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Q FEVER INFECTION IN PATIENTS WITH FEBRILE ILLNESS AT SELECTED HEALTHCARE FACILITIES IN GHANA.

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DECLARATION

I hereby declare that this is the product of my own research undertaken under the supervision of Professor Eric Sampane-Donkor and Professor George Armah and that references made to other people's work have been duly acknowledged. I also declare that this work has neither been presented in whole nor in part for another degree elsewhere.

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DEDICATION

I dedicate this work to my family and most especially, to my loving parents Mr and Mrs Yeboah, whose prayers and wonderful words of encouragement have given me the strength to complete this work.

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BACKGROUND

Q fever is a zoonotic infection transmitted by an intracellular bacterium *Coxiella burnetii*. The most frequently observed clinical feature of Q fever is acute febrile illness, or in rare cases, chronic illnesses. This disease is under-diagnosed and under-reported because the symptoms are nonspecific, resembling other febrile illnesses such as malaria. Due to similarity of Q fever clinical symptoms to malaria and other febrile illness aetiologies, misdiagnosis could lead to clinical complications. In Ghana little is known about Q fever, hence the need for the study.

AIM

The aim of this study was to investigate Q fever infection in patients reporting with febrile illness at selected healthcare facilities in Ghana.

METHOD

This was a cross-sectional study conducted at 37 Military hospital, Accra, and three military healthcare facilities in Sekondi-Takoradi. Participants' which included military personnel and civilians, demographic data, clinical features and exposure data were obtained using a questionnaire. Depending on age, between 2 and 10 ml of venous blood was collected from consenting febrile patients at the selected healthcare facilities. Serum was collected and screened for phase II immunoglobulins M and G by Enzyme Linked Immunosorbent Assay (ELISA). Part of the serum was extracted and used for real-time Polymerase Chain Reaction (rt-PCR) and Loop Mediated Isothermal (LAMP) assays.

RESULTS

A total of 117 febrile patients were recruited into the study comprising 64 (54.70%) males and 53 (45.30%) females. An overall seroprevalence of 16.24% was recorded, comprising of 6.83% for IgM (indicating current or acute infection) and 11.11% for IgG (indicating recent or past infection). Clinical manifestation in study participants showed no significant association with the infection. The occupation of the participant was a statistically significant risk factor, with the unemployed having a greater likelihood of acquiring the infection. *C. burnetii* could not be detected by both rt-PCR and LAMP assays, this may have been due to late sample collection after the onset of symptoms.

CONCLUSION

The study clearly suggests the presence of Q fever among febrile patients in Ghana and occupation was a significant risk factor to Q fever exposure. Active surveillance is recommended to properly identify the source of transmission of *C. burnetii.*

LIST OF ABBREVIATIONS

%	Percentages
β	Beta
°C	Degree Celsius
µl	Microlitres
µM	Micro Molar
2MRS 2	Medical Reception Station
AFI	Acute Febrile Illness
bp	Base-pair
CDC	Center for Disease and Control
dNTP	Deoxynucleotide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
GHS-ERC	Ghana Health Service Ethical Review Committee
GPS	Global Positioning System
icd	Isocritrate dehydrogenase
ICU	Intensive Care Unit
IS*1111*	Insertion sequences
LAMP	Loop mediated isothermal amplification
LCV	Large Cell Variant
LPS	Lipopolysaccharide
ml	Mililitres
nM	Nanomolar
NMIMR	Noguchi Memorial Institute for Medical Research
NMIMR-IRB	Noguchi Memorial Institute for Medical Research Institutional Review Board
NMRC	Naval Medical Research Center

Pmol	Picomole
PFGE	Pulse – field gel electrophoresis
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
tRNA	Transfer Ribonucleic Acid
rpm	Revolution per minute
rt-PCR	Real Time Polymerase Chain Reaction
SCV	Small Cell Variant
TMH	37 Military Hospital
USAMRIID	U.S Army Medical Research for Infectious Diseases

CHAPTER ONE

1.0 BACKGROUND

1.1 INTRODUCTION

Acute Febrile Illness (AFI) is a syndrome which refers to the rapid onset of fever and symptoms such as chills, headaches, muscle and joint aches. In tropical and developing countries, such as Ghana, fever is usually one of the most common presenting complaints of patients seeking healthcare (Murray *et al*., 2012). This illness is predominant in the tropics and subtropics and is caused by a diversity of aetiological agents including bacteria, parasites and viruses (Acestor *et al*., 2012).

Morbidity and mortality in developing tropical and sub-tropical counties are mostly caused by different aetiological agents which first present as febrile illness (Animut *et al*., 2009). Acute Febrile Illness poses a public health challenge in areas where diagnosis of illnesses are only done symptomatically and laboratory test are seldom done (Archibald & Reller, 2001). This is because symptomatic diagnosis alone is insufficient to distinguish between aetiologies that result in febrile illness due to their similarities in clinical presentations. Hence, clinical management of AFI is often inadequate and this has resulted in the paucity of information on the importance and prevalence of their aetiologies, especially AFI associated with other aetiologies order than *Plasmodium falciparum*

In malaria endemic countries such as Ghana, most AFIs are attributed to *Plasmodium falciparum* infection and presumptively treated with expensive combination therapies (Snow *et al.*, 2003).Studies in The Gambia have shown that only 11% (24/224) of febrile episodes were due to malaria (Ceesay *et al*., 2010). Similar studies in the sub-region showing similar results have heightened the interest in research studies into the other causes of febrile illness

(Vanderburg *et al*., 2014). Therefore, the diagnosis, treatment and control of non-malarial febrile illnesses such as Query (Q) fever are emerging as new public health priority (Crump *et al*., 2013).

Query fever has been reported to be a common cause of febrile illness and community acquired pneumonia in resource limited settings (Manock *et al.*, 2009). The infection is a zoonosis, occurring worldwide and has long been recognised as an under diagnosed and underreported illness because symptoms are usually non-specific, making clinical diagnosis difficult (Derrick, 1983). Q fever is caused by an obligate intracellular bacterium *Coxiella burnetii*. The reservoir for *C. burnetii* is broad and includes livestock (sheep, goats, and cattle), domestic pets (dogs and cats), reptiles, birds as well as arthropods (mainly ticks) (Badudieri, 1959). Transmission of the disease to humans is mainly by contact with infected animals, mostly from inhalation of contaminated aerosols. Transmission from person to person is also possible but is seldom reported (Anderson *et al*., 2013). Sexual and trans-plancetal transmission, bone marrow transplants and blood transfusion have also been reported as source of human transmissions (Kanfer*et al*., 1988; Kruszewska *et al*., 1996).

Animals that are known to harbour the causative bacteria are mostly asymptomatic but shed the bacteria in their urine, faeces, and milk. However, clinical manifestation are only associated with reproductive disorders such as abortion and infertility (Lang, 1990). High concentrations of *C. burnetii* are known to be found in the placenta and amniotic fluid of these animals (Rodolakis *et al.*, 2007).

Symptomatic infection in humans could be self-limiting but acute or chronic forms exist (Anderson *et al*., 2013). Acute infection is characterised by febrile illness which involves a sudden onset of fever, chills, myalgia, headache, atypical pneumonia and hepatitis; chronic infection includes sub-acute endocarditis and chronic stress syndrome (Badudieri B., 1959).

Query fever is usually benign but could be fatal with a mortality of 1 to 11% in patients with chronic infection (Raoult, 1990). The non-specificity of the clinical manifestation makes the diagnosis difficult, especially in non-endemic areas (Baumbach *et al*., 1992). Treatment with appropriate antibiotics during early phase is effective but disease could progress to the chronic phase or even death if treatment is delayed (Anderson *et al*., 2013). Hence early diagnosis and treatment is highly recommended. For in-patients with chronic Q fever infection, early diagnosis and treatment maybe lifesaving. Immediate diagnosis and appropriate treatment can shorten the term of illness and the risks of life threatening complications due to Q fever infection (Kampschreur & Dekker, 2012).

Q fever has been reported in both developing and developed countries. In Southern France, seroprevalence of 5 - 8% of human endocarditis cases were due to *C. burnetii* and a prevalence of acute Q fever of 50 cases per 100,000 inhabitants was reported (Dupont *et al*., 1994).

Studies conducted in Africa have recorded prevalence of *C burnetii* infection between 5 and 10% amongst pregnant women (Anstey *et al*., 1997).In Burkina Faso and Tanzania a prevalence of 5% was recorded amongst febrile patients (Ki-Zerbo *et al*., 2000; Prabhu *et al*., 2011). Additionally, in Niger a prevalence of 10% was recorded in children (Julvez*et al*., 1997). Tunisia has also reported a prevalence of 3% and 9% amongst endocarditis patients (Znazen *et al*., 2009) and febrile patients (Kaabia *et al*., 2006) respectively.

Laboratory diagnosis of the infection is by histology, serology, cell culture and molecular methods (Fournier *et al*., 1998). Serological diagnosis which is often used could be ineffective during early diagnosis of the infection because specific antibodies can only detected two to three (2 -3) after infection. Nucleic acid amplification methods are however, useful in early diagnosis of Q fever because it can detects the DNA of the bacteria. It is

important to note that this is only possible if the samples are collected 1 – 5 days of the acute infection stage (Anderson *et al*., 2013).

The drug of choice for the management of Q fever infection is doxycycline and is most effective during acute infection. Prevention of the infection is by observing proper hand hygiene after contact with animals or the animals birthing products since transmission is by contact or inhalation of contaminated animal excrete. Additionally, dairy products should be pasteurized before consumption.

Vaccines against *C. burnetii* for both humans and animals, which have helped prevent the infection, exist but are not commercially available worldwide. Two antigenic phases exist in *C. burnetii* (phase I and II). During acute infection, phase II antibody to *C. burnetii* response appears first and Phase 1 during chronic infection (Maurin & Raoult, 1999). The only commercially available human vaccine for Q fever is Q-Vax. It is a formalin inactivated whole cell preparation. This vaccine has been licensed for use only in Australia and is given only to at-risk population; the vaccine has also demonstrated high efficacy and safety. A formalin inactivated phase 1 Q fever vaccine was previously available from the Special Immunizations Program of U.S Army Medical Research for Infectious Diseases (USAMRIID) as an experimental new drug. However, due to its potency issues with the skin test, this vaccine is currently unavailable (Kersh *et al*., 2013).

1.2 Problem Statement

Q fever gained renewed attention after the largest ever recorded outbreak which involved over 3,500 human cases in the Netherlands from 2007 to 2010 (Van der Hoek *et al*., 2010). Large outbreaks of Q fever have also been reported in Great Britain (Guigno *et al*., 1992), Berlin in Germany (Schneider *et al*., 1993), in Switzerland (Dupuis *et al*., 1987) and in Spain (Aguirre Errasti *et al.*,1984*)*. In a study carried out on hospitalized patients in northern

Tanzania, Q fever was identified as a more common cause of severe febrile illness than malaria (Crump *et al*., 2013). In Ghana, there have been a documented seroprevalence rate of 16.9% and 8.9% of anti-Q fever IgG antibodies in children and adults respectively (Kobbe *et al*., 2008). Additionally, other countries in sub-Saharan Africa, the seroprevalence of Q fever in apparently healthy people varies between 1 – 37% (Klaasen *et al.*, 2014). In the United States, it is estimated that 50 – 60 cases of Q fever are recorded annually (CDC, 2008).

Although Q fever has been described in almost every country, the incidence of human infection has not been properly accessed in most countries (Maurin & Raoult, 1999). In Ghana, preliminary data on Q fever suggest that the infection maybe an important public health problem as stated earlier. However, the incidence of Q fever among humans with febrile illness is probably underestimated, since the clinical presentation is very pleomorphic and nonspecific.

In Ghana a lot of febrile illnesses have been attributed mostly to *Plasmodium* sp (malarial parasite) as the causative agent. However, the last decade has observed a decline in the incidence and prevalence of malaria in most African countries (Rodrigues *et al*., 2008). Additionally, there is paucity of data on Q fever in Ghana.

1.3 Study Justification

Generally, several studies conducted on Q fever in several African countries, highlights the infection as an important public health problem (Vanderburg *et al*., 2014). There is limited data on Q fever infection in Ghana. A study by (Kobbe *et al*., 2008) showed a relatively high seroprevalence of 16.9% in children in rural communities in the Ashanti region of Ghana. This is of concern and highlights the need to screen patients in other parts of Ghana for Q fever infection.

Despite the existence of some data on Q fever in Ghana, little is known about the epidemiology of *C. burnetii infection* in the country. This has created a major hindrance to the effective control of *C. burnetii* in the country. Information from this study will provide the needed data on the epidemiology and the acknowledgement of *C. burnetii* as a causative agent of febrile illness in Ghana and its associated risk factors. Finally, the study will assist in creating the much needed awareness of the public health importance of *C. burnetii* infection in Ghana.

1.5 Aim

To investigate the prevalence of Q fever infection in patients reporting with febrile illness at the only military hospital in Ghana (37Military Hospital (TMH), Accra) and three military clinics (2 Medical Reception Station (2MRS), the Naval Health Centre and the Air Force Medical Centre at Sekondi-Takoradi in the Western region of Ghana.

Specific Objectives

1. To determine the seroprevalence in both hospitalised (in-patients) and non-hospitalized (out-patients) with febrile illness at the selected healthcare facilities.
2. To determine the risk factors associated with Q fever infection among the study participants.
3. To compare the use of Serological (ELISA) and Molecular Methods (rt-PCR and LAMP assays) for the detection of *C. burnetii*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Acute Febrile illness

Acute Febrile Illness (AFI) is medically used to describe an elevated rise in body temperature. It is characterized by a rapid onset of fever, including symptoms like chills, aches and pains. The illness (AFI) originates from a legion of causes and a wide range of microbes are implicated (Liang & McDonald, 2012).

Acute febrile illness (AFI) is a common presentation to health care facilities globally, including Ghana. Limited resources and the diverse causative agents have made the diagnosis and treatment of these types of infection challenging in the developing world. Patients presenting with AFI syndromes are often empirically treated without supportive laboratory evaluation, i.e. symptomatically (Crump *et al*., 2013). The lack of resources in developing countries such as Ghana, limits support for comprehensive diagnostic testing. This practice has often led to inferior clinical care and treatment efficacy while propagating the evolution of antimicrobial resistance.

Common microbes implicated in AFI includes parasites (such as *Plasmodium* sp, *Entamoeba* sp, *Leishmania* sp, *Toxoplasma gondii*, etc), viruses such as Dengue virus, West Nile virus, Yellow Fever virus, Chikungunya virus, Rift Valley Fever Virus, Crimean Congo Hemorrhagic Fever virus, Lassa viruses, Hanta Virus, etc (Lanciotti *et al.,* 1998; Morrill *et al.,* 1991)] and bacteria such as; *Leptospira*, *Brucella*, *Rickettsia*, *C. burnetii*, and *Salmonella typhi* (Magill, 1998; Ostroff & Kozarsky, 1998).

2.2 The organism *Coxiella burnetii*

Coxiella burnetii is the aetiological agent of Q fever, it is a short (0.4 – 1µm long and 0.2 – 0.4µm wide), pleomorphic rod bound by a membrane similar to gram negative bacteria

(Burnet & Freeman, 1937) (Figure 2.1 shows a diagram of *C. burnetii*). *C. burnetti* is an obligate intracellular bacterium, known to grow exclusively in eukaryotic cells.

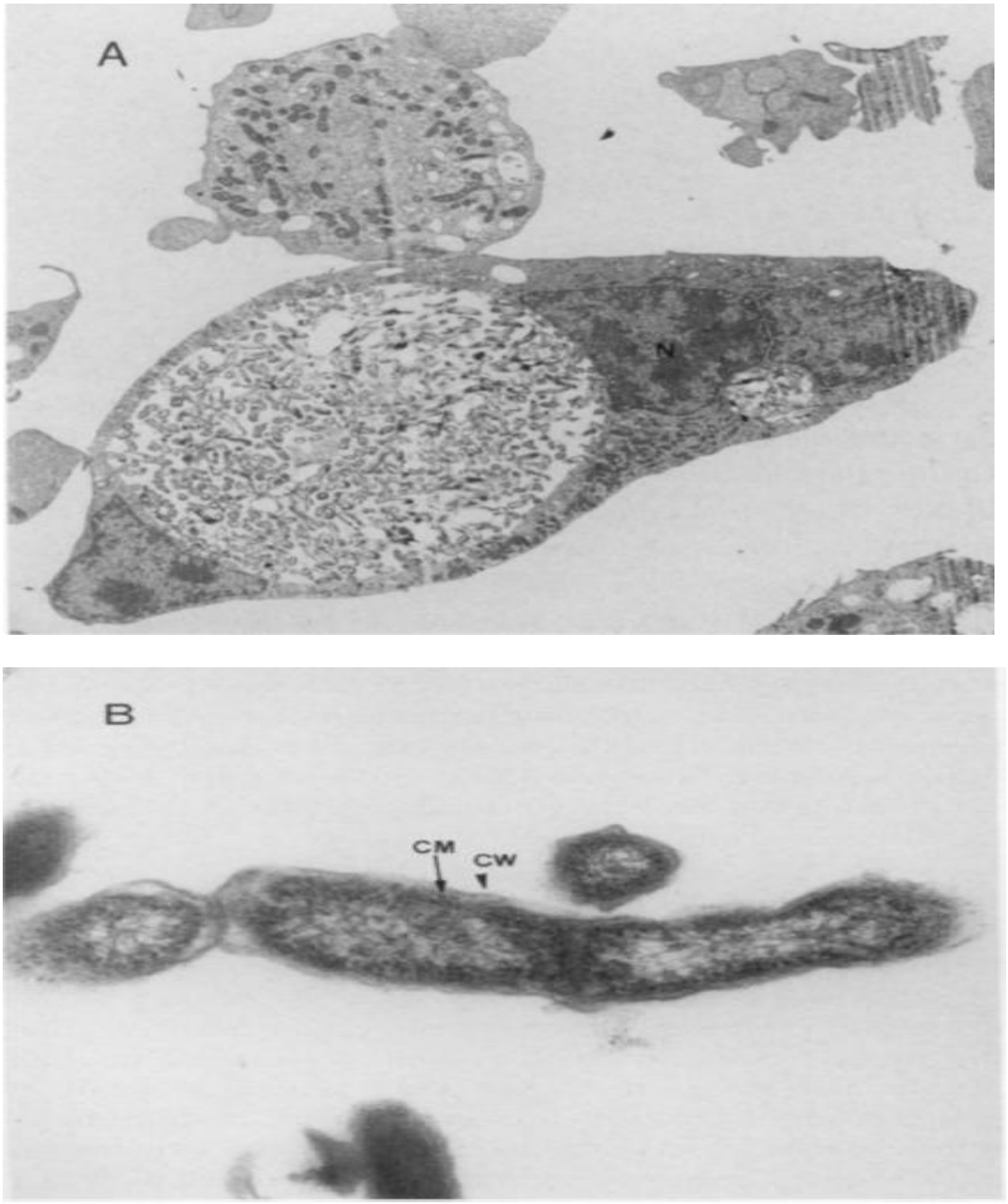

Figure 2.1: Micrograph and high power view of *Coxiella burnetti*

A. Electron micrograph of *Coxiella burnetii* growing in the phagolysosome of a eukaryotic cell @ magnification X 10,000 and N= Nucleus.

B.A high-power view of *Coxiella burnetii*; noting the characteristic of a gram-negative cell wall. Original magnification, X200; C M= cytoplasmic membrane; CW = cell wall.

Source: (Raoult & Marrie, 1995).

It shares similar characteristics with the bacteria of the genus *Rickettsia*, such as its small genome, ability to stain with Gimenez, its strict intracellular growth in eukaryotic cells and its association with arthropods (Heinzen *et al*., 1990; Giménez 1964; Weiss & Moulder 1984)**.** However, the 42.2% guanine-plus-cytosine content of *C. burnetii* DNA is strikingly different fromthat of typical rickettsiae (Baca & Paretsky, 1983).

Coxiella burnetii is the only species of the genus and have been classified into the γ subdivision of family *Proteobacteria*, close to *Rickettsiella grylli*, *Legionella* spp., and *Francisella* spp., on the basis of a comparison of the sequences of the 16S rRNA-encoding gene (Figure. 2.2).

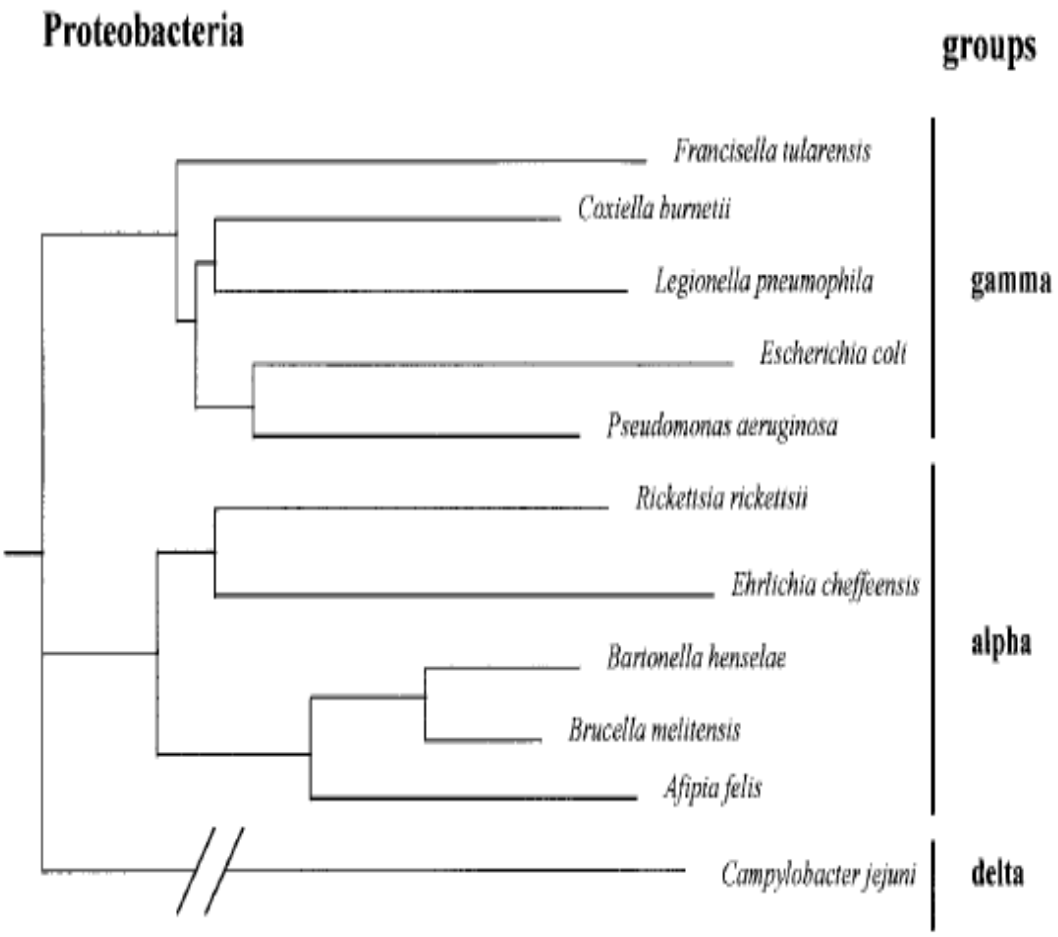

Figure 2.2 Phylogenetic tree showing the relationships of *C. burnetii* to other species belonging to the *Proteobacteria*.

The tree was constructed by the neighbour-joining method with 16S rRNA gene sequences.

Source: (Maurin & Raoult, 1999)

In a suitable eukaryotic host cell *C. burnetii* undergoes a developmental cycle in which small cell variant (SCV) and large cell variant (LCV) exist. In the host, the infecting SCV evolves to LCV that are metabolically active. The SCV and LCV are antigenically different from each other (Honarmand, 2012). The SCV attaches itself to the host cell (mainly the macrophages) and it is ingested by phagocytosis. The development of *C. burnetii* occurs in the phagolysosome, where the acidic pH activates its metabolic enzyme. Maturation of small cell variants to large cell variants occurs and sporogenesis commences. Spore formation of *C. burnetii* explains its success as a pathogen (Fournier *et al*., 1998).

An important immunological characteristic of *C. burnetii* is its antigen variation known as the phase variation (phase I and II). This process is due to the partial loss of the lipopolysaccharide (LPS) and is similar to the smooth to rough transition of the family *Enterobacteriaceae*. The LPS represents a major virulent determinant of the *C. burnetti* (Hackstadt, 1990)**.** When freshly isolated from animals and humans, phase I antigen exist, which is highly infectious and it represents the smooth LPS. Phase I LPS has an extended carbohydrate structure, that sterically blocks access of antibodies to the surface of proteins (Hackstadt, 1990). However, after continued sub-culturing in cells or embryonated egg the LPS results in an antigenic shift to phase II, which is less infectious. Phase II LPS structure is more accessible to antibodies and it represents the rough LPS. Lipopolysaccharide structure seems to be the only antigen and immunogen differing between phase I and II in *C. burnetii* (Amano & Williams, 1984). This antigenic distinctive feature is extremely valuable for the serological differentiation between acute and chronic Q fever.

Immunological response and clinical characteristic of Q fever infection resembles other infections of intracellular microorganisms. During an acute infection, the host immune

response is directed mainly to *C. burnetii* phase II antigen and a cellular response results in immunity. In Q fever infection, relapses occur and this is because immunity does not last.

Heterogeneity among *C. burnetii* is low, however, variation in the LPS have been described. Pulse – field gel electrophoresis (PFGE) and Restriction Fragment Length Polymorphism (RFLP) have been used to delineate about 20 different genotypes (Fournier *et al*., 1998). Genomic groups I, II and III are referred to as acute strains because they have been associated with animal or human acute isolates whilst groups IV and V are referred to as chronic strains because they are associated with human Q fever endocarditis isolates.

The bacterium is resistant to extreme environmental conditions due to its extracellular spore – like life form. It can survive up to 10 months at 15-20°C, for up to 1 month on meat in cold storage and more than 40 months in skimmed milk kept at room temperature (Christie, 1987). It has a low infection dose (even a single organism can cause an infection). Due to its environmental stability, widespread availability and low infective dose, the Centre for Disease Control and Prevention Atlanta has classified *C. burnetii* as a group B organism (a potential bioterrorist agent) (Madariaga *et al*., 2003).

The genomic size of *C. burnetii* ranges from 1.5 – 2.4Mbp. The genome of the bacteria contains two single circular plasmids, a chromosomal circular plasmid (measuring 1,995,281 base-pairs) and a QpH1 circular plasmid (measuring 37,393 base-pairs) (Seshadri *et al*., 2003). In the chromosome 1,022 genes codes for proteins of known functions and 179 gene codes for proteins of unknown functions. There are 3 and 42 stable rRNAs and tRNAs respectively. In addition, the chromosome contains 42.4% G+C contents and approximately 89.9% codes for proteins. In the QpH1, 11 genes codes for functional proteins and 5 proteins of unknown functions. No stable ribonucleic acids (RNAs) are found in this plasmid. The

G+C content is 39.3% and 78.8% codes for proteins. The genome also contains about 29 insertion sequences (IS), this a remarkably high number because other obligate bacteria are known to have very few or none of these element or sequences.

The target sequences for nucleic acid detection originates from singular chromosomal genes like *com1* or *htpB,* on plasmids (QpH1, QpRS) or on the transposase gene of insertion element IS*1111* (Hoover *et al*., 1992) that is present in 20 copies in the genome of the *C. burnetii* Nine Mile RSA493 strain (Seshadri *et al.,* 2003)**.**Based on the high copies of IS*1111* element present in *C. burnetii* the corresponding PCR nucleic acid detection is highly sensitive. Also the *icd* gene (isocritrate dehydrogenase) has been sequenced in 19 strains and shows to be a conserved region (Nguyen & Hirai, 1999). These conserved sequences in *C. burnetii* forms the molecular basis for diagnosis.

2.3 Historical Background of Q fever**.**

The disease was first described in 1935 after an outbreak of fever of unknown origin amongst abattoir workers in Brisbane, Queensland, Australia. The name was suggested as Q fever "Q" which means Query by Edward Holbrook Derrick the then director of the Laboratory of Microbiology and Pathology of the Queensland Health Department at Brisbane in his classic paper (Derrick, 1983). In this investigation, Derrick speculated that the aetiological agent was a virus because he failed in his attempt to isolate it from infected guinea pigs. He sent some infectious material to Macfarlane Burnet and his associate Mavis Freeman, who were able to reproduce the disease in animals such as mice, guinea pigs and monkeys. Burnet and Freeman were able to visualize numerous small rods which seem rickettsial in nature when stained with Castaneda's method or Giemsa, and made them to hypothesize the rickettsial origin of the disease (Burnet & Freeman, 1937). The information derived by Derrick and his collaborators was used to investigate the epidemiology of the disease. They concluded that

wild animals were the natural reservoir, domestic animals were the secondary reservoirs and the disease could be transmitted by arthropods such as ticks.

Independently of Derrick and collaborators works in 1935, Gordon Davis at the Rocky Mountain Laboratory in Hamilton, Montana, USA whilst studying the ecology of Rocky Mountain spotted fever was able to establish febrile illness in guinea pigs which were fed on by ticks collected from Nine Mile, Montana. Symptoms such as lack of testicular swellings, was not suggestive of Rocky Mountain spotted fever which was being studied at the time. The agent lacked the ability to grow in axenic media. In 1936 Herald Rea Cox joined Davis to study the Nine Mile agent and in 1938 Cox successfully grew the infectious agent in embryonated eggs (Cox & Beli, 1939). In 1938 Rolla Eugene Dyer was able to establish a definitive link between Nine Mile agent and the Australian Q fever agent that the two aetiological agents were one and the same.

Rickettsia burnetii was the first name given to the aetiological agent of Q fever, however in 1938 the formation of a new genus "*Coxiella*" was proposed by Cornelius Philip and the renaming of the aetiological agent as *Coxiella burnetii,* to acknowledge both the work of Burnet and Cox, who discovered the novel rickettsia agent.

2.4 Clinical Manifestation

Symptom of the Q fever infection ranges from mild to subclinical. Clinical symptoms differ from individual to individual and also from one environment to another. Incubation period ranges from 2 – 6 week (-40 days) and there is no clear clinical picture during acute infection because symptoms may resemble infection of any infectious agent. Hence during acute infection epidemiological data is a very important diagnostic clue. In most reported cases two out of three patients report contact with a reservoir of the aetiological agent. Q fever has self -

limiting febrile symptoms, which is known to last 1 – 3 weeks. This form is usually followed by a sudden onset of fever with temperature of about 40°C, headaches, fatigue and myalgia. In Spain, self – limiting febrile syndromes have been demonstrated in about 20% of febrile episodes due to Q fever (Honarmand, 2012). The duration of fever usually increases with age. Atypical pneumonia is another frequently observed symptom and this is a major clinical manifestation of acute Q fever infection in countries like Canada (Nova Scotia), Switzerland and Spain (Basque country) (Fournier *et al*., 1998). Studies conducted in Nova Scotia in Canada within a five year period, showed 3.7% of patients with community acquired pneumonia were due to *C. burnetii* (Langley *et al*., 1988).Most clinical cases are asymptomatic, mild or severe, usually characterised by unproductive cough, fever and breath abnormalities. In asymptomatic patients, pneumonia can go unnoticed except a chest radiograph is performed to show the clear picture of the affected lungs. In contrast some patient present acute respiratory discomfort. A radiograph image is usually nonspecific and is similar to infection caused by viruses, *Mycoplasma pneumoniae and Chlamydia pneumoniae*. The duration of symptoms normally ranges from 10 to 90 days and depending on the series of infection mortality ranges from 0.5 – 1.5% (Dupont *et al.*, 1995; Derrick, 1983).

In some cases hepatitis may occur when the liver gets infected with the bacteria. Hepatitis caused by *C. burnetii* is usually revealed by increased levels of hepatic enzymes, alkaline phosphatase levels are also slightly elevated to two or three times the normal levels (Langley *et al*., 1988). Usually Q fever hepatitis is accompanied with fever and less often with abdominal pains, nausea, anorexia, vomiting and diarrhoea. Three forms of hepatitis could exist from *C. burnetii* infection. These include, clinically asymptomatic hepatitis, infectious hepatitis (like forms with hepatomegaly but rarely with jaundice) and sustained fever of

unknown origin usually characterised by granulomas on liver biopsy. In countries like Australia, France and USA (Ontario, California), this is the major clinical presentation.

Many other clinical manifestations could occur in Q fever infection. Exanthema (which could be either maculopapular or purpuric) do occur in contrast to what was formally believed. Aseptic encephalitis and or meningitis, occurs in 0.2 to 1.3% of patients with Q fever (Marrie & Raoult, 1992)and it is not commonly accompanied with coma or seizures. Pericarditis and or myocarditis could also occur in Q fever infection and it is often fatal. Haemolyticanaemia, thyroiditis, gastroenteritis, pancreatitis, lymphadenopathy resembling lymphoma, bone marrow necrosis, inappropriate secretion of antidiuretic hormone and a host of many other clinical manifestations (Honarmand, 2012).

Chronic infection may exist in approximately 5% of patients infected with *C. burnetii*. Chronic form of Q fever develops within 6 months to years after an acute disease encounter. During chronic infection the bacteria multiplies in the macrophages and permanent bacteraemia which is detected by very high levels of antibodies could be found. The heart is the most affected organ and the arteries, bones and liver may also be affected (Brouqui *et al*., 1993). Endocarditis which is the most common form of chronic Q fever infection, usually is known to occur in patients with previously damaged valvular and immunocompromised individuals (Raoult *et al*., 1993). In Lyon, France and England 3% of endocarditis is due to *C. burnetii* infection and 15% in Marseille, France (Palmer & Young, 1982). Endocarditis due to Q fever infection has an annual incidence of 0.75 cases per 1 million individuals in Israel (Siegman-Igra *et al*., 2009). Early diagnosis is crucial but more often diagnosis is delayed because of the unspecific clinical presentation, thereby increasing mortality rate. Other Q fever chronic infections include, pulmonary interstitial fibrosis, pseudotumor of the lungs,

mixed cryoglobinemia and many others have been reported in literature (Fournier *et al*., 1998).

Acute and chronic Q fever infection has been reported during pregnancy. In humans, the infection might seem asymptomatic but complications can lead to death of neonate, low birth weight, placentitis or thrombocytopenia. Miscarriage can be as a result of untreated infection during the first trimester and later during pregnancy premature delivery is likely to occur. Women infected with acute Q fever including those with asymptomatic pregnancy or those who experienced no adverse pregnancy outcomes, might be at risks of recrudescent infection during subsequent pregnancy (Maurin & Raoult, 1999). It is therefore important that pregnant women, who have been infected with Q fever during a former pregnancy, should be monitored closely to prevent recrudescent infection.

It is important to note that depending on one's immunological status infection could go unnoticed, acute or could be life threatening.

2.5 Epidemiology of Disease

The infection is geographically distributed widely (Hilbink *et al*., 1993). Identified cases of the disease are reported periodically as outbreaks. Large outbreaks of Q fever have been reported in the Netherlands from 2007 to 2010 which involved more than 3700 human cases (Honarmand, 2012; Schimmer *et al*., 2008). Large outbreaks of Q fever have also been reported in Spain *(*Cutler *et al.*, 2007), Switzerland (Langley *et al*., 1988), Great Britain (Guigno *et al*., 1992) and Berlin, Germany (Schneider *et al*., 1993).

Animals are normally the source of human infection. The reservoir for the bacterium is broad, it includes mammals and small mammals such as rodents, birds and arthropod mainly ticks. The commonest identified source of human infection is farm animals such as goats, sheep and cattle. Pets such as dogs, rabbits and cats have been demonstrated as potential sources of urban outbreaks and cats are suspected as an important reservoir of *C. burnetii* in urban areas and may be the source of urban outbreaks (Langley *et al.,* 1988). A study conducted in Canada reported that 6 to 20% of cats had anti–*C. burnetii* antibodies (Higgins & Marrie, 1990). However in Great Britain wild rats are an important reservoir for *C. burnetii* (Webster *et al*., 1995). Animals when infected shed the bacterium in their excreta and milk but large amounts of the bacterium are shed in their birthing products. Animals appear asymptomatic but the infection is reactivated during pregnancy and causes abortion and other reproduction problems. *Coxiella burnetii* is able to survive in harsh conditions for long periods of time in the environment, this might be due to the spore production of the organism (McCaul *et al*., 1991) and dust and wind may be the source of the spread of the bacteria. Infection in humans results from inhalation of contaminated aerosols from animal birthing products and animal straws. Consumption of unpasteurized raw milk could also be a source of infection (Fishbein & Raoult, 1992).

Human prevalence of *C burnetii* infection is globally unknown because symptomatic infection is similar to other infectious diseases. However, the determination of the prevalence is highly based on the physicians or researchers awareness and interest in the disease and the availability of a reliable diagnostic laboratory. In areas where the infection is extensively studied it has been observed that the prevalence can be high. In the United States about 3% of the healthy population and 10 – 20% of at–risk population have antibodies for *C. burnetii* suggesting past infection (CDC, 2008). Since 1999, Q fever became a noticeable disease in

the United States, the number of reported cases to the Center for Disease Control (CDC) increased from 17 cases in 2000 to 167 cases by 2007 (CDC, 2008).

In Southern France the prevalence of acute Q fever is 50 cases per 100,000 inhabitants and also 5 to 8% of cases of endocarditis are due to *C. burnetii* (Dupont *et al*., 1994).

Febrile illness studies conducted in some African countries have demonstrated *C. burnetii* as the causative pathogen. In Bobo-Dioulasso in Burkina Faso 5% of hospitalised patients with acute febrile illness was due to Q fever (Martens *et al*., 1994) and in northern Tanzania, 3% of paediatric and 8% of adult admissions for severe febrile illness at two referral hospitals were due to Q fever (Prabhu *et al*., 2011). Two serological studies of Q fever in Tunisia identified acute Q fever in 2% and 9% of hospital admissions respectively (OmezzineLetaief *et al*., 1997; Kaabia *et al*., 2006). In Cameroon, two studies of patients admitted for community-acquired pneumonia report a seropositive of 6% and 9% of persons aged >15 years (Koulla-Shiro *et al*., 1996; Koulla-Shiro *et al*., 1997). In these studies, Q fever was the third most common etiologic agent of pneumonia, after *Streptococcus pneumoniae* and *Mycoplasma pneumoniae*. Most recently, a study conducted in Northeast Kenya, reported a seroprevalence of 16.2% amongst febrile patients (Njeru *et al*., 2016).Survey among blood donors has shown 18.3% in Morocco (Kaplan & Bertagna 1955), 37% in Zimbabwe (Kelly *et al*., 1993), 44% in Nigeria (Blondeau *et al*., 1990), 26% in Tunisia (Letaief *et al*., 1995), 10 to 37% in the northeast Africa and 15 to 36.6% in different areas of Canada (Brouqui *et al*., 1993) had anti-*C. burnetii* antibodies. Studies in Tanzania, have shown that Q fever caused by *C. burnetii* is a more common cause of AFI than malaria (Crump *et al.*, 2013).

2.6 Risk Factors Associated with Q fever

Some factors can increase the risk of been infected with *C. burnetii*:

1. Occupation: Epidemiological studies have identified certain occupations as risk factors for Q fever. These includes; livestock farming, veterinary medicine, meat processing and animal research. The first record case of Q fever infection was amongst abattoir workers.
2. Location: Proximity to a livestock farms or a farming facility may increase one's risk of being infected because transmission occurs through the inhalation of contaminated aerosols even from a far distance. For example, during the outbreak of Q fever in 2010 in the Netherlands, the source of the outbreak was a contaminated goat farm close to the affected community.
3. Sex: Men are more likely to develop symptomatic acute Q fever infection than females (Maurin & Raoult, 1999), this may be partly explained by sex-associated occupational exposures or the protective effect of 17β – estradiol in female(a powerful female hormone that occurs naturally), which has been validated in animal models (Leone *et al*., 2004).
4. Seasonality: The infection is known to occur at any time in the year but in countries like USA the peak time of the year is April and May which coincide with increases in human outdoor activity, and with the birthing season for a number of domestic animals.
5. Recent travel to high risk areas for Q fever, such as rural, agricultural communities (international or domestic) and highly endemic areas, such as places with recent outbreaks (Netherlands and regions in the Middle East).
6. Persons who have family members or partners who has been diagnosed of Q fever.

7. Persons with history of Q fever infection might develop chronic infection especially persons with vascular graft or arterial aneurysm, valvular heart disease, immunosuppressed individuals and pregnant women.

2.7 Laboratory Diagnostic Methods

Acute Q fever infection in most persons shows nonspecific clinical symptoms. More often clinicians do not suspect Q fever during acute infection and hence disease is under-diagnosed. Although laboratory diagnosis of acute Q fever can be made on the basis of serological results, a four-fold titre rise for phase II IgG is recommended between acute (serum taken during acute infection) and convalescent (serum taken 3 to 6 weeks apart) samples for definitive diagnosis, this makes diagnosis a retrospective one. However for early diagnosis of Q fever serology in combination with PCR is recommended. Polymerase Chain Reaction (PCR) of whole blood or serum can be positive very early after symptom onset but becomes negative as the antibody titre increases and after administration of antibiotic (Anderson *et al*., 2013).

2.7.1 Serological Diagnosis

Serological tests are the commonly used methods for the diagnosis of Q fever. The complement fixation, microagglutination test, indirect immunoflourescent antibody (IFA) and Enzyme Linked Immunosorbent Assay (ELISA) have been used for the serological diagnosis of *C. burnetii* (Wegdam-Blans *et al.,* 2012). Enzyme Linked Immunosorbent Assay is preferred for practical reasons and because of their higher sensitivity (Wegdam-Blans *et al.,* 2012). A commercially available ELISA test kit (PanBio *Coxiella burnetii* ELISA QFM and QFG 200, Brisbane, Australia) has been used for the serological detection of Q fever, especially for the detection of IgM and IgG antibodies (Foucault *et al.*, 2004).

Two antigenic phases exist in *C. burnetii* (phase I and II). During acute infection phase II antibody to *C. burnetii* response appears first and is higher than phase I antibodies (Maurin & Raoult, 1999). Hence phase II antigen is important in early diagnosis of *C. burnetii* antibodies. The commonly used confirmatory diagnosis for acute Q fever is the demonstration of a four-fold titre rise in phase II IgM or IgG by serology between serum samples from acute and convalescent taken 3 -6 weeks apart. The acute serum is ideally taken during the first week of infection; more often when tested, results might be negative or too low for detection of measurable antibodies. Therefore serum samples from acute phase might not be helpfully for immediate treatment.

Typically, seroconversion occurs within 7–15 days of illness; hence 90% of patients seroconvert within 3 weeks. Immunoglobulin M (IgM) antibodies against phase II antigen is known to develop within 2 weeks of acute infection. This poses a limitation in using serology for acute diagnosis because some seropositive cases could be missed during the first few weeks of acute infection.

Antibodies can be present for many months, years or for life after infection. Despite these limitations, single serum titres are the most frequently used diagnostic criterion among cases reported in most diagnostic laboratories and this might be because clinical suspicion for Q fever is uncommon for patients who initially seek care for symptoms.

2.7.2 Nucleic Acid Detection

(a) Polymerase Chain Reaction PCR (PCR)

Highly sensitive, specific and rapid quantitative PCR methods have been developed for the detection of *C. burnetii*. Multiple gene sequence like Isocitrate dehydrogenase (*icd*), insertion sequence (IS*1111*), 16S rRNA, 23S rRNA, superoxide dismutase and others has been used

for the detection of *C. burnetii* and might differ in their sensitivity and specificity (Klee *et al*., 2006). A study conducted by Klee *et al*., (2006) evaluated the precision of *icd* and IS*1111* real-time PCR assay and concluded that the assays were shown to be highly specific, sensitive and efficiently reproducible.

Whole blood collected in anticoagulant-treated tubes or serum can be used for PCR testing. Whole blood might have a higher concentration of *C. burnetii* DNA than serum because *C. burnetii* is a strict intracellular organism, but is also likely to have more PCR inhibitors. For PCR results to be useful, the clinical sample must be obtained in the acute phase of infection (optimally during the first 2 weeks of symptom onset) and either before or shortly after (within 24–48 hours) antibiotic administration. When suitable samples are collected (i.e., during the acute infection and before or shortly after the administration of antibiotics), PCR results are positive in almost all patients with early acute Q fever before the antibody response originates (Schneeberger *et al*., 2010).

(b) Nucleic Acid Detection (Loop mediated Isothermal Amplification (LAMP)

Loop mediated isothermal amplification (LAMP) assay is a rapid DNA amplification technique first developed by Notomi, (2000)and it has been used for the detection of several *Rickettsia* pathogens (Pan *et al*., 2013). The method requires specially designed sets of primers that recognize at least six independent regions of the target genes, thereby increasing the sensitivity and rapidity of the technique. The DNA polymerase used in LAMP assay allows strand displacement-DNA synthesis. Hence, making it is an isothermal cycling method that can be performed at a single temperature around 60°–65°C. LAMP reactions are performed under isothermal conditions using a simple incubator, such as a water bath or a heating block as the source of heat. The results could be visualized by turbidity that can be seen by the naked eye; turbidity is proportional to the amount of DNA amplified(Mori *et al*., 2001). The results can also be visualized by agarose gel electrophoresis or by addition of

fluorescent dyes, such as hydroxynaphthal blue (HNB) and visualized under UV light (Qiao *et al*., 2007; Tomita *et al*., 2008). Hence this assay can be deployed as a point-of-care diagnosis technique in low income laboratory settings for easy and quick diagnosis of Q fever.

LAMP assay has been designed by Chen & Ching, (2014) to detect the presence of *C. burnetii* in plasma and have proved to be highly sensitive, producing 25 copies of bacterial DNA (equivalent to one organism).

2.8 Treatment and Prevention of Q fever Infection

2.8.1 Treatment

Early administration of antibodies is essential in the effective management of Q fever. Doxycycline (a class of tetracycline) is the drug of choice for the management of Q fever in all age groups. In the absence of doxycycline and in cases of allergic reaction the drugs of choice aremoxifloxacin, clarithromycin, trimethoprim/sulfamethoxazole, and rifampin(Anderson *et al*., 2013).It is important to initiate treatment immediately Q fever is clinically suspected even before laboratory results are obtained. Treatment within the first three days of disease and fever automatically subsides within 72 hours. Failure to respond to doxycycline indicates that patient's condition might not be Q fever. It is also important to note that severely ill patients may require a longer period for the fever to resolve. The recommended dose of doxycycline is 2.2 mg/kg per dose twice daily for 5 days (that is a maximum of 100 mg per dose). If Q fever is diagnosed and patient remains febrile after 5 days of treatment, trimethoprim/ sulfamethoxazole 4 – 20 mg/kg twice daily for a maximum of 14 days (that is a maximum of 800 mg per dose) may be administered. Infected pregnant women should be treated with trimethoprim/ sulfamethoxazole with dose of 160mg/ 800mg twice daily throughout pregnancy (CDC, 2008). In chronic Q fever, the recommended

treatment for adults is 100 mg of doxycycline every 12 hours and hydroxychloroquine 200mg every 8 hours. The standard duration for treatment is 18 months (CDC, 2008).

Treatment should never be withheld pending receipt of diagnostic results or discontinued because serological or PCR results are negative. It is important to note that antibodies might remain detectable for months to years after infection, treatment should not be provided based solely on elevated titres (such as those detected through routine screening or baseline occupational assessments) without clinical manifestation of acute illness (e.g., fever, pneumonia, hepatitis, or other acute symptoms).

2.8.2 Prevention

Outbreaks of Q fever infection have happened mainly from occupational exposure involving meat processing plant workers, researchers at facilities housing animals, veterinarians, dairy workers and livestock farmers. Prevention effort of Q fever should be made primary towards these groups and environment. A variety of control and prevention measures should be carried out:

1. The public should be educated about the source of infection
2. Hand washing should be practiced after working with animals.
3. *Coxiella burnetii* is known to be in high amounts in birthing products (such as, placenta, foetal membrane, aborted foetuses, etc) of infected animals, hence the appropriate disposal of such products.
4. Milk and milk products should be pasteurized before consumption.
5. Imported animals should be quarantined to ensure new animals are not infected with the infection.

6. Ensure animal holding facilities are not located in close proximity to populated areas. Routine testing of animal for *C. burnetii* antibodies should be conducted and measures should be ensured to prevent airflow to populated areas.
7. Persons at high risk of developing chronic Q fever should be counselled especially those with pre-existing cardiac diseases.
8. At-risk populations should be vaccinated. In Australia and some European countries there is an available vaccine which has aided in the protection of humans and animal against *C. burnetii*. However, there is no commercially available vaccine for Q fever infection in most countries in the world including the United States.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

This was a cross-sectional study, which involved the recruitment of febrile patients who sought healthcare at both the in and out patient's departments at the selected healthcare facilities [the 37 Military Hospital, Accra, (Naval sick bay, 2 Medical Reception Station (2MRS) (Army Forces clinic) and Air Force Medical Centre) in Sekondi-Takoradi)]. The study was conducted over a one year period as part of an ongoing larger study, titled 'Acute Febrile Illness Military to Military' at NMIMR.

3.2 Study sites

The study was carried out at the 37 Military Hospital and three military clinics at Sekondi-Takoradi. The 37 Military Hospital located in Accra is one of largest hospital in Ghana. It has a bed capacity of 400, and is both a teaching and referral hospital in the Accra Metropolitan District of the Greater Accra Region and sees a highly diverse patient population including both military and civilian patients living around the hospital and all over Accra. The Hospital has a polyclinic and an emergency care unit for outpatient cases. There are about 14 wards including an Intensive Care Unit (ICU) in the hospital.

The clinics at Sekondi-Takoradi included the Naval sick bay located at Global Positioning System (GPS) quadrant N 04° 56.188' and W 001° 42.438' Sekondi, 2 Medical Reception Station (2MRS) (Army Forces clinic) located at GPS quadrant N 04° 54.858' and W 001° 48.339' Takoradi, and the Air Force Medical Centre located at GPS quadrant N 04° 53.628' and W 001° 46.373' Takoradi. These healthcare facilities are mainly on-outpatients basis but may detain patients for few hours depending on the severity of the disease, hence, forming a

minor in-patient department. They mainly serve the military and their families but also attend to the civilian population.

These sites were chosen because of their participation in an on-going AFI surveillance study. Furthermore, the funding institution for the study [Global Emerging infectious Surveillance (GEIS)] supports only military surveillance research.

3.3 Recruitment of study participants

All patients with febrile illness seeking medical care at the 37 Military Hospital in Accra and the three military clinics at Sekondi-Takoradi were eligible for participation in the study. Upon reporting, individuals were consented for their participating in the study. Participants were given consent forms to complete either on their own or were assisted by a research assistant. In the case of participants below the age of eighteen years, a child consent form with an additional parental/guardian consent was administered. Questionnaires were administered to participants to capture basic demographic data and clinic/visit information to determine possible risk factors. Participants were allocated a unique study number to ensure confidentiality. No names or identifiers linking participants to data were included on the questionnaire.

3.3.1 Case definition

Case definitions for febrile illness was a patient presenting with fever ≥ 38°C without a localizing aetiology (i.e. otitis media, meningitis, diarrheal syndromes).

3.3.2 Inclusion and exclusion criteria

The inclusion criteria were patients:

- Who were within the ages of 5-65 years
- Having documented fever of ≥38°C at presentation to the hospital or clinics
- Who agreed to consent to the study

The exclusion criteria included patients with evidence of underlying infections such as:

- Urinary tract infection
- Cellulitis
- Otitis media
- Septic arthritis
- Pyogenic soft tissue infection and
- Other obvious localized source of infection.

Informed consent (or parental consent and child assent for participants under 18 years of age) was obtained from participants who met the Acute Febrile Illness case definition, and participants were asked to complete the demographic and epidemiologic questionnaires. Subsequently, their laboratory specimens (venous blood) were acquired by a trained phlebotomist.

Overall, one hundred and seventeen (117) consecutive patients were enrolled.

3.4 Ethical Clearance

Ethical approval for the larger study was obtained from Ghana Health Service Ethical Review Committee (GHS-ERC), Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB) (Appendix III), and for this study ethical approval was obtained from the Ethical and Protocol Review Committee of the School of Biomedical and Allied Health Science, University of Ghana (Appendix IV).

3.5 Sample collectionand processing

For all inpatients/outpatients, 2-10 ml (depending on the age of the patients) intravenous blood was collected from consenting individuals at the healthcare facilities using sterile disposable needles and syringes and dispensed into vacutainers.

Serum was obtained by centrifuging the blood sample at 10,000 rpm (revolution per minute) for 10 minutes. Serum was then stored at -20°C at Noguchi Memorial Institute for Medical Research (NMIMR) for further laboratory analysis.

In the AFI laboratory housed within NMIMR, the samples (serum) were further tested for Q fever (*C. burnetii*), using serology (phase II Enzyme Link Immunosorbent Assay ELISA), PCR and LAMP assays.

3.6 Laboratory procedures

3.6.1.1 Q fever Diagnosis

Serology

Serum was tested by ELISA for the presence of *C. burnetti* antibodies (IgM and past IgG) using Panbio *Coxiella burnetii* IgM and IgG commercial ELISA kit with all procedures performed according to Manufacturer's instructions.

The test samples, controls (positive and negative controls) were diluted in suitable microtitre plates and the calibrator reagent (which was diluted in triplicates). This was done by adding 90µl of sample diluent to 10µl serum, after which 20µl of the diluted serum was added to 180µl sample diluent and mixed well. One hundred microlitres (100µl) of the diluted sample, controls and the calibrator reagent were then pipetted into their respective microwells, covered and incubated for 30 minutes at 37 ºC ± 1ºC. After the incubation period was over, the plate was washed six times with diluted wash buffer. In each well, 100µl of HRP conjugated anti-human IgG or HRP conjugated anti-human IgM was added for the IgG and IgM assays respectively. The plate was then covered and incubated for 30 minutes at 37 ºC ± 1ºC, after which it was washed six times using the wash buffer. One hundred microlitres (100µl) of TMB was pipetted into each well and incubated for 10 minutes at room

temperature (20-25ºC), timing from the first addition. A blue colour was developed. Stop solution (100µl) was then added to all the wells in the same sequence and timing as the TMB addition. After mixing well, the blue colour changed to yellow, the absorbance of each well was read within 30 minutes using an ELISA reader (BioTek ELx808) at a wavelength of 450 nm with a reference filter of 600-650 nm.

Calculation and interpretation of assay results

To determine the cut-off value, the average absorbance of the triplicates of the calibrator was first calculated and multiplied by the calibrator factor (which is found on the calibrator vial in the kit). The index value was then calculated by dividing the sample absorbance by the cut-off value as shown below;

$$\textbf{Index value} = \frac{\boldsymbol{Sampleabsorbance}}{\boldsymbol{Cut-offvalue}}$$

The panbio units were calculated by multiplying the index value obtained by 10.

Panbio unit = Index value x 10

Table 3.1: Interpretation of ELISA results

Index value	Panbio units	Results
<0.9	<9	Negative
0.9 – 1.1	9 - 11	Equivocal
>1.1	>11	Positive

3.6.2 Molecular Techniques

DNA extraction

DNA for both PCR and LAMP assays were extracted from serum. DNA was extracted from the serum samples using chelex method as described by Woodene*t al.*,(1993). All procedures were carried out according to the manufacturer's instructions.

DNA extraction from serum using chelex method:

Fifty microlitres (50µl) of serum was transferred into a 1.5 ml eppendorf tube, after which 1ml of 1x PBS (pH 7.4) and 50µl of 10% saponin were added. The tube was inverted several times to mix the solution and then incubated at 4ºC for overnight. After incubation, the tube was centrifuged at 8000 rpm for 1 minute and the supernatant (PBS and saponin) aspirated from the tube. Subsequently, 1 ml of 1x PBS was added, and the tube inverted several times to mix the solution before incubating at 4ºC for 15-30 minutes. The tube was then centrifuged and the supernatant discarded. About 50µl of sterile distilled water was then added to the tube, followed by 50µl of 20% chelex, and the tube vortexed and incubated at 95ºC for 10 minutes and vortexed at 2 minutes intervals. After incubation the tube was centrifuged for 5 minutes at 12000 rpm, and the supernatant transferred to a clean and sterile 0.5 ml Eppendorf tube, making sure the chelex was not carried over. The DNA was quantified using a Thermo Scientific Nanodrop 2000 Spectrophotometer (appendix II). The resulting DNA was then stored at -20ºC until further use.

3.6.3 Nucleic Acid Amplification (Detection and identification)

3.6.3.1 Real Time Polymerase Reaction

DNA extracts from the samples were used in a 25µL rt-PCR reaction mix. The rt-PCR mix constituted Taqman Universal PCR Master Mix (6.25 µM), nuclease free water (11.0 µM), Cox-F primer (0.3µM), Cox-R primer (0.3µM), probe (0.1µM), 50mM $MgCl_2$ (1.0 µM and a template DNA (5µl). Two rt-PCR assays were employed in the detection of *C. burnetii*

infection in the samples. The *icd* assay targeted a 76 bp fragment of the *C. burnetii icd* gene whereas the IS*1111* assay targeted a 295 bp fragment of the transposase gene of the *C. burnetii*IS*1111a* element present within the *C. burnetii* genome(Klee *et al.*, 2006). Vircell *C. burnetii* DNA control [(which was commercially purchased) (1/10 dilution)] was used as a positive control and nuclease free water was used as negative control. The primers and probes used are as listed in Table 3.2.

Table 3.2: Primers and probes used

The *icd* assay
Primers: forward, *icd*-439F = CGTTATTTTACGGGTGTGCCA (439–459) reverse, *icd*-514R = CAGAATTTTCGCGGAAAATCA (494–514) TaqMan probe: icd-464TM = FAM-CATATTCACCTTTTCAGGCGTTTTGACCGT-TAMRA-T (464–492).
The IS*1111*assay
Primers: forward, Cox-F = GTCTTAAGGTGGGCTGCGTG (219–238) reverse, Cox-R = CCCCGAATCTCATTGATCAGC (493–513) TaqMan probe: Cox-TM = FAM-AGCGAACCATTGGTATCGGACGTTTAMRA-TATGG (259–287).

(Klee *et al*., 2006)

Amplification was carried out in a 7300 Real time PCR system (Applied Biosystems) as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles at 15 s 95°C and 30 s at 60°C. Safety precautions were strictly followed to prevent cross contamination during the PCR analysis. All procedure employed was as describe by Klee *et al.*,(2006).

3.6.3.2 Loop Mediated Isothermal Amplification (LAMP) Assay.

The LAMP assay was performed according to an in-house protocol from the Naval Medical Research Center (NMRC) Maryland USA(Chen & Ching, 2014).The assay was evaluated using 5 sets of primer mixture, which were obtained from NMRC USA at the protein chemistry laboratory. A 1:100 dilution was done from the provided stock positive control and a 4 fold serial dilution was also done. Oligonucleotide primers used for the LAMP assays were designed based on the transposase gene insertion element IS*1111* of *C. burnetii.* Sterile 0.2µlPCR tubes were labelled and a heating block set up to 60ºC. The reaction mix for all samples was prepared, vortexed and span down. It constituted 2x buffer (12.5µl), primer mix (1.2µl), Bst (1.0µl), and water (5.3µl) in a total reaction mix of 20µl. Twenty microlitres (20µl) of the reaction mix was then dispensed into each of the 0.2µl PCR tubes and 5µl of DNA extract was added and tubes were closed tightly. Five microlitres of nuclease free water was added to the negative control tube whiles 5µl of positive control into the positive control tube. All the tubes were then placed on a dry heating block for 60 minutes at 60ºC. After the incubation period the tubes were span briefly and placed on ice. Constituents of the 2x buffer can be found in appendix I.

Table 3.3: Primer sequence

	Primer sequence
QF3-F3	GTGGCAAAAGCCAATGAGG
QF3-B3	CCGCGTTTACTAATCCCCAA
QF3-FIP	GCATAAACCGAGAGCGCCGTTATTGTCAACGGGTA CAGAGC
QF3-BIP	TCATCGTTCCCGGCAGTTGTCCACCTCCTTATTCCC ACTCG
QF3-LB	GGGTTGGTCCCTCGACAACAT

(Chen and Ching, 2014)

3.6.3.3Gel electrophoresis

To prepare the gel, 100ml of 1X Tris Acetate EDTA (TAE) buffer was measured into a conical flask and 2g of agarose powder was weighed and added to the buffer. The suspension was then heated (about 90º C) in a microwave to get a uniform solution and allowed to cool before being transferred into a gel cast system. The cast gel was then transferred into a gel tank filled with 1XTAE buffer. 10x blue loading buffer (0.5µl) of was mixed thoroughly with 5µlof the LAMP products (positive and negative controls) and samples on a sterile parafilm before carefully loading into the respective gel wells. After loading the 100bp ladder, the gel was allowed to run for about 45 minutes and visualized under a UV Transilluminator (Model TM-20) and photographed.

3.7 Statistical Analysis

All the laboratory results were documented using Microsoft word and Excel 2010.The STATA software version 13 was used to analyse the data collected. For the test of association, Fisher's exact test and chi square were used for test of association. The risk factors were analyzed using multiple logistic regression. Significance level was set at a P value < 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic and clinical features of study participants.

A total of 117 febrile patients attending the selected health facilities were enrolled in the study. Out of these 33 were in patients and 84 out patients. The details of their socio-demographic characteristics are summarized in Table 4.1. The study participants comprised 64 (54.70%) males and 53 (45.30%) females, and the age group with the highest enrolment was 25-34 years. The most common occupation among the study participants was 'student' [n=45 (38.46%)], followed by 'government workers' (which comprised of military personnel and civilians [n=30 (25.64%)]. Whereas 42 (35.99%) of the participants had tertiary education 4(3.42%) were illiterates. Majority of the study participants [n=63 (53.84%)] were Akans compared to other tribes in Ghana. This supports the population distribution of Akans in Southern Ghana.

Figure 4.1 summarizes the symptoms reported by study participants. The most common clinical symptom among the study participants was fever (n=114) followed by headaches (n= 106), chills (n= 90), joint and muscle pain (n= 69 and n=57 respectively).

Table 4.1: Socio-demographic characteristics of study participants.

Variable	Frequency	Percentages (%)
Sex		
Male	64	54.7
Female	53	45.3
Age group (Years)		
05-14	24	20.51
15-24	27	23.08
25-34	33	28.2
35-44	19	16.24
>45	14	11.97
Total	117	100
Occupation		
Unemployed	6	5.13
Student	45	38.46
Trader	8	6.84
Government Worker	30	25.64
Private Company Worker	6	5.13
Other	22	18.8
Education		
No school	4	3.42
Primary	22	18.8
JHS	20	17.09
SHS	29	24.79
Tertiary	42	35.9
Ethnicity		
Akan	63	53.84
Ga/Dangme	10	8.55
Ewe	25	21.37
Northerners	9	7.69
Other	10	8.55

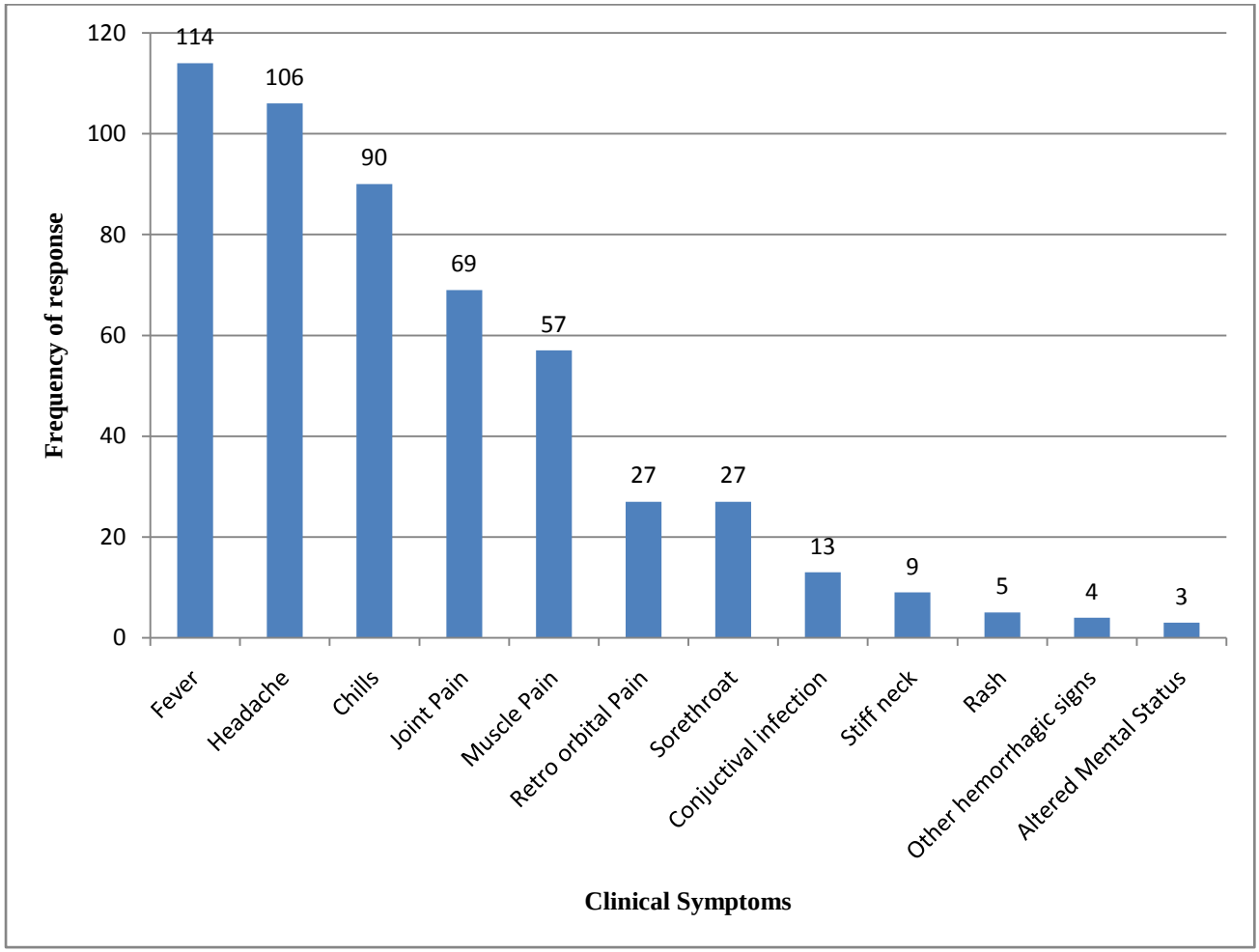

Figure 4.1: Symptoms reported by study participants

4.2 Prevalence of *Coxiella burnetii* infection

Nineteen (16.24%) out of 117 study participants tested seropositive to phase II *Coxiella burnetii* antibodies (IgM and IgG) by Enzyme-Linked Immunosorbent Assay (ELISA) (Table 4.2). Positive cases were identified by either the presence of IgM or IgG antibodies. These included 8 patients who were positive for IgM, 13 patients who were positive for IgG and 2 patients who were positive for both IgM and IgG. Eighty-four study participants were out-patients, and of these, 6 (7.14%) tested seropositive for IgM whilst 11 (13.10%) tested seropositive for IgG. Of the 33 in-patients, 2 (6.06%) were sero-positivity for IgM and IgG. The distribution of *C. burnetii* antibody in relation to the health facilities as well as the type of patients (out-patient and in-patients) is summarized in Table 4.3.

Table 4.2: Overall seroprevalence of *C. burnetii* among study participants.

	IgM / IgG(%)	IgM(%)	IgG(%)	IgG & IgM(%)
No. tested	117	117	117	
No. positive	19 (16.24)	8 (6.84)	13 (11.11)	2 (1.71)

Table 4.3: Seroprevalence of *C. burnetii* in relation to healthcare facilities and patient type.

	IgM			IgG		
Parameters	No. tested	Positive (%)	P value	No. tested	Positive (%)	P value
Health facilities and location						
37 Military Hospital (Accra)	78	7 (8.97)		78	10 (12.82)	
Military Clinics (Sekondi-Takoradi)	39	1 (2.56)	0.266	39	3 (7.69)	0.54
Type of patients						
Out-patients	84	6 (7.14)		84	11 (13.10)	
In-patients	33	2 (6.06)	1	33	2 (6.06)	0.346
Overall	117	8 (6.84)		117	13 (11.11)	

4.3 Association of *C. burnetii* with predisposing factors

4.3.1 Association of *C. burnetii* infection with socio-demographic, clinical and other features of the study participants.

The relationship between seroprevalenceof *C. burnetii* and the socio-demographic features of study participants is summarized in Table 4.4. Twelve (22.64%) females and 7 (10.94%) males were seropositive for *Coxiella burnetii* infection. Age group The highest and least number of seropositive were observed in age groups 15-24 years and 45 years and above respectively [n=2 (14.29%)]. People with no formal education recorded a high seropositivity (50.00%) as compared to those with Tertiary education (11.90%). In the terms of tribe, Northerners had the highest proportion of seropositives (44.44%), while unemployed participants had the highest proportion of seropositives (66.67%) in terms of occupation. In the bivariate analysis of the socio-demographic status of the study participants, only occupation showed a significant association with *C. burnetii* infection (P value = 0.048).

Table 4.5 summarizes the association of *C. burnetii* with clinical features of study participants. Seropositivity to *Coxiella burnetii* was highest in participants who reported to the healthcare facilities with a fever (19/114), followed by headache (18/106), and chills (15/90) and sore throat (1/27). However, there was no association between *C. burnetii* infection and any clinical feature.

A large number of study participants (71/117) were not exposed to any animal (Figure 4.2). Of the 46 participants exposed to animals, most were exposed to cats [28 (60.87%)] whilst the least exposure was to donkeys [1 (2.08%)].The association of *C. burnetii* infection with exposure to several animals is summarized in Table 4.6. Seropositivity was high for patients exposed to cats [6/28 (21.43%)], dogs [3/15 (20.00%)] and sheep [1/6 (16.67%)] though in all cases, this proved insignificant (P=0.391, P=0.709 and P=1 respectively). No seropositive was detected in participants who had been exposed to cattle, donkeys, pigeons and ducks.

Nineteen of 98 (19.39%) study participants who were not exposed to someone with a similar illness were seropositive for *Coxiella burnetii* infection. However, all the 19 (100%) study participants who were exposed to someone with a similar illness were seronegative. The association between *C. burnetii* and exposure to someone with similar illness was significant (P value = 0.04). Of the 33 respondents who had noticed any rodent inside or around the household, 6 (18.18%) were seropositive.

Table 4.4: Association between *Coxiella burnetii* seropositivity and socio-demographic features

Variable	Negative (%)	Positive (%)	P value
Sex			
Male (n=64)	57(89.06)	7 (10.94)	0.13
Female (n=53)	41 (77.36)	12 (22.64)	
Age (Years)			
5-14 (n=24)	20 (83.33)	4 (16.67)	
15-24 (n=27)	21 (77.78)	6 (22.22)	
25-34 (n=33)	30 (90.91)	3 (9.09)	0.659
35-44 (n=19)	15 (78.95)	4 (21.05)	
45 and above (n=14)	12 (85.71)	2 (14.29)	
Education			
No school (n=4)	2 (50.00)	2 (50.00)	
Primary (n=22)	18 (81.82)	4 (18.18)	
JHS (n=20)	17 (85.00)	3 (15.00)	0.389
SHS (n=29)	24 (82.76)	5 (17.24)	
Tertiary (n=42)	37 (88.10)	5 (11.90)	
Ethnicity			
Akan (n=63)	51 (80.95)	12 (19.05)	
Ga/Dangme (n=10)	10 (100)	0 (0)	
Ewe (n=25)	23 (92.00)	2 (8.00)	0.075
Northerners (n=9)	5 (55.56)	4 (44.44)	
Other (n=10)	9 (90.00)	1 (10.00)	
Occupation			
Unemployed (n=6)	2 (33.33)	4 (66.67)	
Student (n=45)	39 (86.67)	6 (13.33)	
Trader (n=8)	6 (75.00)	2 (25.00)	0.048*
Government worker (n=30)	27 (90.00)	3 (10.00)	
Private worker (n=6)	5 (83.33)	1 (16.67)	
Other (n=22)	19 (86.36)	3 (13.64)	

***indicates a statistically significant P value**

Table 4.5: Association between *Coxiella burnetii* seropositivity and clinical symptoms

Variable	Frequency	Positive (%)	P value
Fever			
No	3	0	1
Yes	114	19 (16.67)	
Headache			
No	11	1 (9.09)	0.69
Yes	106	18 (16.98)	
Chills			
No	27	4 (14.81)	1
Yes	90	15 (16.67)	
Joint Pain			
No	48	7 (14.58)	0.801
Yes	69	12 (17.39)	
Muscle Pain			
No	60	9 (15.00)	0.804
Yes	57	10 (17.54)	
Retro orbital Pain			
No	90	13 (14.44)	0.376
Yes	27	6 (22.22)	
Sore throat			
No	90	18 (20.00)	0.07
Yes	27	1 (3.70)	
Conjuctival infection			
No	104	18 (17.31)	0.69
Yes	13	1 (7.69)	
Stiff neck			
No	108	16 (14.81)	0.161
Yes	9	3 (33.33)	
Rash			
No	112	18 (16.07)	1
Yes	5	1 (20.00)	
Other hemorrhagic signs			
No	113	18 (15.93)	0.513
Yes	4	1 (25.00)	
Altered Mental Status			
No	114	18 (15.79)	0.415
Yes	3	1 (33.33)	

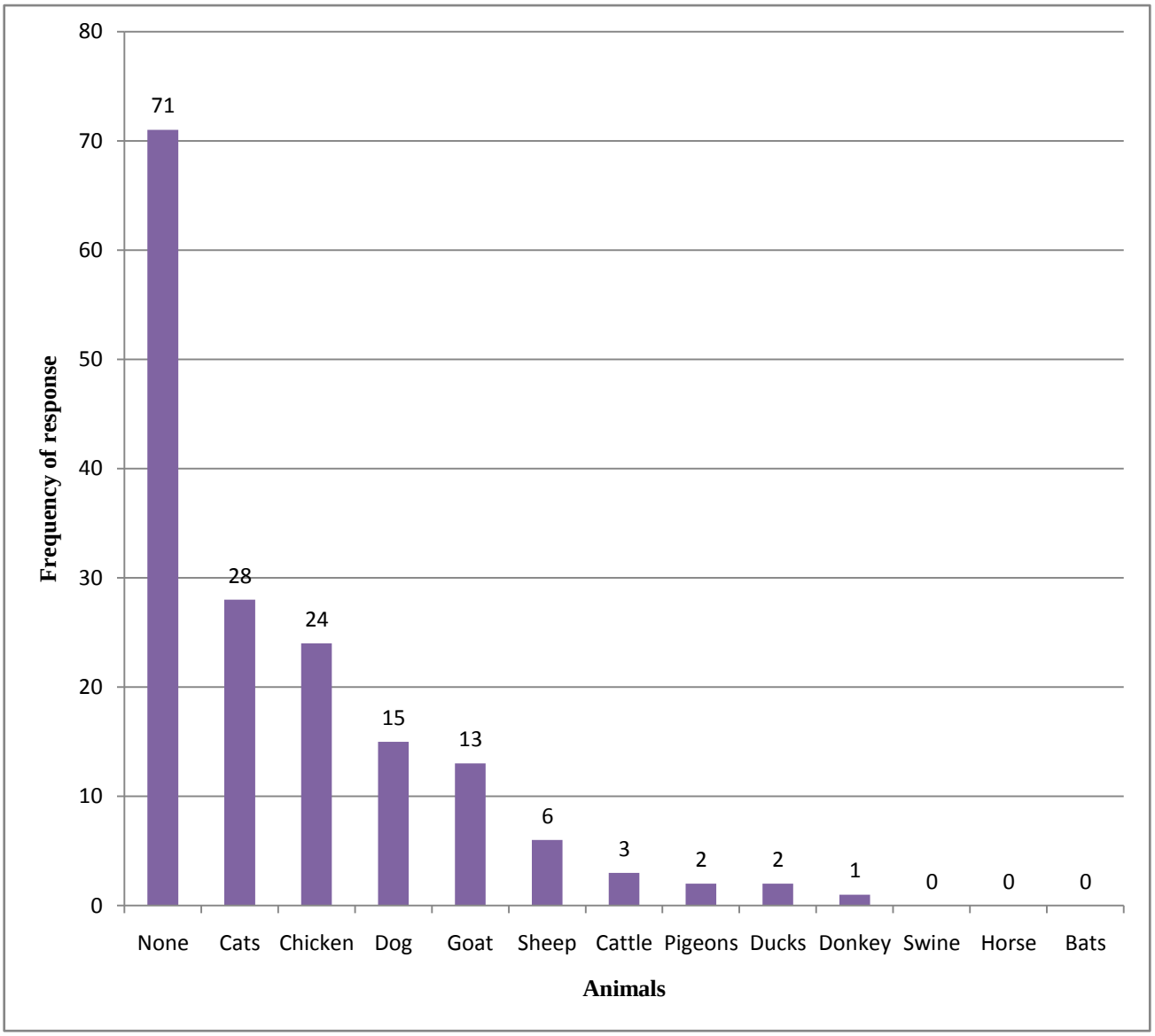

Figure 4.2: Exposure of study participants to animals

Table 4.6: Association between *Coxiella burnetii* seropositivity and animal exposure

Variable	Frequency	Positive (%)	P value
None			
No	46	8 (17.39)	0.802
Yes	71	11 (15.49)	
Cats			
No	89	13 (14.61)	0.391
Yes	28	6 (21.43)	
Chicken			
No	93	18 (19.35)	0.117
Yes	24	1 (4.17)	
Dog			
No	102	16 (15.69)	0.709
Yes	15	3 (20.00)	
Goat			
No	104	18 (17.31)	0.69
Yes	13	1 (7.69)	
Sheep			
No	111	18 (16.22)	1
Yes	6	1 (16.67)	
Cattle			
No	114	19 (16.67)	1
Yes	3	0 (0)	
Pigeons			
No	115	19 (16.52)	1
Yes	2	0(0)	
Ducks			
No	115	19 (16.52)	1
Yes	2	0(0)	
Donkeys			
No	116	19 (16.38)	1
Yes	1	0 (0)	

Table 4.7: Association between *Coxiella burnetii* seropositivity and other forms of exposure

Variable	Number	Positive (%)	P value
Has the patient been involved in the slaughter of animals?			
No	111	19 (17.12)	0.587
Yes	6	0 (0)	
Has the patient been exposed to animal abortus?			
No	116	18 (15.2)	0.162
Yes	1	1 (100.0)	
Has the patient consumed raw or unpasteurized milk product?			
No	112	18 (16.07)	1
Yes	5	1 (20.00)	
Has the patient noticed any rodent inside or around the household?			
No	84	13 (15.48)	0.781
Yes	33	6 (18.18)	
Was the patient in contact with someone with a similar illness?			
No	98	19 (19.39)	0.04*
Yes	19	0 (0)	

***indicates a statistically significant P value**

4.3.2 Risk factor analysis for Q fever by logistic regression

A multiple logistic regression analysis was used to analyze the risk of *C. burnetii* infection with occupation, clinical symptom and animal exposure. In the multiple logistic regression analysis model illustrate in Table 4.8, occupation remained a significant risk factor, with students (OR 0.047, 95% CI 0.005 to 0.541), government workers (OR 0.029, 95% CI 0.002 to 0.397) and other forms of employment (OR 0.046, 95% CI 0.003 to 0.610) showing relevant P values of 0.014, 0.008 and 0.02 respectively. However, the likelihood of an unemployed person having Q fever is higher than any other occupation. Though there appear to be a relation between *C. burnetii* infection and exposure to chicken, this was not significant (P value = 0.057) when added to the multiple logistic models. The model had a significant strength of 0.0072 (P value).

Table 4.8: Multiple logistic regression

Variable	Standard Error	Odds Ratio	95% Conf. Interval	P value
Occupation				
Unemployed	Reference			
Student	0.059	0.047	0.004 - 0.541	0.014*
Trader	0.155	0.107	0.006 - 1.834	0.123
Government Worker	0.039	0.029	0.002 - 0.397	0.008*
Private Company Worker	0.114	0.07	0.003 - 1.730	0.104
Other	0.06	0.046	0.003 - 0.610	0.02*
Clinical feature				
Sore throat	0.226	0.21	0.025 - 1.733	0.147
Animal exposure				
Chicken	0.109	0.084	0.007 - 1.078	0.057

***indicates a statistically significant P value**

4.4. Comparing serology (ELISA) with other diagnostic methods (rt-PCR and LAMP assay).

Serology (IgM or IgG), which is the gold standard for the diagnosis of Q fever infection, was compared to other diagnostic methods (nucleic acid detection methods). The ELISA results for *C. burnetii* infection were compared to results from real-time polymerase chain reaction (rt-PCR) and Loop-mediated Isothermal amplification (LAMP) assay. Two sets of primers were used for rt-PCR to detect *icd* and IS*1111* genes (conserved regions in *C. burnetii*), while the LAMP assay targeted the IS*1111* region of *C. burnetii*. Out of the 117 samples tested, 19 were sero-positive by ELISA. However, rt-PCR and LAMP assays did not amplify any *C. burnetii* DNA. The results are summarized in Table 4.9. Figures 4.3, 4.4 and 4.5 shows examples of the rt-PCR (IS*1111*), rt-PCR (*icd*) and 2% agarose gel photograph of LAMP assay respectively.

Table 4.9: Comparison of ELISA results with rt-PCR and LAMP assay

	ELISA IgM & IgG	rt-PCR *icd* & IS*1111*	LAMP Assay
Positives	19	0	0
Negatives	98	117	117
Total no. Tested	117	117	117

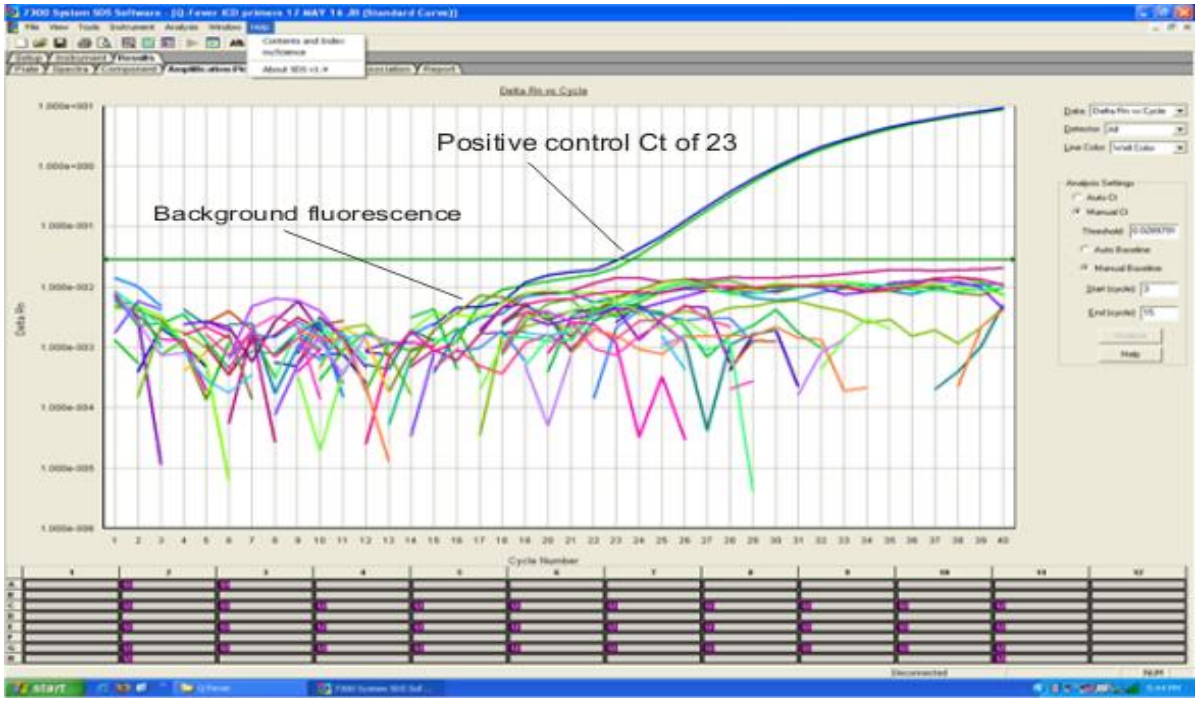

Figure 4.3: rt-PCR using Isocitrate dehydrogenase (*icd*) primers and probe (Ct: Cycling Threshold)

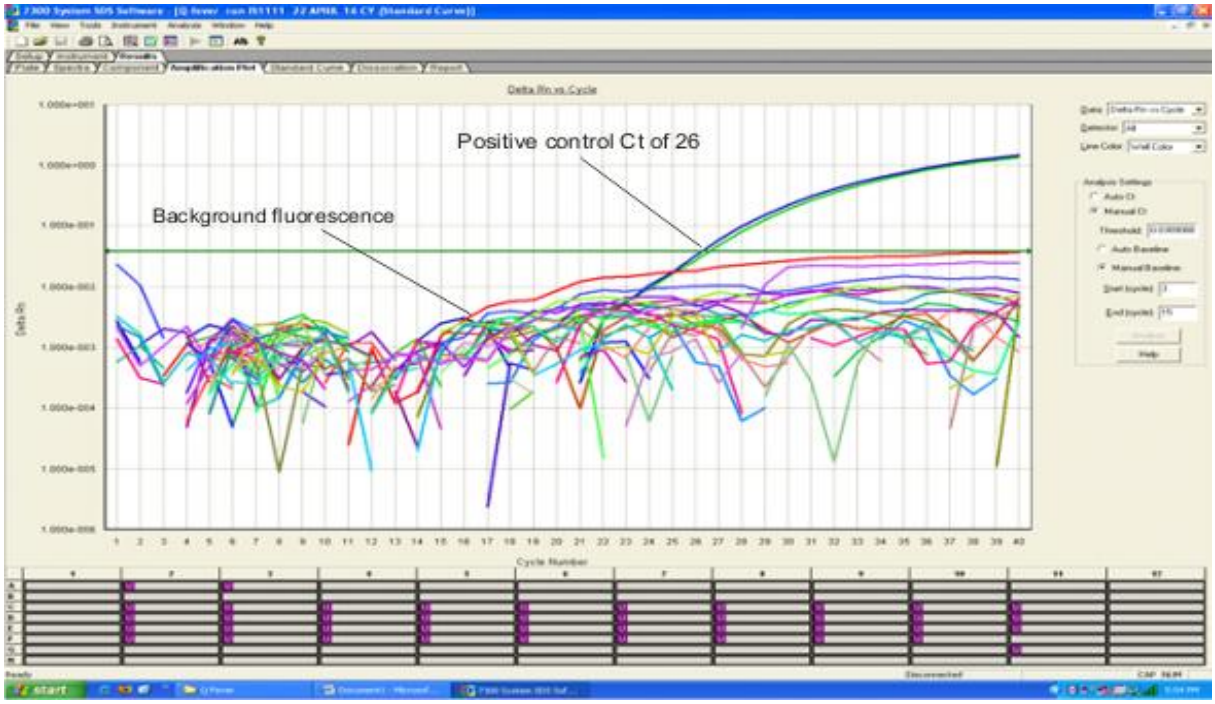

Figure 4.4: rt-PCR using Insertion sequence *(IS1111)* primers and probe (Ct: Cycling Threshold)

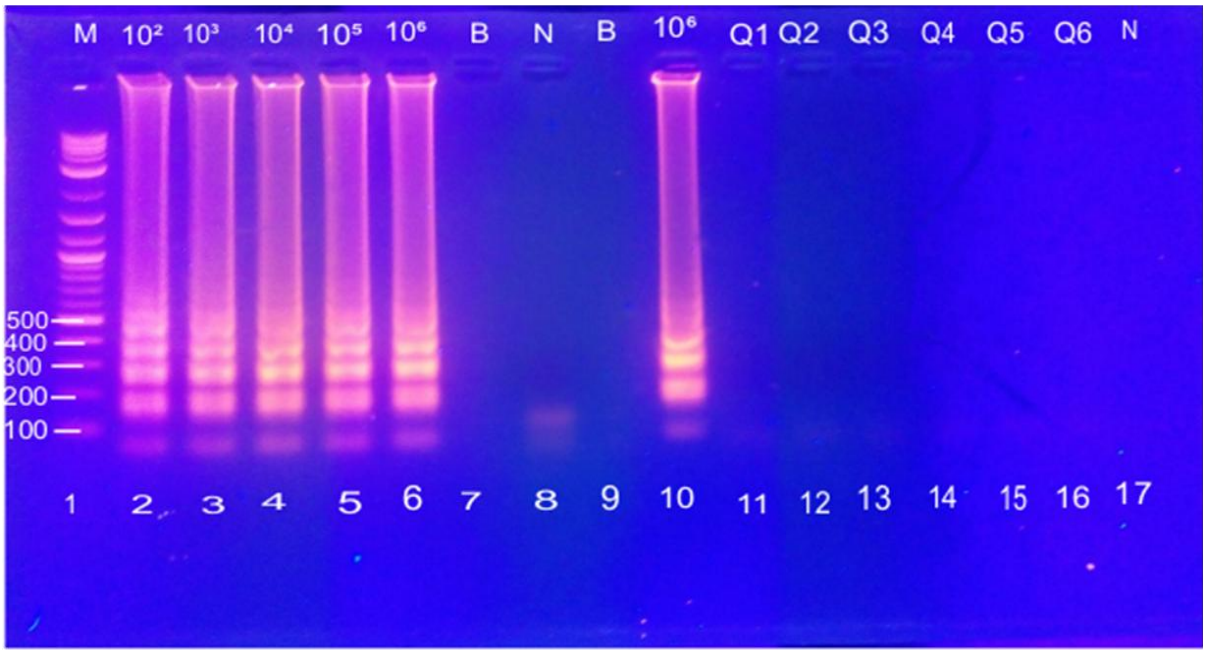

Figure 4.5: 2% agarose gel photograph of LAMP assay for the detection of *C. burnetii,* displaying the typical ladder-like pattern.

Lane 1, M = 100 bp weight molecular marker; Lanes 2, 10^2 positive control; Lanes 3, 10^3 positive control; Lane4, 10^4 positive control; Lane 5, 10^5 positive control; Lane 6, 10^6 positive control; Lane 7, blank well; Lane 8, negative control; Lane 9, blank well; Lane 10, 10^6 positive control; Lane 11 – 16, samples Q1 – Q6 and lane 17, negative control respectively.

The LAMP assay was evaluated using 5 sets of primer mixture, which were obtained from NMRC USA at the protein chemistry laboratory. A 1:100 dilution was done from the provided stock positive control and a 4 fold serial dilution was also done.

4.5 Analysis of Q fever co-infection with malaria

Malaria data was obtained from 101/117 study participants. A total of 29 (28.71%) were positive for malaria with 5 (17.2%) having a co-infection with Q fever and 14 (19.4%) had only Q fever. There was no significant association between having malaria and Q fever (P value = 1.0) as summarized in Table 4.10 below.

Table 4.10: Q fever co-infection with malaria

		Malaria			
	Variable	Negative (%)	Positive (%)	Total	P value
Q fever	Negative	58 (80.6)	24 (82.8)	82 (81.2)	1.0
	Positive	14 (19.4)	5 (17.2)	19 (18.8)	
	Total	72	29	101	

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of Q fever

The human prevalence of Q fever is not precisely known. This is because about 50-60% of infected individuals have no symptoms at all or show symptoms that mimic other infections and are therefore misdiagnosed (Vilibic-Cavlek *et al.,* 2012). None of the 19 seropositive cases detected in this study were clinically diagnosed with Q fever. They were rather diagnosed for known tropical aetiologies such as malaria and typhoid fever, or pyrexia of unknown origin, suggesting that the incidence of Q fever is probably underestimated in Ghana. It must be noted that in this study there was no significant association between malaria and Q fever.

This study sought to investigate the prevalence of Q fever amongst patients with febrile illness in two urban cities in Ghana and a considerable proportion of febrile patients (16.2%) showed antibodies (IgM or IgG) for *C. burnetii*. The seroprevalence observed in this study is comparable to that of Njeru *et al.*,(2016), who reported a seroprevalence of 16.2% among febrile patients in Kenya. Similar, albeit slightly lower seroprevalence data (12% and 13.1%) has been reported from similar studies in Egypt and Burkina Faso respectively (Ali-Eldin *et al.,* 2011; Ki-Zerbo *et al.,* 2000). Though the seroprevalence reported in this study was substantially higher than the 5% documented in Tanzania (Prabhu *et al.,* 2011), it was lower than that reported in a similar study conducted in Mali, which recorded a seroprevalence of 50% (Steinmann *et al.,* 2005). In Mali, 51.28% (80/156) of participants in the two study sites reported contact with animals, compared to 39.31% (46/117) in this study. Interestingly, seropositivity in Mopti (43/84, 51.19%) was much higher than in Bamako (20/72, 27.78%). Mopti is a centre for trade and has the largest livestock production in Mali, suggesting a

greater degree of exposure, compared to Bamako. Considering that Q fever is a known zoonosis, it is plausible that the seroprevalence detected in our study is, among others, a reflection of the generally limited degree of exposure within our study population, where reported daily contact with animals was likely to be with pets.

The type of antibodies detected (Immunoglobulin M and G) enabled the identification of acute and past infection. Immunoglobulin M (IgM) was used to identify suspected acute infection and IgG was used to identify evidence of exposure (past infection).Acute Q fever (IgM) was documented in 6.83% of febrile patients, which is comparable to that reported by Steinmann *et al.*,(2005) which recorded 9.6%. In this study, 11.11% of study participants showed exposure (IgG) to *C. burnetii*. This finding is similar o that reported by Kobbe *et al.*,(2008)which recorded an IgG seroprevalence of 8.9% among healthy adults.

A higher seropositivity was recorded at 37 military hospital, Accra [IgM 7 (8.97%) and IgG 10 (12.82%)], compared to that observed at the military clinics in Sekondi-Takoradi [IgM 1 (2.56%) and IgG 3 (7.69%)]. It is difficult to explain this observation. However, this data suggest that exposure to *C. burnetii* may be higher in Accra compared to Sekondi-Takoradi.

More out-patients than in-patients participated in the study. The seroprevalence of *Coxiella burneti* infection was also higher in out-patients than in-patients in the study sites. Out-patients recorded IgM 6 (7.17%) and IgG 11 (13.10%) whilst 2 (6.06%) seropositivity was reported for both IgM and IgG amongst in-patients. The observation was not entirely surprising considering the self-limiting nature of the infection, given that it resolves within the first few days after onset.

5.2 Association of *C. burnetii* with predisposing factors

Seropositivity was higher in females (22.64%) compared to males (10.94%). However, there was no significant association between the infection and gender. The observed higher seropositivity in females maybe due to some activities they routinely perform, such as sweeping. It is known that *C. burnetii* can be transmitted by inhalation of contaminated dust particles. This finding is contrary to other studies where males predominated, having higher seroprevalence than female (Coulombier, 2010; Vilibic-Cavlek *et al.,* 2012). The high seroprevalence in males were explained in such studies by greater occupational exposure. Nonetheless, the findings of this study are similar to that observed by Njeru *et al.,* (2016) where gender was not a significant factor for infection. Similarly, there was no significant association of the infection with age in this study. By comparison,Cardeñosa *et al.*,(2006) reported an increase of seropositivity with age and explained their findings were due to longer exposure by older individuals. These observations imply that gender and age might not be a contributing factor to *C. burnetii* exposure in the study areas.

Data from this study showed no significant association of Q fever with the educational status of study participants, though it was observed that illiterates (no school) had a higher proportion of seropositivity (50%). Usually, individuals with lower educational background might engage in activities associated with soil, animals and generally poor hygiene. Similarly, higher seropositivity was observed among people from the northern part of Ghana (44.44%) although this association was statistically insignificant (P value 0.075).

Q fever has been associated with certain types of occupations, especially those that involve contact with animals. Direct contact with animals increases the risk of transmission with dust-contaminated by animal excreta (Steinmann *et al*., 2005).

More than 60% of study participants had no daily contact with animals. This result was not unexpected because the study was conducted in an urban setting where daily contact with animals might be minimal. However the 40% that where in daily contact with animals did not show any significant association with Q fever. Additionally, there was no association with exposure to animal slaughter, the presence of rodents in participant's households, exposure to animal abortus and consumption of raw or unpasteurized milk. These findings are in contrast to another study where contact with animal and animal products has shown high statistical significance, probably due to an increased risk of exposure (Njeru *et al.,* 2016).

Occupation played a significant role in acquiring Q fever (P value 0.048) and unemployed people had the highest prevalence of seropositive samples (66.67%). Though this finding is interesting, it is difficult to explain and further studies are needed to throw more light on factors that account for the relationship between Q fever infection and unemployment in the study area.

Clinical features of participants observed in this study were pleomorphic and did not clearly differentiate Q fever from other types of febrile illness. Participants manifested a range of clinical characteristics with fever, chills, aches and pains being the most common complaints. Clinical data obtained from this study could not directly predict *C. burnetii* as the cause of the infection. This finding confirms the nonspecific nature of the infection (Fournier *et al.,* 1998).

Contact with persons with similar illness has been documented as a rare means of Q fever transmission (Anderson *et al.,* 2013). Participants, who responded yes to being in contact with someone with similar illness, were all seronegative and this association was significant. This means that there was no transmission by human contact.

A multiple logistic regression was used to assess the risk of Q fever among the study participants in addition to bivariate analysis. This confirms employment as an independent prediction of Q fever infection. The unemployed had higher odds of acquiring Q fever than any other occupation (students, government workers and other lines of work).

5.3 Comparing Serology and Nucleic Acid Amplification Method for diagnosis of Q fever.

Surprisingly the molecular methods (rt-PCR and LAMP assays) did not detect any positive sample though several positive samples were detected by serology (ELISA).This study could not therefore, address the comparison between serology and nucleic acid amplification methods in the diagnosis of Q fever. The findings of the study confirm serology (ELISA) as a gold standard for the diagnosis of Q fever.

Nucleic acid amplification methods for the detection of *C. burnetii* are highly sensitive during early diagnosis of Q fever [within the first week of infection (onset of symptoms)] and before the administration of appropriate antibiotic (Anderson *et al.*, 2013).However, the mean time from onset of illness until presentation to the healthcare facilities was 14 days. This may probable explain why *C. burnetii* was not detected by either real-time PCR (rt-PCR) or LAMP assays, although primers employed in this study were known to be highly specific and sensitive (Chen & Ching, 2014; Klee *et al.*, 2006).

It is important to note that the presence of detectable antibodies (IgM and IgG) indicates a recent or past infection and does not necessarily indicate the presence of the bacteria. Moreover the serological method employed in this study was not aimed at the detection of the bacterial antigen. It has also been documented that as antibodies builds, nucleic acid detection decreases (Schneeberger *et al.*, 2010). This may explain the disagreement between nucleic acid amplification methods and ELISA.

Acute phase of Q fever is often characterised by a self-limiting febrile illness which could resolve within few days of illness onset. It is also highly observed in low-resource settings that readily use of tetracycline (the class of the drug of choice for Q fever) which is an inexpensive and easily accessible (Prabhu *et al.,* 2011). Therefore, the use of certain antibiotics (such as tetracycline) could have probably rendered the nucleic acid amplification method useless.

In a study conducted by Schneeberger *et al.*,(2010), PCR negative results of some serologically positive samples were attributed to delayed sample collection for diagnosis, and minimal levels of *C. burnetii* in some patients. Though Njeru *et al.*,(2016) reported a seroprevalence of 16.2%, detection by quantitative PCR was only 2.2%. Similarly, a study conducted among febrile patients in Ghana recorded a seroprevalence of 10.9% but yielded no PCR positives (Puplampu *et al.* 2014 unpublished data). As was observed in this study, it is possible that *C burnetii* was either not present or present in levels below PCR detection limits.

CHAPTER SIX

6.0 Conclusion, Recommendation and Limitations

6.1 Conclusion

This study reported an appreciably high seroprevalence of Q fever (16.24%) among febrile patients in Accra and Sekondi-Takoradi and Q fever could probably be a cause of hospitalization, as the infection was detected in some in-patients. The clinical symptoms of study participants were nonspecific and there was no association between Q fever and any of the clinically presented symptoms. Occupation showed significant association with Q fever and the likelihood of the unemployed getting infected was greater than any other type of occupation. Serology (ELISA) is superior and was effective in the diagnosis of Q fever than nucleic acid amplification methods (rt-PCR and LAMP assays).

6.2 Recommendation

1. Awareness of Q fever should be raised among clinicians and laboratory personnel, and there should be accessible laboratory testing capacity to enable instant detection and early treatment of the infection to prevent severe complications.
2. Community based studies of the infection should be conducted, comprising of febrile and asymptomatic participants in all age groups.
3. Active surveillance maybe necessary to identify the source and control the disease in humans. In identified endemic areas, Q fever diagnosis should be considered in cases of fevers of unknown origin.

6.3 Limitations

1. This study solely concentrated on recruiting patients with febrile illness presenting to the selected health facilities. Therefore, those who were unable to come to the selected health facilities were not captured in the study.

2. The age group considered in this study was 5-65 years, due to ethical reasons ages 1-4 years were exempted from this study. However, studies conducted by Kobbe *et al.,* 2008and Hoek *et al.,* 2013reported high seroprevalence of Q fever in children within the ages of 1-4 years. Therefore that information was missed in this study.

3. Convalescent serum samples were not taken from study participants. This is done 2-6 weeks after the first sample is taken. It helps observe if there was seroconversion because antibodies are known to appear within 1-2 weeks after the onset of an infection. Therefore, it is possible that some seropositive results were missed.

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APPENDICES

APPENDIX I: Materials and Preparation of Reagents

1. Reagents and volumes used for LAMP assay

a. Primer set for Q Fever DNA detection:1.2µl pre-prepared primer mix to each 25µl of reaction

b. Bst DNA polymerase 8 u/µl (New England Biolabs, catalog # MO275L)

c. Nuclease free water (Promega, REF # P119C)

d. 2X buffer

The 2X buffer consist of the following reagents and Volumes:

5 M betaine (Sigma-Aldrich, catalog #B0300-1VL)-1280 µl

10X Thermo Pol reaction buffer (New England Biolabs, catalog #B9004S)-800 µL

1M $MgSO_4$ (Sigma-Aldrich, catalog #M3409-1ML)-48 µL

dNTP 10 mM solution mix (New England Biolabs, catalog #N0447L)-1120 µLH_2O-752 µl

2. Preparation of 1X TAE Buffer from 50X Stock Solution

Twenty millilitres (20mL) of 50X TAE was diluted with 980mL of distilled water in order to obtain a one litre (1L) volume of 1X TAE buffer.

3. Reconstitution of lyophilized primers and probe.

a. Forward primer: Five hundred and twenty nine (529µl) of nuclease free was added to the lyophilized primer to make a concentration of 100 pmol/µl which is

equivalent to 100 µM (formed the stock). Working concentration was 300 nM which is equivalent to 0.3 µM.

b. Reverse primers: Seven hundred and sixty six (766 µl) of nuclease free was added to the lyophilized primer to make a concentration of 100 pmol/µl which is equivalent to 100 µM (formed the stock). Working concentration was 300 nM which is equivalent to 0.3 µM.

c. Probe: Three hundred and eight four (384 µl) of nuclease free was added to the lyophilized primer to make a concentration of 100 pmol/µl which is equivalent to 100 µM (formed the stock). Working concentration was 100 nM which is equivalent to 0.1 µM.

APPENDIX II: Quantified DNA (Nanodrop 2000 spectrophotometer readings)

Sample ID	DNA Concentration (ng/µl)
Q01	4.3
Q02	4.2
Q03	2.9
Q04	4.3
Q05	2.9
Q06	3.3
Q07	3.5
Q08	6.1
Q09	5.7
Q10	3.3
Q11	3.2
Q12	6.8
Q13	6
Q14	3.5
Q15	7.4
Q16	5.1
Q17	5
Q18	5.2
Q19	2.5
Q20	3.9
Q21	6.8
Q22	7.8
Q23	4
Q24	5
Q25	4.7
Q26	5.3
Q27	5.2
Q28	3
Q29	6.1
Q30	6.1
Q31	4
Q32	3.1
Q33	3.1
Q34	2
Q35	3.5

Sample ID	DNA Concentration (ng/µl)
Q36	15.6
Q37	3.3
Q38	6.4
Q39	3.7
Q40	6
Q41	5.7
Q42	5.3
Q43	4.5
Q44	3.9
Q45	5
Q46	3.8
Q47	4.3
Q48	4.9
Q49	7
Q50	5
Q51	5.2
Q52	2.7
Q53	3.2
Q54	6.1
Q55	3.4
Q56	6.5
Q57	4.5
Q58	4
Q59	5.8
Q60	11.4
Q61	8.8
Q62	5.1
Q63	5.8
Q64	5.9
Q65	5.7
Q66	3.7
Q67	6.9
Q68	11
Q69	7.7
Q70	2.8

Sample ID	DNA Concentration (ng/µl)
Q71	3.7
Q72	6.8
Q73	6.5
Q74	6.6
Q75	8.7
Q76	5.3
Q77	3.4
Q78	4.7
Q79	3.9
Q80	6.8
Q81	5.6
Q82	11.1
Q83	2.5
Q84	3
Q85	2.8
Q86	4.3
Q87	3.1
Q88	3.6
Q89	3.8
Q90	2.8
Q91	1.9
Q92	4.8
Q93	6.6
Q94	3.8
Q95	4.1

Sample ID	DNA Concentration (ng/µl)
Q96	4.6
Q97	5.5
Q98	4.2
Q99	5.2
Q100	3.5
Q101	6.1
Q102	5.5
Q103	4.7
Q104	4.4
Q105	5.2
Q106	5.6
Q107	4.8
Q108	5.8
Q109	4.1
Q110	3.3
Q111	4.7
Q112	5.1
Q113	3.3
Q114	3.9
Q115	4.2
Q116	6.7
Q117	5.2
Q118	8.1
Q119	8.3
Q120	7.1

APPENDIX III: Ethical Clearance Obtained from NMIMR-IRB

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

INSTITUTIONAL REVIEW BOARD

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-302-502182/513202
E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

2nd July, 2014

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 126/12-13 ***revd. 2014*** **IORG 0000908**

On 2nd July 2014, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting conducted continuing review and renewed your protocol titled:

TITLE OF PROTOCOL	:	**Acute Febrile Illness Surveillance in Ghana, Military to Military**
PRINCIPAL INVESTIGATOR	:	**LT Nehkonti Adams, MD USN**
CO – INVESTIGATORS	:	**Naiki Puplampu, Christopher Duplessis, MD, Dr. Habib Sahnoon, Prof. K. Bosompem et al.**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 1st July, 2015. You are to submit annual reports for continuing review.

Signature of Chair: .....
Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Gha

INSTITUTIONAL REVIEW BOARD

Phone: +233-302-916438 (Direct)
+233-289-522574
Fax: +233-302-502182/513202
E-mail: nirb@noguchi.mimcom.org
Telex No: 2556 UGL GH

Post Office Box LG 581
Legon, Accra
Ghana

My Ref. No: DF.22
Your Ref. No:

6th May, 2015

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 00001276**

NMIMR-IRB CPN 126/12-13 *amend. 2015* **0000908 IORG**

On 6th May 2015, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB) at a full board meeting conducted continuing review and and amended your protocol titled:

TITLE OF PROTOCOL	:	**Acute Febrile Illness Surveillance in Ghana, Military to Military**
PRINCIPAL INVESTIGATOR	:	**LT Nehkonti Adams, MD USN**
CO – INVESTIGATORS	:	**Prof. Kwabena Bosompem, CDR Christopher Duplesis, Dr. Rania Abedel Khalek, Ms. Naiki Puplampu Shirley Nimo Paintsil, LCDR Edward Nyarko et al**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 5th May, 2016. You are to submit annual reports for continuing review.

Signature of Chair: .....

Mrs. Chris Dadzie
(NMIMR – IRB, Chair)

cc: Professor Kwadwo Koram
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon

APPENDIX IV: Ethical Clearance Obtained from College of Health Sciences Ethical and Protocol Committee

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.....

5th January, 2016.

Clara Ngyedua Yeboah
Department of Medical Microbiology
School of Biomedical and Allied Health Science
University of Ghana
Korle-Bu,

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.4 – P 3.3/2015-2016**

The Ethical and Protocol Review Committee of the College of Health Sciences unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Q Fever Infection in Patients with Febrile Illness, Ghana"**

PRINCIPAL INVESTIGATORS: **Clara Ngyedua Yeboah**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

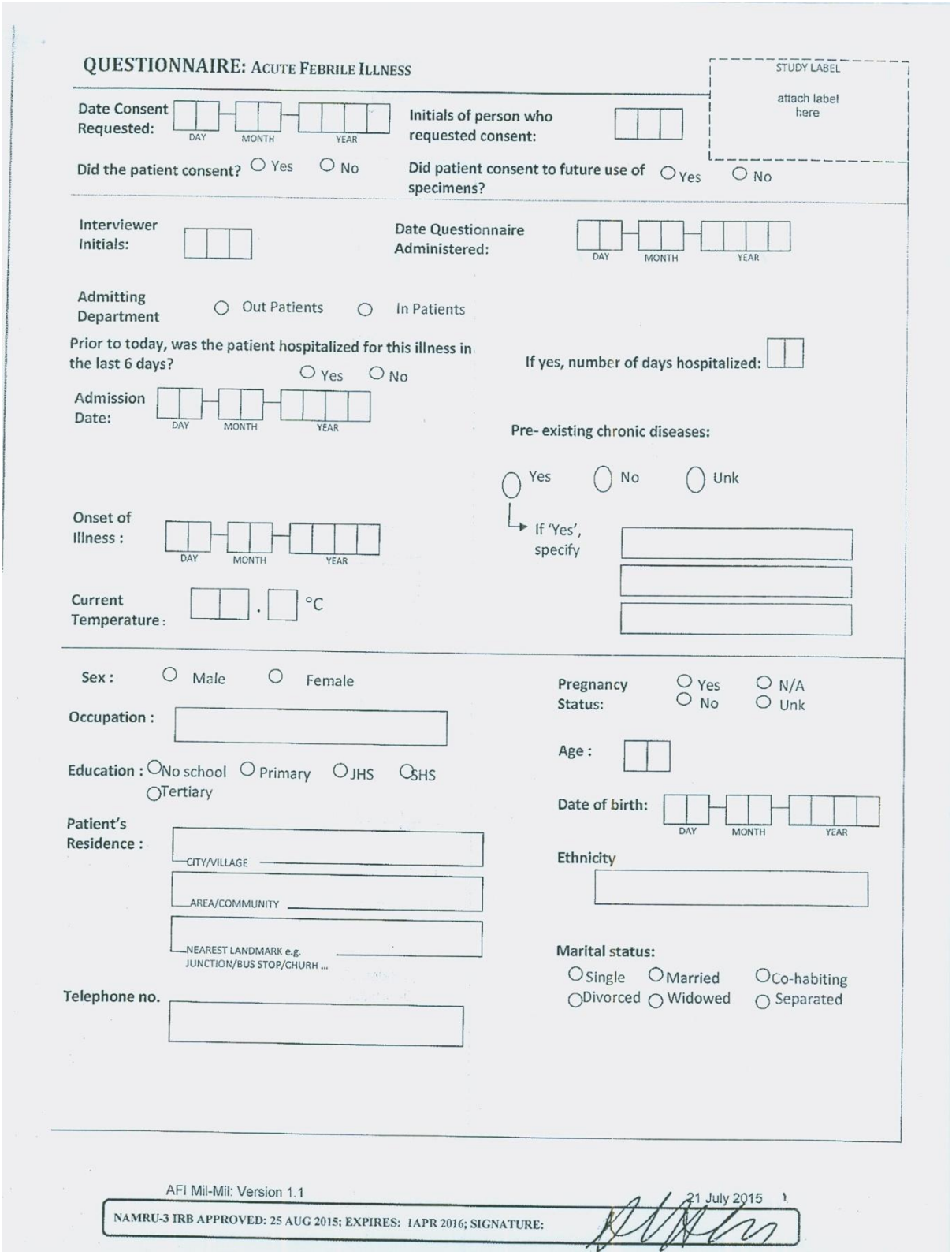
This ethical clearance is valid till 30th June, 2016.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed:.....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS

APPENDIX V: Questionnaire



QUESTIONNAIRE: ACUTE FEBRILE ILLNESS

STUDY LABEL
attach label here

Date Consent Requested: DAY MONTH YEAR

Initials of person who requested consent:

Did the patient consent? ○ Yes ○ No

Did patient consent to future use of specimens? ○ Yes ○ No

Interviewer Initials:

Date Questionnaire Administered: DAY MONTH YEAR

Admitting Department ○ Out Patients ○ In Patients

Prior to today, was the patient hospitalized for this illness in the last 6 days? ○ Yes ○ No

If yes, number of days hospitalized:

Admission Date: DAY MONTH YEAR

Pre- existing chronic diseases:

○ Yes ○ No ○ Unk

If 'Yes', specify

Onset of Illness : DAY MONTH YEAR

Current Temperature : . °C

Sex : ○ Male ○ Female

Pregnancy Status: ○ Yes ○ No ○ N/A ○ Unk

Occupation :

Age :

Education : ○ No school ○ Primary ○ JHS ○ SHS ○ Tertiary

Date of birth: DAY MONTH YEAR

Patient's Residence :

CITY/VILLAGE

AREA/COMMUNITY

NEAREST LANDMARK e.g. JUNCTION/BUS STOP/CHURH ...

Ethnicity

Marital status:

○ Single ○ Married ○ Co-habiting ○ Divorced ○ Widowed ○ Separated

Telephone no.

AFI Mil-Mil: Version 1.1 21 July 2015

NAMRU-3 IRB APPROVED: 25 AUG 2015; EXPIRES: 1APR 2016; SIGNATURE:

Please check symptom that apply to patient.

	YES	NO	UNK
Fever	○	○	○
Chills	○	○	○
Headache	○	○	○
Retro orbital pain	○	○	○
Conjunctival injection	○	○	○
Sore throat *(Pharyngitis)*	○	○	○
Rash	○	○	○
Stiff neck	○	○	○
Hemorrhagic spot-*Petechiae*	○	○	○
Other hemorrhagic signs*	○	○	○
Joint pain *(Arthralgia)*	○	○	○
Muscle pain *(Myalgia)*	○	○	○
Altered mental status	○	○	○
Other _____			

ANTIBIOTIC USE

Did the patient take antibiotics in the 3 days before admission?

○ Yes ○ No ○ Unk

↳ If yes, specify:

Date when antibiotics intake started:

DAY MONTH YEAR

Duration (no. days administered): ☐☐

*Erythema-redness of the skin. Bruising. Melena-blood in stool. Hemoptysis- coughing up blood. Hematuria-blood in urine.

1. Is the patient or his/her family in daily contact with any of the following animal(s)? Circle all that apply

None Sheep Goats Cattle Swine Donkeys Horse Chicken Pigeons Ducks Cats Dogs Bats

In the last 2 weeks has the patient been exposed to the following?	YES	NO	UNK
2. Has the patient been involved in the slaughter of animals?	○	○	○
3. Has the patient been exposed to animal abortus?	○	○	○
4. Has the patient consumed raw or unpasteurized milk products?	○	○	○
5. Has the patient noticed any rodent inside or around the household?	○	○	○
6. Has the patient been bitten by (fleas, ticks, lice)?	○	○	○
Other_____			
7. Has the patient been bitten by mosquitoes?	○	○	○
8. Was the patient in contact with someone with a similar illness?	○	○	○
9. Does the patient have contact with water from rivers, canals or ponds?	○	○	○
10. Does the patient have tap water in the home?	○	○	○
11. Does the patient store water before use?	○	○	○

12. If yes, what is the mode of water storage?_____

DISCHARGE QUESTIONS

1. Did the patient survive? ○ Yes ○ No
2. Date of discharge or death ☐☐ ☐☐ ☐☐☐☐ DAY MONTH YEAR
3. What was the patient discharge diagnosis? _____
4. Special comments

_____

APPENDIX VI: Consent Form

CONSENT FORM

You have been asked to volunteer to participate in a research study entitled

Acute Febrile Illness Surveillance in Ghana Military to Military

1. General Information about Research
The purpose of this study is to identify the cause of your fever. We will draw blood (about 2 tablespoons) on the day that you present with your fever to look for infections that may be the cause. We will like to repeat the testing in 2 - 6 weeks to search for the same infections. If you are in the hospital, we may ask to repeat the testing, if you are admitted for more than 5 days. These repeated blood draws will only be about one tablespoon total of blood, for each blood draw. Your blood WILL NOT be tested for HIV/AIDS. We may also ship some of your blood specimen outside of Ghana for further testing to help identify the cause of your fever. Your blood sample will not be accompanied with any of your personal information.

We will ask you questions about exposures to animals or vegetation to identify risk factors. This process will take about 30 minutes.

We will enroll a maximum of 450 patients in this study.

This study is a collaborative effort by the Naval Medical Research Unit Number 3 (NAMRU-3), the Ghana Military Hospitals and Clinics, the Noguchi Memorial Institute for Medical Research (NMIMR) and the Global Emerging Infectious Disease and Surveillance (GEIS) agencies.

We will acquire your home number and address so the investigators may contact you to remind you of your appointments for follow up visits to the clinics. This information will only be used by study team for contacting you.

2. Possible Risks and Discomforts
The investigators believe that the risks or discomforts to you involve the risk associated with blood draws which includes infections, pain, and bleeding. We will minimize these risks by utilizing meticulous care when drawing blood by trained staff according to the standards of care of medical practice. We are limiting the overall blood volumes drawn to ensure we haven't placed you at risk from any excessive blood removal.

3. Possible Benefits
The benefit(s) that you may expect from your participation in this research is the benefit of possibly identifying the potential cause of the fever, which will guide physicians when treating you and other patients (if it is an infection). Some of the results will be made available to the treating physician in two weeks while others may take 3-4 weeks or longer. A copy of the results will be kept in your folder/military medical record for future reference if you so wish.

4. Alternatives to Participation
The alternative for participation in the study is not to participate… and instead receive the standard care provided at the military clinic.

5. Confidentiality
Your confidentiality during the study will be ensured by using only numbers, not using your name on forms. The confidentiality of the information related to your participation in this research will be ensured by maintaining all records in sealed areas with no identifiers. The data will be accessed by the participating agencies [NAMRU-3, Noguchi, Military Clinics, and GEIS].

6. Compensation
Fifteen Ghana cedis for the follow up visit for transportation fees.

7. Additional Cost
No costs involved in participating in this study.

8. Voluntary Participation and Right to Leave the Research
Your participation in this study is completely voluntary. If you do not want to participate, there will be no penalty and you will not lose any benefit to which you are otherwise entitled. You may discontinue your participation in this study at any time you choose. If you do stop, there will be no penalty and you will not lose any benefit to which you are otherwise entitled. Your specimen and data will also be destroyed.

9. Termination of Participation by the Researcher
Your participation in this study may be stopped by the researchers without your consent, if the risk to your health outweighs the benefits that you may get from participating.

10. Notification of Significant New Findings
Significant new findings developed during the course of the research will be provided for the duration of your participation.

11. Contacts for Additional Information
If you have questions about this study, you should contact the following individuals: for questions about research (science) aspects contact Dr. Nehkonti Adams at 0244333027 in Ghana or CDR Edward Nyarko at eonyarko@yahoo.co.uk. For questions about medical aspects, injury, or any health or safety questions you have about your or any other volunteer's participation, contact CDR Edward Owusu Nyarko, M.D. email: eonyarko@yahoo.co.uk. For questions about the ethical aspects of this study, your rights as a volunteer, or any problem related to protection of research volunteers, contact the NMIMR IRB at 0302916438 in Ghana or via email: nirb@noguchi.ug.edu.gh or contact the "Human Protection Office" in Cairo, Egypt at +20-2-2348-0345

12. You have been informed that Dr. Adams from NAMRU-3 Detachment in Ghana is responsible for the long-term storage of your/your child's consent form and the research records related to your/your child's participation in this study. You understand that these records are stored in a secure place at NAMRU-3 Detachment in Ghana.

13. All answers that were provided by the researcher(s) are understandable to you and are satisfactory. You understand what has been explained in this consent form about your participation in this study. By your signature or thumbprint below, you give your voluntary informed consent to participate in the research as it has been explained to you, and acknowledge receipt of a copy of this form for your own personal records.

Signature/Thumbprint of participant

Date (DD/MM/YY)

NAMRU-3 IRB APPROVED: 2 APR 2015; EXPIRES: 1 APR 2016; SIGNATURE:

Printed name of Investigator | Signature of Investigator | Date (DD/MM/YY)

15. I was present during the consent process and I have actually viewed the signing of the form by the participant. "NOTE: ***Witness signature*** *is required* ***IF: a)*** *a short form (oral script) is used,* ***b)*** *subject is illiterate or blind, or* ***c)*** *if consent/assent form is translated into another language at the time of the consent process.* ***The witness must be present*** *during the consenting process.* **The witness must be** someone other than the person who obtains the informed consent/assent or someone who is NOT involved in the day-to-day operations of the research study."

Printed name of witness | Signature of Witness | Date (DD/MM/YY)

FORM FOR DISPOSITION OF BLOOD SAMPLES

I **authorize the use of** all biological material (blood,) collected from me as part of this research study to NAMRU-3. I understand that these samples may be used in the future for other testing as determined by NAMRU-3, but that my name and any information that could link my identity to these samples will be removed.

Signature/Thumbprint of participant | Date

APPENDIX VII: Parental Consent Form

AFI-NAV-026

PARENTAL CONSENT FORM

Title: Acute Febrile Illness Surveillance in Ghana Military to Military

Principal Investigator: Dr. Nehkonti Adams

Address: NAMRU-3 Ghana Detachment, Noguchi Memorial Institute for Medical Research

General Information about Research
Your child has been asked to volunteer to participate in a research study entitled "Acute Febrile Illness Surveillance in Ghana Military to Military"
The purpose of this study is to identify the cause of your child's fever. We will draw blood (about 2 tablespoons) on the day that your child presents with fever to look for infections that may be the cause. Your child's blood WILL NOT be tested for HIV/AIDS. We may also ship some of your child's blood specimen outside of Ghana for further testing to help identify the cause of your child's fever. Your child's blood sample will not be accompanied with any of his/her personal information.

We will like to ask your child questions about your exposures (animals, vegetation) to identify risk factors for infection. This process will take about 30 minutes.

We will enroll a maximum of 450 patients in this study.

This study is a collaborative effort by the Naval Medical Research Unit-3 (NAMRU-3), the Ghana Military Hospitals and Clinics, and the Noguchi Memorial Institute for Medical Research (NMIMR) and the Global Emerging Infectious Disease and Surveillance (GEIS) agencies.

We will acquire your home number and address so the investigators may contact your child to remind him/her of his/her appointments for follow up visits to the clinics. This information will only be used by study team for contacting you.

Possible Risks and Discomforts
The investigators believe that the risks or discomforts to your child involve the risk associated with blood draws which includes infections, pain, and bleeding. We will minimize these risks by utilizing meticulous care when drawing blood by well trained personnel according to the standards of care of medical practice. We are limiting the overall blood volumes drawn to ensure we have not placed your child at risk from any excessive blood removal.

Possible Benefits
The benefit(s) that you may expect from your child's participation in this research is the benefit of possibly identifying the potential cause of the fever (if it is an infection) which will guide physicians when treating your child and other patients. Some of the results will be made available to the treating physician in two weeks by phone, while other test may take 3-4 weeks or longer. A copy of the results will be kept in your child's folder for future reference if he/she so wishes.

Alternatives to Participation
The alternative for participation in the study is not to participate… and instead receive the standard of care provided at the military clinics.

NAMRU-3 IRB APPROVED: 2 APR 2015; EXPIRES: 1 APR 2016; SIGNATURE:

Confidentiality
Your child's confidentiality during the study will be ensured by using only numbers, not using your name on forms. The confidentiality of the information related to your child's participation in this research will be ensured by maintaining all records in sealed areas with no identifiers. The data will be accessed by the participating agencies [NAMRU-3, Noguchi, Military Clinics, and GEIS].

Compensation
Fifteen Ghana cedis for the follow up visit for transportation fees.

Additional Cost
No costs involved in participating in this study.

Voluntary Participation and Right to Leave the Research
Your child's participation in this study is completely voluntary. If you do not want your child to participate, there will be no penalty and your child will not lose any benefit to which your child is otherwise entitled. You may discontinue your child's participation in this study at any time you choose. If you do stop, there will be no penalty and your child will not lose any benefit to which your child is otherwise entitled also, his/her specimen and data will be destroyed.

Termination of Participation by the Researcher
Your child's participation in this study may be stopped by the researchers without your/your child's consent/assent, if the risk to your child's health outweighs the benefits that he/she may get from participating.

Notification of Significant New Findings
Significant new findings developed during the course of the research will be provided for the duration of your child's participation.

Contacts for Additional Information
If you have questions about this study, you should contact the following individuals: for questions about research (science) aspects contact CDR Edward Nyarko at eonyarko@yahoo.co.uk or Dr. Nehkonti Adams at 0244333027. For questions about medical aspects, injury, or any health or safety questions you have about your or any other volunteer's participation, contact CDR Edward Owusu Nyarko, M.D. email: eonyarko@yahoo.co.uk. For questions about the ethical aspects of this study, your child's rights as a volunteer, or any problem related to protection of research volunteers, contact the NMIMR IRB at 0302916438 or via email: nirb@noguchi.ug.edu.gh or contact the "Human Protection Office" in Cairo, Egypt at +20-2-2348-0345

Your Child's Rights as a Participant
This research has been reviewed and approved by the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB). If you have any questions about your child's rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh.

NAMRU-3 IRB APPROVED: 2 APR 2015; EXPIRES: 1 APR 2016; SIGNATURE:

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title "***Acute Febrile Illness Surveillance in Ghana Military to Military***" has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree that my child should participate as a volunteer.

Date _____ Name and signature or mark of parent or guardian _____

If participant cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the child's parent or guardian. All questions were answered and the child's parent has agreed that his or her child should take part in the research.

Date _____ Name and signature of witness _____

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Date _____ Name Signature of Person Who Obtained Consent _____

FORM FOR DISPOSITION OF BLOOD SAMPLES

You authorize the use of all biological material (blood,) collected from your child as part of this research study to NAMRU-3. You understand that these samples may be used in the future for other testing as determined by NAMRU-3, but that your child's name and any information that could link your child's identity to these samples will be removed.

Signature/Thumbprint of parent or guardian _____ Date _____

APPENDIX VIII: Child Assent Form

CHILD ASSENT FORM

Acute Febrile Illness Surveillance in Ghana Military to Military

Introduction
My name is Dr. Nehkonti Adams and I am from the Naval Medical Research Unit # 3 located at the Noguchi Memorial Institute for Medical Research at University of Ghana, Legon. I am conducting a research study entitled "Acute Febrile Illness Surveillance in Ghana Military to Military"
I am asking you to take part in this research study because I am trying to learn more about the other germs apart from the malaria germ that cause fevers. This will take about 30 minutes of your time.

General Information
If you agree to be in this study, you will be asked to allow us draw blood (about 2 tablespoons) on the day that you present with your fever to look for infections that may be the cause. Your blood WILL NOT be tested for HIV/AIDS. We may also ship some of your blood specimen outside of Ghana for further testing to help identify the cause of your fever. Your blood sample will not be accompanied with any of your personal information.

We will like to ask you questions about your exposures to (animals, vegetation) to identify risk factors for infection.
We will acquire your home number and address so the investigators may contact you to remind you of your appointments for follow up visits to the clinics.

Possible Benefits
Your participation in this study will result in possibly knowing the potential cause of the fever that you are suffering from (if it is an infection). Some of the results will be made available to the treating physician in two weeks by phone, while others may take 3-4 weeks or longer. A copy of the results will be kept in your folder for future reference if you so wish.

Possible Risks and Discomforts
However, the risks associated are infections, pain, and bleeding. We will minimize these risks by being very careful when drawing blood by well trained personnel according to the standards of care of medical practice. We will make sure to draw the most minimal amount of blood that we need for testing.

Voluntary Participation and Right to Leave the Research
You can stop participating at any time if you feel uncomfortable and your specimen and data will be destroyed. No one will be angry with you if you do not want to participate.

Confidentiality
Your information will be kept confidential. No one will be able to know how you responded to the questions and your information will be anonymous.

Contacts for Additional Information
You may ask me any questions about this study. You can call me at any time 0244333027 or talk to me the next time you see me.

NAMRU-3 IRB APPROVED: 25 AUG 2015; EXPIRES: 1APR 2016; SIGNATURE:

Please talk about this study with your parents before you decide whether or not to participate. If you cannot write or sign your name but agree to participate, that is also fine. I will also ask permission from your parents before you participate in the study. Even if your parents say "yes" you can still decide not to participate.

Your rights as a Participant
This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research titled **"Acute Febrile Illness Surveillance in Ghana Military to Military"** has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate.
If less than 5 years old:

☐ **Child verbal assent provided**

Researcher's Name:.....

Child's Signature/Thumbprint..... **Researcher's Signature:**.....

Date: ..... **Date:** .....

I was present during the consent process and I have actually viewed the signing of the form by the participant. "NOTE: ***Witness signature*** *is required* ***IF: a)*** *a short form (oral script) is used,* ***b)*** *subject is illiterate or blind, or* ***c)*** *if consent/assent form is translated into another language at the time of the consent process.* ***The witness must be present*** *during the consenting process.* **The witness must be** someone other than the person who obtains the informed consent/assent or someone who is NOT involved in the day-to-day operations of the research study."

Printed name of witness	Signature of Witness	Date (DD/MM/YY)

NAMRU-3 IRB APPROVED: 25 AUG 2015; EXPIRES: 1APR 2016; SIGNATURE: