacted on the overall sero-prevalence. Nevertheless, the data generated demonstrates that SFG and ST rickettsiae, but not TG, are common in patients presenting with fever in Kenyan hospitals and are likely to be endemic infections in the areas specific areas.

5.1 Sero-prevalence of Spotted fever

In this study the sero-prevalence of SFG was (9.5%). This was similar to sero-prevalence that was reported among febrile patients in Northern Tanzania: SFG (8%) (Prabhu *et al.*, 2011). Significant differences in sero-prevalence were observed among the different surveillance sites across different geographic locations in Kenya. The highest sero-prevalence (25.2%) was recorded in Garissa. The inhabitants are predominantly semi-nomadic pastoralist population of Somali origin who keep cattle, sheep, goats, and camels. The region also carries a wide range of wildlife, including zebras, antelopes, waterbucks, giraffes, warthogs, monkeys, gerenuks, dik diks, lions, hyenas, and cheetahs. Livestock interacts freely with wildlife, with which they share pasture and watering holes, increasing the risk of *Rickettsia* transmission since wildlife act as a reservoir for *Rickettsia* gene pool in nature (Macaluso *et al.*, 2003). Alupe had a prevalence of (15.3%), Alupe is a border town with Uganda, located in Busia County and the main economic activities include trade, subsistence farming, tourism, fishing and livestock rearing. Marigat with a prevalence of (11.6%) is located in Rift valley province and it's in main economic activities is semi-nomadic livestock rearing. The presence of these livestock rearing activities and probable contact with in these areas could explain the high prevalence rate of *Rickettsia* transmitted through ticks of the genus *Amblyomma*, which is the main *Rickettsia* vector in these areas (Mutai *et al.*, 2013). The detection of IgG titers as high as 1:1600 and 1:6400 in majority of patients who were sero-positive indicate re-current SFG infections.

The sero-prevalence of TG was low (0.2%), but similar to sero-prevalence that was reported among febrile patients in Northern Tanzania (0.5%) (Prabhu *et al.*, 2011).

5.2. Sero-prevalence of Scrub Typhus rickettsia

In this study the prevalence of ST was (4.8%). This is the first report of ST in Sub-Saharan Africa. However, there is a reported case study of scrub typhus on a 46-year-old male from the United States who had visited Cameroon, West Africa and circumstantial evidence supported its origin in Africa (Osuga *et al.*, 1991). The sero-prevalence reported here (4.8%) differs from that reported among febrile patients in Sri Lanka (13.7%). ST is transmitted through larval mites (chiggers) to human and rodent hosts. Disease transmission occurs primarily in rural areas, and is therefore likely that these areas with the highest sero-prevalence in this study have peridomestic rodents and vectors that are infected with causative agents of ST. The detection of IgG titers of 1:400 and 1:1600 in majority of patients suggest the likelihood of continuous transmission.

5.3 Risk factors for Rickettsioses

Important risk factors for SFG sero-positivity were age and animal contact. Persons > 26years were more likely to be seropositive than persons in lower age groups. This finding was similar to a study done in Kenya (Richards *et al.*, 2010) and Sri Lanka (Reller *et al.*, 2012) where the older populations were reported to have higher sero-prevalence than the younger age groups. This finding appears to signify the role of certain behavioral and occupational factors that increase exposure to rickettsiae. There was a reported higher odds of sero-positivity with contact with camels, donkeys and dogs. This association could be due to the fact that communities from Garissa keep camels and dogs are used as guards to the animals. These animals act as hosts to the vectors such as ticks, fleas, mites and lice that may be infected with *Rickettsia* subsequently infecting humans. Contact with animals has been demonstrated as a risk factor associated with rickettsioses in the study carried out Sri Lanka (Reller *et al.*, 2012)

Similarly, sero-positivity to ST increased with age, with persons > 26years more likely to be seropositive than persons of all other age groups. This result was similar to a study carried out in Sri Lanka (Reller *et al.*, 2012) where the older populations

were reported to have higher sero-prevalence of ST than the younger ones. This in part could be explained by cumulative exposure to the pathogen and lifelong persistence of IgG. Significant association was found between ST sero positivity and contact with goats. This could be due to the fact that goats are the major livestock found in the area and can harbor vectors such as ticks, fleas and mites increasing *Rickettsia* transmission.

5.4 Clinical characteristics associated with rickettsioses

In this study, clinical characteristics were nonspecific and did not segregate with SFG or ST indicating difficulties of relying on clinical presentations when making a diagnosis of rickettsioses. (Prabhu *et al.*, 2011). Presence of eschars, which are a common sign of SFG and ST are less well examined in local populations and may be difficult to observe in darker skinned persons (Jenseni *et al.*, 2003). Nevertheless, when the levels of antibody titers were factored in, it emerged that headache, joint pains and muscle ache are important in making a diagnosis of SFG especially for titers of 1:1600 and 1:6400. Similar findings were found for ST .It is worth noting that this symptoms overlap with those of malaria. Majority of AFI patients who may have rickettsioses are never identified and are nearly always misdiagnosed on clinical grounds as having malaria. Inappropriate treatment is given as patients are given anti-malarial drugs which in turn can cause drug resistance delayed diagnosis of rickettsioses leading to morbidity

5.5 Limitations of the study

This study had a number of limitations. The serum samples available for testing were from a one-time encounter with the patient. Convalescent-phase serum samples would have helped in defining acute versus chronic cases. The ELISA for SFGR was specific for *R. conorii* despite the fact that recent reports have documented human infections with *R. felis* (Richards *et al.*, 2010) and tick infections with *R. africae* (Macaluso *et al.*, 2003).

5.6 Conclusions

- 1) This study demonstrates that Spotted Fever Group and Scrub typhus rickettsiae are common among acute febrile illness patients in Kenya. As in other reports, the typhus group rickettsiae are uncommon. This is the first sero-prevalence report of scrub typhus in Kenya and Sub-Saharan Africa.
- 2) Several risk factors are apparent for STG and ST sero-positivity. These include age and animal contact with presumably because older individuals are more likely to encounter rickettsioses by contact animals while herding.
- 3) Symptoms of headache, muscle ache and joint pain that are similar to those of malaria have a higher odds of sero-positivity to rickettsioses.

5.7 Recommendations

- 1) The findings of this study suggest that rickettsial infections should be considered in the differential diagnosis of febrile cases in Kenya and that diagnostic capacity should be established.
- 2) Greater awareness among clinicians and the availability of a reliable diagnostic test would improve patient management resulting in better patient outcomes, and would assist with estimating the disease burden for these infections.
- 3) Future sero-prevalence studies should include convalescent samples to allow determination of acute and chronic infections.
- 4) Additional entomological investigations are needed to identify the vectors for ST since chigger mites have not been reported in Kenya.

REFERENCES

- Amano, K., Hatakeyama, H., Okuta, M., Suto, T. & Mahara, F. (1992a).** Serological studies of antigenic similarity between Japanese spotted fever rickettsiae and Weil-Felix test antigens. *J Clin Microbiol*, **30**, 2441-2446.
- Amano, K., Suzuki, N., Hatakeyama, H., Kasahara, Y., Fujii, S., Fukushi, K., Suto, T. & Mahara, F. (1992b).** The reactivity between rickettsiae and Weil-Felix test antigens against sera of rickettsial disease patients. *Acta Virol*, **36**, 67-72.
- Anacker, R. L., Philip, R. N., Thomas, L. A., & Casper, E. A. (1979).** Indirect hemagglutination test for detection of antibody to *Rickettsia rickettsii* in sera from humans and common laboratory animals. *J Clin Microbiol*, **10(5)**, 677-684.
- Anderson, B. E., Regnery, R. L., Carlone, G. M., Tzianabos, T., McDade, J. E., Fu, Z. Y. & Bellini, W. J. (1987).** Sequence analysis of the 17-kilodalton-antigen gene from *Rickettsia rickettsii*. *J Bacteriol*, **169**, 2385-2390.
- Andersson, S. G., Zomorodipour, A., Andersson, J. O., Sicheritz-Ponten, T., Alsmark, U. C., Podowski, R. M., Naslund, A. K., ... & Kurland, C. G. (1998).** The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature*, **396**, 133-140.
- Archibald, L. K., McDonald, L. C., Nwanyanwu, O., Kazembe, P., Dobbie, H., Tokars, J., Reller, L. B. & Jarvis, W. R. (2000).** A hospital-based prevalence survey of bloodstream infections in febrile patients in Malawi: implications for diagnosis and therapy. *J Infect Dis*, **181**, 1414-1420.
- Beati, L., Meskini, M., Thiers, B. & Raoult, D. (1997).** *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. *Int J Syst Bacteriol*, **47**, 548-554.
- Clements, M. L., Dumler, J. S., Fiset, P., Wisseman, C. L., Jr., Snyder, M. J. & Levine, M. M. (1983).** Serodiagnosis of Rocky Mountain spotted fever:

- comparison of IgM and IgG enzyme-linked immunosorbent assays and indirect fluorescent antibody test. *J Infect Dis*, **148**, 876-880.
- Crum, J. W., Hanchalay, S. & Eamsila, C. (1980).** New paper enzyme-linked immunosorbent technique compared with microimmunofluorescence for detection of human serum antibodies to *Rickettsia tsutsugamushi*. *J Clin Microbiol*, **11**, 584-588.
- Dasch, G. A., Halle, S. & Bourgeois, A. L. (1979).** Sensitive microplate enzyme-linked immunosorbent assay for detection of antibodies against the scrub typhus rickettsia, *Rickettsia tsutsugamushi*. *J Clin Microbiol*, **9**, 38-48.
- Dong, X., El Karkouri, K., Robert, C., Raoult, D. & Fournier, P. E. (2012).** Genomic analysis of *Rickettsia japonica* strain YHT. *J Bacteriol*, **194**, 6992.
- Dumler, J. S., Taylor, J. P. & Walker, D. H. (1991).** Clinical and laboratory features of murine typhus in south Texas, 1980 through 1987. *JAMA*, **266**, 1365-1370.
- Dunster, L. M., Sanders, E. J., Borus, P. & Tukei, P. M. (1997).** Yellow fever in Kenya: the need for a country-wide surveillance programme. *World Health Stat Q*, **50**, 178-184.
- Eremeeva, M. E., Roux, V. & Raoult, D. (1993).** Determination of genome size and restriction pattern polymorphism of *Rickettsia prowazekii* and *Rickettsia typhi* by pulsed field gel electrophoresis. *FEMS Microbiol Lett*, **112**, 105-112.
- Faccini-Martinez, A. A., Alvarez, L. G., Hildago, M. & Oteo, J. A. (2014).** Syndromic classification of rickettsioses: an approach for clinical practice. *Int J Infect Dis*, **28**, 126-139.
- Feng, H. M., Whitworth, T., Popov, V. & Walker, D. H. (2004).** Effect of antibody on the rickettsia-host cell interaction. *Infect Immun*, **72**, 3524-3530.
- Fiset, P., Ormsbee, R. A., Silberman, R., Peacock, M. & Spielman, S. H. (1969).** A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta Virol*, **13**, 60-66.

- Fournier, P. E., Gouriet, F., Brouqui, P., Lucht, F. & Raoult, D. (2005).** Lymphangitis-associated rickettsiosis, a new rickettsiosis caused by *Rickettsia sibirica* mongolotimonae: seven new cases and review of the literature. *Clin Infect Dis*, **40**, 1435-1444.
- Fournier, P. E., Zhu, Y., Yu, X. & Raoult, D. (2006).** Proposal to create subspecies of *Rickettsia sibirica* and an emended description of *Rickettsia sibirica*. *Ann N Y Acad Sci*, **1078**, 597-606.
- Furuya, Y., Yoshida, Y., Katayama, T., Yamamoto, S. & Kawamura, A., Jr. (1993).** Serotype-specific amplification of *Rickettsia tsutsugamushi* DNA by nested polymerase chain reaction. *J Clin Microbiol*, **31**, 1637-1640.
- Gage, K. L., Gilmore, R. D., Karstens, R. H. & Schwan, T. G. (1992).** Detection of *Rickettsia rickettsii* in saliva, hemolymph and triturated tissues of infected *Dermacentor andersoni* ticks by polymerase chain reaction. *Mol Cell Probes*, **6**, 333-341.
- Gage, K. L., Schrumpf, M. E., Karstens, R. H., Burgdorfer, W. & Schwan, T. G. (1994).** DNA typing of rickettsiae in naturally infected ticks using a polymerase chain reaction/restriction fragment length polymorphism system. *Am J Trop Med Hyg*, **50**, 247-260.
- Graf, P. C., Chretien, J. P., Ung, L., Gaydos, J. C. & Richards, A. L. (2008).** Prevalence of seropositivity to spotted fever group rickettsiae and *Anaplasma phagocytophilum* in a large, demographically diverse US sample. *Clin Infect Dis*, **46**, 70-77.
- Graves, S. & Stenos, J. (2003).** *Rickettsia honei*: a spotted fever group Rickettsia on three continents. *Ann N Y Acad Sci*, **990**, 62-66.
- Halle, S., Dasch, G. A. & Weiss, E. (1977).** Sensitive enzyme-linked immunosorbent assay for detection of antibodies against typhus rickettsiae, *Rickettsia prowazekii* and *Rickettsia typhi*. *J Clin Microbiol*, **6**, 101-110.
- Harries, A. D., Speare, R. & Wirima, J. J. (1990).** Medical admissions to Kamuzu Central Hospital, Lilongwe, Malawi in 1986: comparison with

admissions to Queen Elizabeth Central Hospital, Blantyre in 1973. *Trop and geog med*, **42**, 274-279.

- Hechemy, K. E., Anacker, R. L., Carlo, N. L., Fox, J. A. & Gaafar, H. A. (1983).** Absorption of *Rickettsia rickettsii* antibodies by *Rickettsia rickettsii* antigens in four diagnostic tests. *J Clin Microbiol*, **17**, 445-449.
- Hechemy, K. E., Anacker, R. L., Philip, R. N., Kleeman, K. T., MacCormack, J. N., Sasowski, S. J. & Michaelson, E. E. (1980).** Detection of Rocky Mountain spotted fever antibodies by a latex agglutination test. *J Clin Microbiol*, **12**, 144-150.
- Heinzen, R. A., Hayes, S. F., Peacock, M. G. & Hackstadt, T. (1993).** Directional actin polymerization associated with spotted fever group *Rickettsia* infection of Vero cells. *Infect Immun*, **61**, 1926-1935.
- Hersey, D. F., Colvin, M. C. & Shepard, C. C. (1957).** Studies on the serologic diagnosis of murine typhus and Rocky Mountain spotted fever. II. Human infections. *J Immunol*, **79**, 409-415.
- Higgins, J. A. & Azad, A. F. (1995).** Use of polymerase chain reaction to detect bacteria in arthropods: a review. *J Med Entomol*, **32**, 213-222.
- Holman, R. C., Paddock, C. D., Curns, A. T., Krebs, J. W., McQuiston, J. H. & Childs, J. E. (2001).** Analysis of risk factors for fatal Rocky Mountain Spotted Fever: evidence for superiority of tetracyclines for therapy. *J Infect Dis*, **184**, 1437-1444.
- Huebner, R. J., Stamps, P. & Armstrong, C. (1946).** Rickettsialpox; a newly recognized rickettsial disease; isolation of the etiological agent. *Public Health Rep*, **61**, 1605-1614.
- Jensenius, M., Fournier, P. E., Kelly, P., Myrvang, B. & Raoult, D. (2003).** African tick bite fever. *Lancet Infect Dis*, **3**, 557-564.
- Jiang, J., Karen, J. M., Laurel, A. M., James, B Wilkinson, R., Wei-Mei, C. J. & Richards, A. L. (2003).** Laboratory diagnosis of two scrub typhus outbreaks at camp Fuji, Japan in 2000 and 2001 by Enzyme -Linked ImmunoSorbent assay, rapid flow assay and western blot assay using outer membrane 56-KD recombinant proteins. *Am J Trop Med Hyg*, **69**, 60-66.

- Jiang, J., Soeatmadji, D. W., Henry, K. M., Ratiwayanto, S., Bangs, M. J. & Richards, A. L. (2006).** *Rickettsia felis* in *Xenopsylla cheopis*, Java, Indonesia. *Emerg Infect Dis*, **12**, 1281-1283.
- Kaabia, N., Rolain, J. M., Khalifa, M., Ben Jazia, E., Bahri, F., Raoult, D. & Letaief, A. (2006).** Serologic study of rickettsioses among acute febrile patients in central Tunisia. *Ann N Y Acad Sci*, **1078**, 176-179.
- Kang, J. S. & Chang, W. H. (1999).** Antigenic relationship among the eight prototype and new serotype strains of *Orientia tsutsugamushi* revealed by monoclonal antibodies. *Microbiol Immunol*, **43**, 229-234.
- Kaplan, J. E. & Schonberger, L. B. (1986).** The sensitivity of various serologic tests in the diagnosis of Rocky Mountain spotted fever. *Am J Trop Med Hyg*, **35**, 840-844.
- Kelly, D. J., Wong, P. W., Gan, E. & Lewis, G. E., Jr. (1988).** Comparative evaluation of the indirect immunoperoxidase test for the serodiagnosis of rickettsial disease. *Am J Trop Med Hyg*, **38**, 400-406.
- Kelly, P. J., Beati, L., Mason, P. R., Matthewman, L. A., Roux, V. & Raoult, D. (1996).** *Rickettsia africae* sp. nov., the etiological agent of African tick bite fever. *Int J Syst Bacteriol*, **46**, 611-614.
- Kelly, P. J., Beati, L., Matthewman, L. A., Mason, P. R., Dasch, G. A. & Raoult, D. (1994).** A new pathogenic spotted fever group rickettsia from Africa. *J Trop Med Hyg*, **97**, 129-137.
- La Scola, B. & Raoult, D. (1997).** Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol*, **35**, 2715-2727.
- Lai, C. H., Huang, C. K., Chen, Y. H., Chang, L. L., Weng, H. C., Lin, J. N., Chung, H. C., Liang, S. H. & Lin, H. H. (2009).** Epidemiology of acute q Fever, scrub typhus, and murine typhus, and identification of their clinical characteristics compared to patients with acute febrile illness in southern taiwan. *J Formos Med Assoc*, **108**, 367-376.
- Land, M. V., Ching, W. M., Dasch, G. A., Zhang, Z., Kelly, D. J., Graves, S. R. & Devine, P. L. (2000).** 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**, 2701-2705.

Macaluso, K. R., Davis, J., Alam, U., Korman, A., Rutherford, J. S., Rosenberg, R. & Azad, A. F. (2003). Spotted fever group rickettsiae in ticks from the Masai Mara region of Kenya. *Am J Trop Med Hyg*, **68**, 551-553.

Mahajan, S. K. (2005). Scrub typhus. *J Assoc Physicians India*, **53**, 954-958.

Mediannikov, O. Y., Sidelnikov, Y., Ivanov, L., Mokretsova, E., Fournier, P. E., Tarasevich, I. & Raoult, D. (2004). Acute tick-borne rickettsiosis caused by *Rickettsia heilongjiangensis* in Russian Far East. *Emerg Infect Dis*, **10**, 810-817.

Mokrani, N., Parola, P., Tebbal, S., Dalichaouche, M., Aouati, A. & Raoult, D. (2008). *Rickettsia aeschlimannii* infection, Algeria. *Emerg Infect Dis*, **14**, 1814-1815.

Murai, K., Okayama, A., Horinouchi, H., Oshikawa, T., Tachibana, N. & Tsubouchi, H. (1995). Eradication of *Rickettsia tsutsugamushi* from patients' blood by chemotherapy, as assessed by the polymerase chain reaction. *Am J Trop Med Hyg*, **52**, 325-327.

Murdoch, D. R., Woods, C. W., Zimmerman, M. D., Dull, P. M., Belbase, R. H., Keenan, A. J., Scott, R. M.,... & Reller, L. B. (2004b). The etiology of febrile illness in adults presenting to Patan hospital in Kathmandu, Nepal. *Am J Trop Med Hyg*, **70**, 670-675.

Mutai, B. K., Wainaina, J. M., Magiri, C. G., Nganga, J. K., Ithondeka, P. M., Njagi, O. N., Jiang, J., Richards, A. L. & Waitumbi, J. N. (2013). Zoonotic surveillance for rickettsiae in domestic animals in Kenya. *Vector Borne Zoonotic Dis*, **13**, 360-366.

Nadjm, B., Amos, B., Mtove, G., Ostermann, J., Chonya, S., Wangai, H., Kimera, J., Msuya, W., ...& Reyburn, H. (2010). WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study. *BMJ*, **340**, 1350.

- Ndip, L. M., Bouyer, D. H., Travassos Da Rosa, A. P., Titanji, V. P., Tesh, R. B. & Walker, D. H. (2004).** Acute spotted fever rickettsiosis among febrile patients, Cameroon. *Emerg Infect Dis*, **10**, 432-437.
- Nilsson, K., Elfving, K. & Pahlson, C. (2010).** *Rickettsia helvetica* in patient with meningitis, Sweden, 2006. *Emerg Infect Dis*, **16**, 490-492.
- Ohashi, N., Nashimoto, H., Ikeda, H. & Tamura, A. (1990).** Cloning and sequencing of the gene (tsg56) encoding a type-specific antigen from *Rickettsia tsutsugamushi*. *Gene*, **91**, 119-122.
- Oster, C. N., Burke, D. S., Kenyon, R. H., Ascher, M. S., Harber, P. & Pedersen, C. E., Jr. (1977).** Laboratory-acquired Rocky Mountain spotted fever. The hazard of aerosol transmission. *N Engl J Med*, **297**, 859-863.
- Osuga, K., Kimura, M., Goto, H., Shimada, K. & Suto, T. (1991).** A case of Tsutsugamushi disease probably contracted in Africa. *Eur J Clin Microbiol Infect Dis*, **10**, 95-96.
- Parola, P., Paddock, C. D. & Raoult, D. (2005).** Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev*, **18**, 719-756.
- Perez-Osorio, C. E., Zavala-Velazquez, J. E., Arias Leon, J. J. & Zavala-Castro, J. E. (2008).** *Rickettsia felis* as emergent global threat for humans. *Emerg Infect Dis*, **14**, 1019-1023.
- Pether, J. V., Jones, W., Lloyd, G., Rutter, D. A. & Barry, M. (1994).** Fatal murine typhus from Spain. *Lancet*, **344**, 897-898.
- Phan, J. N., Lu, C R., Bender, W. G., SmoakIII, R. M. & Zhong, J. (1994).** Molecular detection and Identification of *Rickettsia* species in *Ixodes pacificus* in California. *Vector Borne Zoonotic Dis*, **11**, 360-366.
- Philip, R. N., Casper, E. A., Ormsbee, R. A., Peacock, M. G. & Burgdorfer, W. (1976).** Microimmunofluorescence test for the serological study of rocky mountain spotted fever and typhus. *J Clin Microbiol*, **3**, 51-61.
- Prabhu, M., Nicholson, W. L., Roche, A. J., Kersh, G. J., Fitzpatrick, K. A., Oliver, L. D., Massung, R. F., ...& Crump, J. A. (2011).** Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. *Clin Infect Dis*, **53**, 8-15.

- Pretorius, A. M. & Birtles, R. J. (2002).** *Rickettsia aeschlimannii*: A new pathogenic spotted fever group rickettsia, South Africa. *Emerg Infect Dis*, **8**, 874.
- Raoult, D., De Micco, C., Chaudet, H. & Tamalet, J. (1985).** Serological diagnosis of Mediterranean spotted fever by the immunoperoxidase reaction. *Eur J Clin Microbiol*, **4**, 441-442.
- Raoult, D. & Dasch, G. A. (1989).** Line blot and western blot immunoassays for diagnosis of Mediterranean spotted fever. *J Clin Microbiol*, **27**, 2073-2079.
- Raoult, D. & Dasch, G. A. (1995).** Immunoblot cross-reactions among *Rickettsia*, *Proteus* spp. and *Legionella* spp. in patients with Mediterranean spotted fever. *FEMS Immunol Med Microbiol*, **11**, 13-18.
- Raoult, D., Brouqui, P. & Roux, V. (1996).** A new spotted-fever-group rickettsiosis. *Lancet*, **348**, 412.
- Raoult, D., Berbis, P., Roux, V., Xu, W. & Maurin, M. (1997).** A new tick-transmitted disease due to *Rickettsia slovaca*. *Lancet*, **350**, 12-113.
- Raoult, D. & Roux, V. (1997).** Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev*, **10**, 694-719.
- Raoult, D., Fournier, P. E., Fenollar, F., Jensenius, M., Prioe, T., de Pina, J. J., Caruso, G., ...& Marrie, T. J. (2001).** *Rickettsia africae*, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med*, **344**:1504-1510.
- Reller, M. E., Bodinayake, C., Nagahawatte, A., Devasiri, V., Kodikara-Arachichi, W., Strouse, J. J., Flom, J. E.,... & Dumler, J. S. (2012).** Unsuspected rickettsioses among patients with acute febrile illness, Sri Lanka, 2007. *Emerg Infect Dis*, **18**, 825-829.
- Richards, A. L., Jiang, J., Omulo, S., Dare, R., Abdirahman, K., Ali, A., Sharif, S. K., & Njenga, M. K. (2010).** Human Infection with *Rickettsia felis*, Kenya. *Emerg Infect Dis*, **16**, 1081-1086.
- Robertson, R. G. & Wisseman, C. L., Jr. (1973).** Tick-borne rickettsiae of the spotted fever group in West Pakistan. II. Serological classification of

isolates from West Pakistan and Thailand: evidence for two new species.
Am J Epidemiol, **97**, 55-64.

- Romer, Y., Sejjo, A. C., Crudo, F., Nicholson, W. L., Varela-Stokes, A., Lash, R. R. & Paddock, C. D. (2011).** *Rickettsia parkeri* Rickettsiosis, Argentina. *Emerg Infect Dis*, **17**, 1169-1173.
- Roux, V., Fournier, P. E. & Raoult, D. (1996).** Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. *J Clin Microbiol*, **34**, 2058-2065.
- Roux, V. & Raoult, D. (1995).** Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. *Res Microbiol*, **146**, 385-396.
- Roux, V., Rydkina, E., Ereemeeva, M. & Raoult, D. (1997).** Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. *Int J Syst Bacteriol*, **47**, 252-261.
- Rutherford, J. S., Macaluso, K. R., Smith, N., Zaki, S. R., Paddock, C. D., Davis, J., Peterson, N., ...& Rosenberg, R. (2004).** Fatal spotted fever rickettsiosis, Kenya. *Emerg Infect Dis*, **10**, 910-913.
- Sanchez, J. L., Candler, W. H., Fishbein, D. B., Greene, C. R., Cote, T. R., Kelly, D. J., Driggers, D. P. & Johnson, B. J. (1992).** A cluster of tick-borne infections: association with military training and asymptomatic infections due to *Rickettsia rickettsii*. *Trans R Soc Trop Med Hyg*, **86**, 321-325.
- Sarov, B., Manor, E., Hanuka, N., Sikuler, E., Galil, A. & Sarov, I. (1992).** Comparison of structural polypeptides, detected by immunoblotting technique, in the sera of spotted fever group rickettsia positive cases--symptomatic versus asymptomatic. *Acta Virol*, **36**, 57-61.
- Senneville, E., Ajana, F., Lecocq, P., Chidiac, C. & Mouton, Y. (1991).** *Rickettsia conorii* isolated from ticks introduced to northern France by a dog. *Lancet*, **337**, 676.
- Sharma, P., Kakkar, R., Kaore, S., Yadav, V. & Sharma, R. (2010).** Geographical distribution, effect of season & life cycle of scrub typhus *JK Science*, **12**, 63-64.

- Shepard, C. C., Redus, M. A., Tzianabos, T. & Warfield, D. T. (1976).** Recent experience with the complement fixation test in the laboratory diagnosis of rickettsial diseases in the United States. *J Clin Microbiol*, **4**, 277-283.
- Simser, J. A., Palmer, A. T., Munderloh, U. G. & Kurtti, T. J. (2001).** Isolation of a spotted fever group Rickettsia, *Rickettsia peacockii*, in a Rocky Mountain wood tick, *Dermacentor andersoni*, cell line. *Appl Environ Microbiol*, **67**, 546-552.
- Stenos, J., Roux, V., Walker, D. & Raoult, D. (1998).** *Rickettsia honei* sp. nov., the aetiological agent of Flinders Island spotted fever in Australia. *Int J Syst Bacteriol*, **48(4)**, 1399-1404.
- Stover, C. K., Marana, D. P., Carter, J. M., Roe, B. A., Mardis, E. & Oaks, E. V. (1990).** The 56-kilodalton major protein antigen of *Rickettsia tsutsugamushi*: molecular cloning and sequence analysis of the sta56 gene and precise identification of a strain-specific epitope. *Infect Immun*, **58**, 2076-2084.
- Suputtamongkol, Y., Suttinont, C., Niwatayakul, K., Hoontrakul, S., Limpaboon, R., Chierakul, W., Losuwanaluk, K. & Saisongkork, W. (2009).** Epidemiology and clinical aspects of rickettsioses in Thailand. *Ann N Y Acad Sci*, **1166**, 172-179.
- Svraka, S., Rolain, J. M., Bechah, Y., Gatabazi, J. & Raoult, D. (2006).** *Rickettsia prowazekii* and real-time polymerase chain reaction. *Emerg Infect Dis*, **12**, 428-432.
- Tamura, A., Ohashi, N., Urakami, H. & Miyamura, S. (1995).** Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. *Int J Syst Bacteriol*, **45**, 589-591.
- Teyssie, N., Chiche-Portiche, C. & Raoult, D. (1992).** Intracellular movements of *Rickettsia conorii* and *Rickettsia typhi* based on actin polymerization. *Res Microbiol*, **143**, 821-829.
- Todar, K. (2009).** Online textbook of bacteriology. Texas:University of Wisconsin-Madison

- Walker, D. H., Liu, Q. H., Yu, X. J., Li, H., Taylor, C. & Feng, H. M. (1992).** Antigenic diversity of *Rickettsia conorii*. *Am J Trop Med Hyg*, **47**, 78-86.
- Watt, G. & Parola, P. (2003).** Scrub typhus and tropical rickettsioses. *Curr Opin Infect Dis*, **16**, 429-436.
- Wells, G. M., Woodward, T. E., Fiset, P. & Hornick, R. B. (1978).** Rocky mountain spotted fever caused by blood transfusion. *JAMA*, **239**, 2763-2765.
- Yoshikawa, H., Kimura, M., Ogawa, M., Rolain, J. M. & Raoult, D. (2005).** Laboratory-confirmed Mediterranean spotted fever in a Japanese traveler to Kenya. *Am J Trop Med Hyg*, **73**, 1086-1089.
- Yu, X., Jin, Y., Fan, M., Xu, G., Liu, Q. & Raoult, D. (1993).** Genotypic and antigenic identification of two new strains of spotted fever group rickettsiae isolated from China. *J Clin Microbiol*, **31**, 83-88.
- Yun Chan, Y. G., Riley, S. P., & Martinez, J. J. (1978).** Adherence to and Invasion of Host Cells by Spotted Fever Group *Rickettsia* Species *Front Microbiol*, **1**, 139.
- Zhang, J. Z., Fan, M. Y., Wu, Y. M., Fournier, P. E., Roux, V. & Raoult, D. (2000).** Genetic classification of "*Rickettsia heilongjiangii*" and "*Rickettsia hulunii*," two Chinese spotted fever group rickettsiae. *J Clin Microbiol*, **38**, 3498-3501.
- Zinsser, H. & Castaneda, M. R. (1934).** Active and Passive Immunization in Typhus Fever. *Proc Natl Acad Sci U S A*, **20**, 9-11.

Appendix 1: SSC Ethical approval letter.



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website: www.kemri.org

KEMRI/RES/7/3/1

October 17, 2012

TO: DR. JOHN WAITUMBI (PRINCIPAL INVESTIGATOR)

THROUGH: DR. JUMA RASHID,
THE DIRECTOR, CCR,
NAIROBI

Forwarded
to CCR 18/10/2012

Dear Sir,

RE: **SSC PROTOCOL No. 1282 (REQUEST FOR AMENDMENT 3): ACUTE FEBRILE ILLNESS SURVEILLANCE IN KENYA (VERSION 20.2 DATED 05 JULY 2012)**

Reference is made to your letter dated August 13, 2012. The ERC Secretariat acknowledges receipt of the Revised Study Protocol - Version 20.2 dated 05 July 2012 on August 22, 2012.

This is to inform you that at the 208th meeting of the KEMRI Ethics Review Committee held on 9th October 2012, the above referenced application was discussed.

The Committee noted the following proposed amendments:

1. To include assays that will be performed on a diverse range of pathogens that cause fever.
2. To expand the surveillance sites.
3. To collect detailed epidemiological information that will facilitate GPS citing of disease hotspots and allow for more focused disease surveillance and ecological niche modeling.

The Committee concluded that the suggested amendment is justified and is consequently granted approval for implementation. Please obtain a letter of authorization from the management of each of the new facilities before embarking on any surveillance activities and submit copies of the approval letters for ERC records.

Note that you are required to submit any further requests for changes to this version of the protocol to the SSC and ERC for review and approval prior to implementing any additional changes.

Sincerely,

DR. CHRISTINE WASUNNA,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE