SEROPREVALENCE OF COXIELLA BURNETII PHASE I AND PHASE II ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH BRUCELLA-LIKE ILLNESS IN METO HEALTH CENTRE, KAJIADO, KENYA

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DECLARATION AND APPROVAL

Declaration by the Student

I declare that this research proposal has been written by me and has not been submitted for the award of a degree in any university.

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DEDICATION

I dedicate this study to my adored Children, for being my source of motivation and will to complete this research study.

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I am grateful to Almighty God for his grace, and provision of health that has allowed me to complete my research. My sincere gratitude to my supervisors, Dr. Stanley Kang'ethe and Dr. Pauline Gitonga, for their invaluable assistance from ideation to the completion of my studies. I sincerely thank Prof. Richard Bowen and Prof Bosco from Colorado State University, who facilitated the full funding of my studies. I am indebted to the head of the Medical Laboratory Sciences Department and my lecturers for sharing their vast knowledge and expertise. My appreciation to the Research approval bodies, MKU Ethical approval committee, NACOSTI and office of the County Director of Medical Services, Kajiado. My sincere appreciation to my classmates, Laboratory staff at Meto and Kajiado for their support, and Meto community health promoters for their support during sample collection. Lastly, my gratitude to all the study participants.

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ABSTRACT

Coxiella burnetii is a zoonotic bacterial agent responsible for Q fever in both humans and animals. Ruminants are the most common livestock species associated with Q fever infections in humans. The disease presentation in humans range from asymptomatic, non-specific symptoms to fatal illness. In Kenya, no healthcare indicators seek to clinically diagnose and report Q fever majorly because there are no readily available diagnostic technologies. This cross-sectional study leveraged on the existing equipment in the Kajiado County referral laboratory to demonstrate antibodies to *Coxiella burnetii* in sera of febrile patients presenting with Brucella-like symptoms in Meto Health Centre, Kajiado Kenya, using the Indirect Immunofluorescent assay (IFA). A total of 100 paired blood samples were obtained from consenting and assenting study subjects. A pre-tested questionnaire was used to collect patient's socio-demographic information, knowledge of Q fever disease, and community practices that put them at risk of exposure. *Coxiella burnetii* phase I (IgG) and phase II (IgM) antibodies were characterized using IFA, while Brucella spp. IgG antibodies were demonstrated using the Indirect Enzyme-Linked Immunosorbent assay (iELISA), Rose Bengal Test (RBT), and Febrile Brucella Agglutination Test (FBAT). The overall seroprevalence of *C. burnetii* antibodies was 49.0% (95% CI: 39.42-58.65, p-value: 0.920) for Phase I IgG antibodies and 27.0% (95% CI: 19.27-36.43**,** p-value: <0.001) for phase II IgM antibodies. *Brucella* species results varied with the serological test used. RBT demonstrated a seroprevalence of 1.0% (95% CI: 0.18-5.45, p-value <0.001), while FBAT and indirect ELISA had 6.0% (95% CI: 2.78-12.48**,** p-value <0.001), and 13.0% (95% CI: 7.76-20.98, p-value: <0.001) seroprevalence respectively. Study participants' knowledge of Q fever disease was deficient at 0%. The overall co-infections between *C. burnetii* and *Brucella* spp. were 11.0% (95% CI: 8.25-18.63, p-value: <0.001). The chi-square (X^2) values and corresponding p-values for gender, age, and occupation did not exhibit statistical significance. Gender had an X^2 value of 0.160 (p=0.689), age had X^2 of 1.365 (p=0.714), and occupation had X^2 of 3.037 (p=0.219). Multivariable logistic regression analysis was applied to ascertain community-level risk predictors for Q fever disease. The practice of boiling milk at home had 91/100 study participants responding yes, but still exhibited a seropositivity of 59.3% (95%CI = 0.664-156.732. OR=10.201). Assisting a birthing animal and the practice of taking raw blood during community ceremonies were statistically significant both had p-values of less than 0.001. In conclusion, the seroprevalence of acute and chronic Q fever disease was demonstrated. *C burnetii* and Brucella spp. co-infections and exposure cultural practices were reported. The prerequisite equipment for the IFA method used to determine Q fever disease were the fluorescent microscope and water bath, which were resident equipment used in other Lab procedures in the referral Hospital. Cold chain facilities were also available and in use, thus this study strongly recommended the diagnosis of Q fever illness using Indirect Immunofluorescent assay as a point-of-care test at Hospital and peripheral laboratories, likewise, the healthcare workers and community sensitization was strongly recommended.

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LIST OF ABBREVIATIONS AND ACRONYMS

"Q" fever: "Query" Fever

°C: Degrees Celsius

ASAL: Arid and Semi-Arid land

BSL3: Biosafety level three

C. burnetii: *Coxiella burnetii*

CC: Cubic Centimetre

CDC: Center for Disease Control and Prevention

CFT: Complement Fixation Test

CI: Confidence Interval

CPV Coxiella Parasitophorous Vacuole

DNA: Deoxyribonucleic Acid

DTRA Defense Threat Reduction Agency

EDPs: Especially Dangerous pathogens

ELISA: Enzyme-Linked Immunosorbent Assay

FBAT: Febrile Brucella Antibody Test

IFA: Immunofluorescent assay

IgG: Immunoglobulin gamma

IgM: Immunoglobulin Mu

IHC: Immunohistochemical Staining

Km: Kilometre

LPS: Lipopolysaccharide

Nm: Nanometre

NZDs: Neglected Zoonotic Diseases

OD: Optical Density

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OIE: Office International des Epizooties

OR: Odds Ratio

P: Probability

PBS: Phosphate-buffered saline

PCR: Polymerase Chain Reaction

PS I: O-specific chain

PV: Parasitophorous Vacuole

QFS: Q fever Fatigue Syndrome

QFS: Q fever fatigue syndrome

RBT: Rose Bengal Test

S/P: Seropositivity Percentage

SPSS: Statistical Package for Social Sciences

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Increasing numbers of cases of emerging and re-emerging neglected zoonotic diseases continue to pose a global public health problem (Rahman et al., 2020). To eradicate these diseases, which are highly neglected, targeted control is critical. Effective control is only feasible if the burden of these diseases, their spread and transmission, and the associated risk factors in animals and humans is known (Groten et al., 2020). The zoonotic bacteria Coxiella burnetii, which causes Q fever, was the pathogen examined in this cross-sectional investigation. This particular bacterium is pleomorphic, exclusively intracellular, Gram-negative, and is 0.2-0.5 µm in width and 0.4-1.0 µm in length. Bacteria include *C. burnetii* and other members of the Legionellales order, class Gammaproteobacteria, family Coxiellaceae, and genus Coxiella (Abnave et al., 2017). Different doses of infection, different routes of exposure, and different antigenic phases of C. burnetii determine the incubation time in humans, which may be anywhere from two weeks to four weeks or more. This pathogen is easily identifiable by its distinctive cell wall lipopolysaccharide (LPS) molecule, which is both structurally and antigenically distinct (Patil & Rigunath, 2022). *C. burnetii* goes through two separate antigenic stages that are determined by the structure of its LPS: Isolation of the pathogenic, LPS-containing Phase I from infected hosts phase II is a milder variation that lacks the terminal O-antigen and contains a partial or truncated LPS. Repetition of Phase I passage via embryonated eggs or cell cultures is the usual method for obtaining Phase II (Sahu et al., 2020).

The bacterium *Coxiella burnetii* mostly live in domestic animals. Animals with the disease often pollute the ground with their excrement, which carries the disease with it, urine, and birthing

products (Byeon et al., 2022).The latter has been reported to have an extremely high bacteria load that persists in soil for months, hence rendering the environment infectious. Therefore, Q fever is mostly transmitted by inhalation of infected dust, particularly in pastoralist communities in Sub-Saharan Africa (Muema et al., 2022; Robi et al., 2023).

Africa and many other places with low resources suffer from inadequate Q fever illness preventive and control efforts, majorly attributed to the lack of diagnostic capacities, coupled with the non-specific and self-limiting nature of *C. burnetii* infection. The unavailability of the gold-standard tests locally and variations in test specificity and sensitivity contribute to the problem of under diagnosis and under reporting (Lemtudo et al., 2021; Muema et al., 2022; Robi et al., 2023). Control of zoonotic diseases requires discussions and collaborations from human health, animal health, and the environment. This means that for interventions to be effective, multidisciplinary involvement is essential. The One Health Approach is a comprehensive strategy that has been shown to effectively control zoonotic diseases (Horefti, 2023; Kiptanui et al., 2022).

In Kenya, Q fever is still not well identified or reported (Wainaina et al., 2024; Cadmus et al., 2021). A thorough understanding of the disease's burden and risk factors, researching the incidence of this infectious zoonotic disease and the variables that put Kenyans at risk is, hence, very important (Kiptanui et al., 2022).

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1.2. Statement of problem

Analysis of 2018 to 2024, laboratory reports obtained from the Kajiado County Director of Veterinary Services (Kajiado-CDVS) indicated that most aborting animals were strongly positive for phase I, Immunoglobulin gamma (IgG) antibodies to *Coxiella burnetii*. Despite veterinary laboratory surveillance records indicating high seroprevalence of animal Q fever disease, very few studies in humans have been conducted in Meto area of Kajiado County to confirm if humans are also affected by this zoonotic disease.

1.3. Justification

Meto is located in Matapato South ward, predominantly occupied by a homogenous pastoral community that depend on livestock for their livelihood, and their occupation majorly being livestock keeping further bringing them to close interaction with their livestock. As such Meto becomes a potential high-risk area for zoonotic diseases. Interestingly, the area is unexposed to studies.

Syndromic surveillance reports from the Kenya Animal Bio-surveillance System (KABS) an electronic animal disease reporting system, revealed that between January 2018 and June 2024, abortion in livestock in Kajiado County was the most reported syndrome with 3544 (23%) reports out of a total of 15,320. Goats and sheep were the most common livestock species reported to be experiencing abortions during this period. Furthermore, almost half (44%) of abortion cases were reported from Kajiado Central, specifically, Matapato South ward where Meto Health centre is situated, (Kajiado County Director of Veterinary Services (CDVS) Report-Unpublished).

These reports, therefore, coupled with the occupation of the communities living in Meto, informed the selection of Meto Health Centre for this cross-sectional study.

1.4. Objectives

1.4.1 General Objective

To determine the seroprevalence of *Coxiella burnetii* Phase I and Phase II antibodies and associated risk factors among patients presenting with brucella-like illness in Meto Health Centre, Kajiado county.

1.4.2 Specific objectives

1. To determine the seroprevalence of IgG (phase I) and IgM (phase II) antibodies of *Coxiella burnetii* among patients presenting with Brucella-like symptoms at Meto Health Centre.
2. To determine *Coxiella burnetii* and Brucella spp. co-infection among the study population at Meto Health Centre.
3. To correlate the sociodemographic risk factors associated with *C. burnetii* infection with the study results

1.5. Research Questions

Specific objective 1:

What is the seroprevalence of Q fever among patients presenting with Brucella-like sypmtoms at Meto Health Center, Kajiado County?

Specific objective 2:

Is there co-infection of *Coxiella burnetii* and Brucella spp. among the study population at Meto Health Centre?

Specifice objective 3:

Is there a correlation between the sociodemographic risk factors associated *with Coxiella burnetii* infection with the study results?

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1.6. Study significance

This study provides new insight into introduction of Immunofluorescent Assay as a point of care diagnostic test for Q fever disease by leveraging on resident equipment used for other diagnosis in the peripheral Laboratories.

1.7. Scope of the study

The research study was conducted at Meto Health Center. The Indirect Immunofluorescence Assay (IFA) serological technique was utilized for characterization of *Coxiella burnetii* phase I and II antibodies in sera of eligible consenting and assenting outpatients who sought treatment at Meto Health Centre of Kajiado county. A sample size of 100 acute samples was obtained at the time the study participants visited the health centre and 100 convalescent samples were collected from the same participants two weeks after. Brucella antibodies were also characterized in the acute serum samples using three serological tests, the Rose Bengal Test (RBT). Febrile Brucella Agglutination Test (FBAT) and Indirect Enzyme-Linked Immunosorbent Assay (iELISA). The research was carried out in the Kajiado County Referral Hospital's laboratory.

1.8. Limitations of the Study

The study was limited to consenting and assenting patients only. It was also limited to patients seeking health services in Meto Health Center.

1.9. Delimitation of the Study

Delimitation by introducing routine testing of Q fever in other diagnostic laboratories and inclusion of all age categories.

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1.10. Assumptions of the study

The first assumption was that the responses from the study participants were truthful and honest The second assumption is that the interpretation of the results obtained from the study population is generalizable to the larger population of the pastoralist communities living in Matapato south ward of Kajiado county.

1.11. Operational Definition of Key Terms

"Gray zone'' means results cannot be interpreted as positive or negative because they are below the limit of detection. Reasons may be very early or late in infection or suboptimal sample collection.

Lipemia is the term used to describe visible turbidity in serum or plasma samples brought on by the presence of lipoprotein particles, particularly chylomicrons. High triglyceride levels are the most typical cause of turbidity.

Antigenic phase II antibodies - IgM and IgA *Coxiella burnetii* antigens that are predominant during an acute infection.

Antigenic phase I antibodies - IgG *Coxiella burnetii* antigens that are predominant during chronic infection.

CHAPTER TWO

LITERATURE REVIEW

2.0. Introduction

Q fever and brucellosis are neglected zoonoses that cause acute febrile illness with non-specific clinical presentations in humans (de França et al., 2022; Hirschmann, 2019).

In 1955, researchers in Nakuru, Kenya, examined feverish patients and were able to report the first case of Q fever disease in the country (Mwololo et al., 2022; Craddock & Gear, 1955). Several studies have since been done in the subsequent 69 years since then, with more recent studies indicating a frequency varying between 2.5% and 31.7% (Cook et al., 2021; Wardrop et al., 2016). Following this, Q fever has spread extensively nationwide (Lemtudo et al., 2021; Muema et al., 2022; Njeru et al., 2016). Although the bacteria *Coxiella burnetii* may infect a wide variety of animals, the majority of human infections occur in domestic ruminants. Major routes of infection include breathing in spore-like particles or coming into touch with infected animals' amniotic fluid, placenta, or fetal membranes. Animal items that are not properly prepared, such raw milk, blood, or undercooked meat, may also spread the disease (Tan et al., 2024). Those who deal with animals, their products, or their waste run the risk of contracting Q fever (Robi et al., 2023). The spore-like forms of *C. burnetii* may become airborne, putting laboratory personnel at significant risk during regular sample handling and analysis (Miller et al., 2021b).

Q fever transmission can be prevented by the use of PPE, pasteurization of milk, and correct cooking methods for meat, and proper disposal of animal birthing products (Rahaman et al., 2021).

Inhalation of aerosols is the main method of transmission for *Coxiella burnetii*, however alternative ways include coming into touch with tainted animal products such milk, birthing supplies, and excrement (Patil et al., 2022).

Coxiella burnetii is a strict intracellular Gram-negative pathogen that replicates in myeloid cells such as the monocytes and the macrophages. Its lipopolysaccharide (LPS) membrane structure is the primary mechanism by which it evades host defenses. Phase II is more common during the early stages of infection, whereas Phase I is more common during the latter stages of chronic illness; both types are antigenic variations of the same bacteria. The pathogenicity of the Phase I version is enhanced by its complex, full-length LPS that contains characteristic O-chain saccharidic units. (Sireci et al., 2021). *C. burnetii* may infect the adipocyte cells, which might explain why some patients have Q fever relapses. The bacterium inhabits the Coxiella parasitophorous vacuole (CPV), a membrane-bound compartment that serves as a barrier to shield itself from the host's immune response (Bechah et al., 2014). The CPV is known to have an acidic environment (pH ~5.0) that is necessary for the activation of Coxiella metabolism and critical for its survival (Baca & Mallavia, 2002).

Because of its structure and quantity in the bacterium's cell membrane, the lipopolysaccharide (LPS) of *Coxiella burnetii* is a key virulence factor that is responsible for immune evasion. Phase I and Phase II are the two stages of its antigenicity. The most infectious phase for humans is Phase I, whereas Phase II organisms are non-virulent (Long et al., 2024; Narasaki and Toman, 2012; Sireci et al., 2021). The small cell variation (SCV) is one of two morphological forms of *C. burnetii* and it assumes the vegetative obligate intracellular form that is metabolically active and cannot survive outside the host cells (Ullah et al., 2022). The second form is known as the large cell variation (LCV), a spore-like form that shows remarkable resistance to environmental stresses while remaining metabolically inactive. These variants trigger the immune system of the

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host to produce Immunoglobulin mu (IgM), and immunoglobulin gamma (IgG) respectively (Sireci et al., 2021).

Antibodies become detectable two to three weeks after clinical symptoms are evident, then increase steadily for some months, after which they persist for several years (Gürtler et al., 2014; Patil & Regunath, 2024). The bacterium, can thrive inside scavenger cells such as the monocytes and the macrophages and is able to do so by seizing the important obligations of the immune system (Baca & Mallavia, 2002). Invasion of macrophages and subsequent replication in a vacuole similar to a phagolysosome, which is acidic and degradative, is the primary mechanism of disease pathogenesis (Larson et al., 2016).

Almost half of the people who get infected with *C. burnetii* have subclinical infection and the other half experience non-specific flu-like symptoms that include high fever and headache as the most typical symptoms (Patil & Regunath, 2024). Symptomatic cases may become severe and result in atypical pneumonia, myocarditis. hepatitis, Q fever fatigue syndrome (QFS), and neurologic abnormalities. Q fever may take a chronic form in about 5% of patients where, years after the initial infection, patients develop endocarditis. Other symptoms of chronic infections include vasculitis, hepatitis and osteomyelitis. Additionally, Q fever can cause abortion, neonatal mortality, preterm birth, or fetal growth retardation. However, the danger to pregnancy has only been partially studied (Quijada et al., 2012; Sireci et al., 2021). The obligate intracellular bacterium, *C. burnetii* can thrive inside scavenger cells such as the monocytes and the macrophages and is able to do so by seizing the important obligations of the immune system (Baca & Mallavia, 2002). Invasion of macrophages and subsequent replication in a vacuole similar to a phagolysosome, which is acidic and degradative, is the primary mechanism of disease pathogenesis (Larson et al., 2016).

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The *C. burnetii* reservoirs are predominantly sheep, goats and cows are examples of domestic ruminants. Nonetheless, transmission is supported by evidence in several animals such as wild game, aquatic animals, avian, and reptiles (Tan et al., 2024). *C. burnetii* has been reported to infect ticks and has been isolated from close to forty species of ticks (Getachew et al., 2024). Animal products such as milk, urine, and faeces can contain *C. burnetii*, the concentration of this bacterium is much higher in birthing materials such as the amniotic fluid and fetal membranes(Norlander, 2000; Patil & Regunath, 2024). Due to the non-specific febrile fever and a wide range of Q fever symptoms, a number of differential diagnoses must be considered, including brucellosis, leptospirosis, Rift Valley fever, and dengue fever amongst others(de França et al., 2022; Wainaina et al., 2024). In Kenya, the only primary point of care diagnostic test that is readily available to assist in differentiating these zoonotic diseases is the Brucella antibody testing by Febrile Brucella agglutination test (FBAT) (Glanville et al., 2017; Munyua et al., 2021; Wainaina et al., 2024; Waringa et al., 2023) (The Netherlands).

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2.1 Epidemiology of Q fever disease

The first cases of Q fever were reported in 1935 among slaughterhouse employees in Queensland, Australia. Queensland and New South Wales had the highest incidence of human Q fever, with 50-110 cases per 100,000 populations per year (Hirschmann et al., 2019). A recent study found that the incidence rate in Guiana, France, was much higher with 223 cases per 100,000 (95% CI: 189-258) (Fernandes & Sampaio de Lemos, 2023). World Health Organization (WHO) and the World Organization for Animal Health (WOAH) therefore, denoted Q fever as a notifiable disease that required formal case reporting from signatory nations (Kaufman et al., 2018). A 2022 comprehensive review of serological and molecular Q fever investigations in rudimentary domestic animals and humans in Africa approximated a mean seroprevalence of 16% (95%CI 11–23%) in humans; 14% with a 95% confidence interval ranging from10 to 20% in cattle; 13% in sheep; and 21% in goats with 95% confidence interval of a range of 15 to 29 percent. Contrarily, the average molecular prevalence was 3% with 95%CI ranging from 0–16%, in humans; 9% (95%CI 4–19%) in cattle; 16% (95%CI 5–41%) in sheep; and 23% (95%CI 20–80%) in goats (Bwatota et al., 2022). Several studies in Kenya that have attempted to quantify the human and animal seroprevalence of Q fever, have shown that seroprevalence in domestic ruminants may vary greatly, ranging from 5% to 12% (Kiptanui et al., 2022; Mwololo et al., 2022) and as high as 83% (Muema et al., 2022). Comparatively, seroprevalence in humans have reported ranges from thirteen percent to twenty-four percent (Lemtudo et al., 2021; Muema et al., 2022; Mwololo et al., 2022). More still, human seroprevalence of 45% was reported (Wainaina et al., 2024). Both acute and chronic forms of Q fever may manifest in humans; the former usually may cause localized infection or affect the liver and lungs, while the latter usually may damage multiple organs, most commonly the heart valves, causing cardiovascular infections. Although infected animals may not exhibit any symptoms of Q fever, it is linked to

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intermittent or endemic abortions resulting in discharge of high population of bacteria in the environment, which in turn puts other animals and people at risk of infection (Tan et al., 2024). In its quadripartite structure, the United Nations Food and Agriculture Organization (FAO), Global Organization for Animal Health (WOAH, previously OIE), the United Nations Environment Programme (UNEP) created a One Health Joint Action Plan (2022–2026) in collaboration with the WHO. The goal of this strategy was to improve environmental, animal, and human health by coordinating monitoring efforts and providing compelling data on the patterns of transmission that will guide efforts to eradicate Q fever (Fernandes & Sampaio de Lemos, 2023).

2.2 Pathophysiology of *Coxiella burnetii* infection.

The infectious dosage of *Coxiella burnetii* bacteria needed to infect humans is less than ten, making it a very contagious zoonotic disease (Miller et al., 2021a). Several factors, including the host's immune system, the mode of infection, and the particular strain of *C. burnetii*, may affect the bacterium's pathogenicity and virulence. Reportedly, when administered with the same amount of inoculation, the Priscilla strain is less contagious than the Nine Mile phase I strain. Age and being male or female have also been reported to influence the clinical presentation of infected humans (Eldin et al., 2016). Published papers have reported the proportion of humans infected with *C. burnetii* developing illness to be 40%. However, human-model trials showed that exposure dosage and route of exposure may substantially affect the fraction of infections causing symptoms. When the exposure dose is increased, the probability of the exposed person developing illness becomes higher. The death rate from acute Q fever is low at 1 to 2% of infected persons, while chronic Q fever related mortalities are high, reported at about 26.5% (Heppell et al., 2017; Maurin & Raoult, 1999; Norlander, 2000).

2.3 Clinical Manifestation

Q fever manifests in two distinct forms, the acute disease, normally described as primary infection and chronic disease, termed as localized infection. Acute infections often resolve on their own and asymptomatic in about 50% of infected persons, the other half become symptomatic usually after 2 to 3 weeks' incubation, thus presenting as a non-descript illness (Miller et al., 2021).

2.3.1 Acute Q Fever

Sudden development of a high temperature (40–104 degrees Celsius) is a hallmark of an acute Q fever infection and usually suspected when the fever lasts for more than 10 days, which is accompanied by thrombocytopenia, a normal leukocyte count, and elevated liver enzymes. Half of the infected persons manifest with subclinical infection (Patil & Regunath, 2024) and the other half present with symptomatic illness, that include atypical pneumonia especially in older Adults, headache, fatigue, calf muscle pain, hepatitis, myocarditis, and sometimes neurologic disorders. In around 5% of instances, the condition might worsen and last for months or even years (Quijada et al., 2012; Sireci et al., 2021). The acute sickness in children with Q fever is often less severe than in adults. Additionally, a rash is more likely to appear; in fact, studies show that as many as half of the children who have acute Q fever have a rash (Patil & Regunath, 2022). The risk of miscarriage and preterm birth is higher in pregnant women who have Q fever. Pregnancy testing and counselling can help women of childbearing age who have been diagnosed with Q fever make informed decisions about their care (Patil & Rigunath, 2022).

2.3.2 Chronic Q Fever

Acute infections only account for around 5% occurrences of long-term Q fever, which may manifest from a few months to many decades after the original infection (Eastwood et al., 2020).

After symptomatic or silent infections, chronic illness can manifest. People with previous valvular heart disease, vascular grafts, or arterial aneurysms are particularly vulnerable to getting chronic or persistent Q fever (Miller et al., 2021). The development of persistent Q fever has been linked to both infections contracted during pregnancy and immunosuppression (caused, for example, by chemotherapy). Endocarditis, aneurysm infections, chronic hepatitis, and vascular prosthesis infections are rather common forms of chronic Q fever, all of which are usually fatal if left untreated (Miller et al., 2021; Patil &Rigunath, 2022). Even though individuals are probably immune to reinfection for life, illness recrudescence could happen and has been thoroughly documented (Patil &Rigunath, 2022). Documentation of childhood cases of chronic Q fever is rare. Unlike in adults, osteomyelitis is a common sign of pediatric chronic Q fever in children (Sireci et al., 2021).

When it comes to detecting endocarditis and vascular infections, imaging technologies are invaluable. They are especially important for finding infection foci linked to Q fever. Ultrasound, computed tomography (CT) scans, MRI, and other cutting-edge diagnostic instruments Reliable proof of *Coxiella burnetii* infection may be found by positron emission tomography (PET) and leukocyte scanning (Miller et al., 2021). Although valve lesions in Q fever endocarditis may be diagnosed using echocardiograms, this technique only identifies 12% of cases. The little stature of the bacteria is responsible for its lack of sensitivity. The use of transesophageal echocardiograms as an alternative diagnosis has improved the detection of the lesions (Miller et al., 2021).

2.3.3 Post–Q fever fatigue syndrome

This condition affects 10-15% of individuals after acute Q fever. Patients display a 'chronic fatiguelike' image and the initial infection can be moderate or severe. One frequently mentioned

trait is alcohol intolerance. The intensity of the first acute infection is the post-Q fever weariness has just one identified risk factor (Eastwood et al., 2020).

2.4 Laboratory Findings

Q fever may manifest in two ways: systemically or locally. Although it may be raised in 30% of instances, a complete blood cell (CBC) count usually reveals a normal white blood cell count in 70-90% of patients. Furthermore, 25% of patients have moderate thrombocytopenia, which is followed by reactive thrombocytosis during the convalescent phase. Extremely uncommon cases may also include hemolytic anemia (Ullah et al., 2022; Heo et al., 2019) found that 70–85% of patients had transaminases and alkaline phosphatase levels that were somewhat elevated, without hyperbilirubinemia. In fact, in these individuals, the values were 2–3 times higher than the reference range. On average, the erythrocyte sedimentation rate is quite high, hovering about 55 mm/h ± 30 mm/h. Potentially detectable autoimmune antibodies include those that target smooth muscle and phospholipids. Cultures of blood often come up negative (Ullah et al., 2022)..

2.5. Diagnosis of Q Fever

There are typically four types that have been created for the purpose of diagnosing Q fever, culture and isolation, staining, serological testing, and molecular typing (Ullah et al., 2022).

2.5.1 Culture and isolation methods

Being an obligate intracellular bacterium, *Coxiella burnetii* poses a challenge in to culture and study. This pathogen is unique in that it can survive in a highly acidic environment in a mammalian cell's phagolysosomal compartment, in a relatively low oxygen tension. The availability of an axenic culture medium, which matches the acidic environment of the pathogen's intracellular habitat, has made it possible to grow and study this neglected pathogen. Unlike other

culture media, the axenic medium requires nitrogen gas to maintain the low oxygen levels that under normal circumstances are maintained using carbon dioxide gas (Miller et al., 2021b) Inoculation of embryonated chicken eggs or laboratory animals is another way of isolation that involves the use of Biosafety Level 3 chemicals to proliferate the organism. (BSL-3) facilities because the bacteria are highly infectious and easily aerosolized (Ullah et al., 2022).

2.5.2 Staining Methods

Coxiella burnetii cell membrane is densely packed with lipopolysaccharide, however, The Gram staining technique cannot detect it. The recommended staining technique include Stamp's Modified Ziehl-Neelsen, formerly known as Stamp test. Other commonly used staining techniques are Gimenez, Giemsa and modified Koster method. Gimenez staining is the most preferred because it is quicker, unlike Modified Ziehl-Neelsen technique, it does not require decolorization (Ullah et al., 2022).

Given that positive samples usually contain high concentrations of bacteria, the detection limit is high ($>10^5$ bacteria/ml) and suitable for clinical diagnostics. *C. burnetii* cells are naturally polymorphic, similar in size to *Chlamydia abortus* or *Brucella spp*., with dimensions of 0.3 to 1.5 µm in length and 0.2 to 0.4 µm in breadth under a microscope. However, Chlamydia presents with sharper, rounded, tiny contours that may appear globular with similar staining. Greater size (0.6-1.5 mm length and 0.5-0.7 mm breadth) is characteristic of Brucella species more defined, and stain more intensely. It is crucial to use control slides for *Brucella*, *Chlamydia abortus*, and *C. burnetii* for comparison. Typically, a diagnosis based on microscopy and positive serological results suffices, but if staining results are ambiguous, molecular confirmatory tests like Polymerase Chain Reaction (PCR) should be utilized (Patil & Regunath, 2024; Porter et al., 2011)

2.5.3 Serological Tests

Serology has been indicated to be the most essential laboratory diagnosis of Q fever disease. Two antigenically distinct variants of *Coxiella burnetii* are designated as phase I and phase II. Most serological tests are developed using the *C. burnetii* reference strain, Nile Mile phase I (RSA 493) (Long et al., 2024). Confirmed diagnosis of acute Q fever is based on serological detection of a fourfold rise in IgG titers to phase II between two serum samples using the IFA method. The first sample is the acute and the second one, termed as convalescent, is taken two to three weeks apart. It is recommended that the first sample be taken within the first week when the disease symptoms are evident. After collecting the first sample, testing can be done. However, the results are likely to be negative, possibly because the antibodies may be too low for detection; as such, more time may be required for antibodies to be sufficient for detection (Ullah et al., 2022).

The indirect immunofluorescence assay (IFA) is the gold standard test for Q fever diagnosis in humans, however this method is not often used for primary point of care diagnosis. The assay can potentially differentiate acute infection from a chronic infection. Chronic infections exhibit higher IgG and IgA titers against both Phase I and Phase II antigens, while acute infections primarily show elevated IgM titers against Phase II antigen (Norlander, 2000; Patil & Regunath, 2024).

The convalescent sample should always be analyzed alongside the acute sample to accurately compare antibody titers. Phase I1 IgA titers by IFA should also be requested for suspected chronic Q fever cases. It is advisable to repeat Q fever serology six months after initial infection to monitor for potential chronic disease progression.

Chronic Q fever typically showing high phase I IgG and IgA titers (Sireci et al., 2021). These titers may not decrease over time and may even rise before an effective treatment begins,

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gradually declining over many months or years. No correlation exists between post-Q fever fatigue syndrome and any specific serological pattern (Miller et al., 2021b).

2.5.3.1 Immunofluorescence Assay (IFA)

The immunofluorescence assay is the reference approach for finding anti- *C. burnetii* antibodies in human serum. It is helpful, particularly for monitoring the patient's health and identifying patients at risk for persistent infections (Patil & Regunath, 2024). Determining antibody tiers to phase I and II antigens, the assay is reliably able to characterize the febrile illness and the systemic stages of Q fever. Chronic Q fever will be indicated if the Phase I antibody titer is less than the Phase II titer, and an acute infection will be indicated if the Phase II antibody titer is more than the Phase I titer. An IgG antibody titers of 1:800 against Phase I antigen is suggestive of Q fever endocarditis (Miller et al., 2021b). Even though IFA is denoted as the gold standard for diagnosing Q fever in patients, however, is limited by the delayed formation of the acquired humeral immunoglobulins that are available in serum one or two weeks after start of clinical signs. usually, making it not appropriate for the identification of early acute Q fever cases (de França et al., 2022). In a South Korean study, 35.4% of the initial IFA tests were negative, and these patients were diagnosed by follow-up exams at a late stage when they typically had no clinical symptoms (Heo et al., 2019).

2.5.3.2 Complement Fixation Test (CFT)

The Complement fixation test (CFT) is commercially available as a kit for detecting antibodies against *C. burnetii* in patient serum. The temperature of reaction medium is elevated to allow for deactivation of natural complement before testing for *C. burnetii* specific antibodies, IgM and Ig G complex. This complex binds the complement and prevents the rapture of sheep erythrocytes

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during the reaction. Unavailability of Coxiella bacterium antibodies in the serum, the complement is not bound therefore can freely lyse the sheep erythrocytes. CFT though economical when compared to IFA, it however lacks sensitivity. IFA is able to determine presence of IgM antibodies even within three days of disease onset, unlike CFT that's picks much later. Furthermore, studies have shown that CFT sensitivity is much lower than that of IFA, it may take up to three weeks to show positivity. More still, the procedure is tedious and has a long turn-around time, thus further limiting its usefulness (Miller et al., 2021b).

2.5.3.3 Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA technique determines antibodies in serum against both phase I and phase II antigens of *C. burnetii* and offers an integrated interpretation of the results as being either seropositive, suspicious (commonly referred to as 'Gray Zone') or seronegative. However, it is less sensitive than IFA. Specifically, the low sensitivity of phase II ELISA may lead to underestimation of the true burden of acute Q fever disease (Lemtudo et al., 2021). IFA has remained the most reliable method for Q fever detection, however, ELISA becomes more reliable when dealing with a huge load of samples , may be in large surveys (Miller et al., 2021b). In a standard ELISA test, different antigen-antibody pairs are employed, one of which can be tagged with an enzyme. The optical densities resulting from enzyme-medicated substrate conversion are monitored colorimetrically . This means that the test antigens are added into the reaction chambers in a sequential manner and the optical densities are determined calorimetrically using the appropriate wave lengths as per the manufacturers guidance. The light absorption of the resulting product is then read out and translated into numerical values. Based on the specificity of the antigen-antibody linkage, the technique can be further categorized into direct ELISA, indirect ELISA, sandwich ELISA, and

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competitive ELISA. Further to this, there are two universal forms of ELISA technique: a silica-based ELISA and a non-silica-based ELISA (Hayrapetyan et al., 2023)

2.5.4 Polymerase Chain Reaction (PCR)

Molecular characterization of *C. burnetii* is most commonly accomplished using the polymerase chain reaction (PCR). This diagnostic technology is characterized by high sensitivity and specificity, which sets it apart from other laboratory tests. PCR's heightened sensitivity enables the identification and measurement of extremely small quantities of bacterial DNA, greatly improving diagnostic and research techniques. PCR can utilize a range of body fluids and excretions that may include, foetal tissues and swabs collected from the anal and throat regions that can be used for the genomic identification of *C. burnetii* by quantitative PCR (qPCR) (Bae et al., 2019; Wainaina et al., 2024)

2.5.5 Metagenomic Next-Generation Sequencing (mNGS)

Metagenomic next-generation sequencing is the most upgraded and efficient pathogen identification method that rapidly detects disease etiologies, including *Coxiella burnetii*, thus giving solution to identifying pathogens already known to exist and the unknown pathogens (Cao et al., 2020; Liu et al., 2024). mNGS is a highly multiplexed process that involves phenotypic identification of organisms through sequencing and characterization of nucleic acid and can potentially detect any pathogenic organism, present in a single sample except prions, because prions do not harbor nucleic acids. Bacteria, parasites and viruses can potentially be identified. The variation in nucleic acid sequence gives the basis for the identity of an organism's phenotypic and virulence properties. These variations are used as barcodes and are matched with existing microbial gene banks for identification (Duan et al., 2021).

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2.6. Treatment of Q fever disease.

Quick recovery from acute Q fever is possible with the right antibiotics. whereas the Chronic phase of the disease may require long dosage of antibiotics and may go for up to two years. During treatment continuous serological surveillance is paramount (Miller et al., 2021b; Porter et al., 2011). The antibiotic to treat Q fever must be able to enter the cell, work in the low pH environment where *C. burnetii* replication occurs, and inhibit the bacterium. Even though no individual antibiotic is bactericidal, a synergistic combination of hydroxychloroquine (HCQ) and doxycycline (DOXY has bactericidal effects) is recommended. 100 mg of doxycycline twice day for 2-3 weeksis appropriate for the acute non-complicated Q fever illness. Other indicated forms of medication include trimethoprim-sulfamethoxazole (TMP-SMX) (Bactrim), ofloxacin (Floxin) 200 mg twice day and rifampin (Rifadin) 300 mg twice day. The risk of fetal death can be reduced in pregnant women with Q fever through one or two tablets of double-strength TMP-SMX, 160 mg of trimethoprim, and 800 mg of sulfamethoxazole taken twice day till the mother delivers.

Those with high antiphospholipid antibodies, specifically greater than 40G PLA2, and acute Q fever can be treated with hydroxychloroquine until their values return to normal (Miller et al., 2021b; Porter et al., 2011). For patients with acute and persistent focalized endocarditis, treatment should be continued for a period of 18 months to 2 years and a frequent check on antibody levels is necessary. Patients with prosthetic heart valves may require therapy for up to two years. Two regimens of doxycycline—doxycycline together with hydroxychloroquine one for 1. 5 to 2 years have been observed to achieve a plasma concentration of one 0. 20 g/mL combined with doxycycline plus ofloxacin 1 for 4 years to lifetime- have been investigated. The favorable outcome increases with the doxycycline serum levels, which was recorded at 4. 5 g/mL

This last regimen has recorded better outcomes and thus better suited for treatment and to reduction of the risk of relapse. In turn, the determination of the chloroquine concentration in the retina requires careful ophthalmologic assessment. There is a moderate increased risk of photosensitization with both regimens in the patient. To reduce the risk of developing endocarditis, administration of doxycycline and hydroxychloroquine is recommended in patients with high levels of antiphospholipid antibodies until the antibody level decreases (Al Suqri & Al Brashdi, 2024).

2.7 Prevention and Control

Q fever is known to be an occupation-related disease. Most reported outbreaks have occurred among shepherds, livestock farmers, Laboratory personnel who deal with *C. burnetii*, dairy workers, and those employed in abattoirs (Škultéty L., 2020). So, the pre-exposed should be the primary target of preventative and control efforts groups and the surrounding environment. Use of personal protective attires, pasteurization of milk, proper cooking of animal products (Ullah et al., 2022). A key factor in controlling C. burnetii infection is the prompt treatment of affected animals and people. Vaccination is considered the most effective way of prophylaxis, especially for vulnerable those who have preexisting medical disorders, such as heart valve diseases, vascular aneurysms, and immunosuppression (Škultéty L. (2020).

2.8 Q fever Vaccines

Vaccinating people who work in high-risk areas is the best strategy to control Q fever disease. Out of three human vaccination options, the formalin-killed whole-cell vaccine Q-VAX® has the lowest risk of adverse effects and the highest effectiveness (92% to 98%). When administered to previously exposed people, the formalin-inactivated whole-cell vaccination (WCV) offers long-term protection but is not without considerable adverse effects. vaccines should be preceded

by skin testing (Sam et al., 2023). However, human vaccines are only approved in Australia (Rahaman et al., 2021). On the other hand, vaccines for livestock are approved for use globally. The Coxevac® product is an inactivated vaccine used in livestock shown to reduce bacterial shedding and thus, decrease the likelihood of abortion. This was evidenced in a study involving pregnant ewes (Williams-Macdonald et al., 2023).

2.9 Brucellosis

Brucellosis is a zoonotic disease caused by bacteria of the Brucella genus. They are classified in five species known to cause brucellosis in both animals and humans, namely: *B. abortus, B. melitensis, B. suis, B. canis, and B. ovis.* Brucella is a capsulated aerobic intracellular bacterium (Khurana et al., 2021). In gram stain they appear as short rods or coccobacilli in singles. They are non-motile, and intracellularly parasitic, with a high invasion ability and a wide infection route (Mengele et al., 2023; Liu et al., 2021). Animals serve as natural hosts for Brucella and also act as reservoirs for human infections. *B. melitensis, B. abortus, and B. suis* are known to cause disease in humans. It was originally described as undulating fever, that resembles many diseases that cause high fever in affected persons and may be occasioned by chronic localized or generalized organ infections (Laine et al., 2023). The bacterium often causes infertility and abortion in animals (Deresa et al., 2020; Pal et al., 2016). *B. abortus* is mainly reported in cattle, while *B. suis* and *B. melitensis* mainly affect pigs and goats, respectively (Khurana et al., 2021; Franc et al., 2018).

2.9.1 Epidemiology

Human brucellosis remains a significant global health concern, with evidenced estimation of a global annual incidence of 2.1 million, (Laine et al., 2023) a significantly high figure compared

to a previous approximation of 500,000 people annually (Liu et al., 2021). The global prevalence has been previously estimated at 15.53% (Khoshnood et al., 2022), with a higher occurrence in resource constrained regions compared to developed nations (Dean et al., 2012). Furthermore, it is more common in males across both age groups, including children and adults. In regions where malaria is endemic, brucellosis is often misdiagnosed due to the similarity in clinical symptoms, leading to febrile illnesses being treated as malaria (Mentari et al., 2021). This misdiagnosis is a significant challenge in controlling and eradicating the disease. Moreover, in the underserved low resource countries the exact burden of brucella infection is significantly underestimated this is as a result of suboptimal disease reporting systems (Mentari et al., 2021). Despite these challenges, efforts to prevent and eradicate brucellosis have led to notable changes in the global distribution of the disease over time especially in the European countries, the USA, Canada and Australia that have overtime designed multisectoral and multidisciplinary Brucellosis and other zoonotic diseases control strategies (Moriyón et al., 2023). It has proven a challenge over the years in controlling and eradicating the disease.

The real impact of brucellosis is often underreported in low- and middle-income nations because of insufficient disease monitoring systems (Mentari et al., 2021). Efforts to manage and prevent brucellosis have altered the disease's spread globally, despite these limitations. One Health and targeted eradication initiatives have had a significant influence in many nations, including those in Europe, the United States, Canada, and Australia (Moriyón et al., 2023)

2.9.2 Sources of infection and Risk factors

Brucella species are naturally harbored in a wide range of animal species, including domestic and wild animals. Transmission to humans primarily occurs through the ingestion of milk products from infected animals and coming into contact with sick animals (Laine et al., 2023). Brucella spp. can cause significant abortion in animals, and contact with aborted fetuses increases

the risk of transmission to humans. Although brucella infection originates from animals, horizontal transmission has been reported involving man to man transmission through sexual contact, blood transfusion, and aerosols from infected individuals. However, the majority (6%) of reported cases involve transmission between mothers and children, indicating that human-to-human transmission mainly occurs within this familial context (Zhao et al., 2019; Moriyón et al., 2023).

Previous studies have outlined the risk factors linked to brucellosis across various regions globally. Occupational hazards stemming from handling aborted fetuses, blood tissues, vaginal discharge, and even urine have been well-documented. Among those at highest risk are farmers, veterinary officers, and laboratory personnel due to their frequent exposure to animals (Alzuheir et al., 2022; Pereira et al., 2020). Furthermore, inadequate knowledge regarding risky practices like drinking unboiled milk, aiding birthing animals, and disposing of aborted fetuses has shown significant associations with brucellosis, particularly in resource limited low and middle-income countries (Laine et al., 2021). In Kenya, factors such as raw milk consumption, involvement in animal birthing, occupation, gender, and limited awareness about brucellosis have direct correlation with increased brucella infection (Kahariri et al., 2021). These findings underscore a substantial gap in public education regarding brucellosis prevalence.

2.9.3 Clinical disease

Human brucellosis can present as acute, chronic, or asymptomatic illness. An acute case is characterized by fever, myalgia, malaise, back pain, fatigue, arthralgia, and in men, epididymal-orchitis information were prevoiusly documented (Alzuheir et al., 2022; Dean et al., 2012). Within the first few weeks of acute infection, symptoms may significantly improve without treatment, and within six months, many cases fully resolve. However, delayed or incorrect diagnosis can lead to asymptomatic or chronic infections. Misdiagnosis is common in brucellosis,

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particularly in developing countries due to healthcare system limitations and socioeconomic factors (Dean *et al*., 2012). Persistence of brucellosis infection for over a year is defined as chronic infection (Castano & Solera, 2009). Brucella pathogenesis involves developing chronic infections due to its ability to invade the mononuclear phagocyte system, where they replicate within the endoplasmic reticulum (Qureshi et al., 2023). However, there are no objective laboratory diagnostic criteria for chronic infections, as these patients may have experienced delayed diagnosis or misdiagnosis, as well as delayed treatment. Chronic infection, primarily caused by *B. melitensis*, is characterized by fatigue, myalgia, and chronic fatigue syndrome, which includes symptoms such as arthralgia, depression, and insomnia (Munyua et al., 2021).

Moreover, brucellosis can manifest as asymptomatic or sub-clinical infections. During this phase, individuals do not exhibit brucellosis symptoms; however, under conditions such as compromised immunity, the disease may become symptomatic (Zhen *et al*., 2013). There is no standard criterion for determining asymptomatic infections, but the presence of a positive serum agglutination test (SAT ≥100) without brucellosis-related symptoms, such as fever, malaise, and joint inflammation, has been used before (Yang *et al*., 2020). Asymptomatic infections significantly impact disease control and prevention, yet they are often overlooked. If asymptomatic individuals, are not treated or if the process is not controlled effectively, there may be a risk of development of complications of chronic disease and also potential risk of disease transmission (Li et al., 2023). The limited diagnosis and interventions for asymptomatic infections, point to the research gaps that need to be addressed (Li et al., 2023).

2.9.4 Diagnosis and Treatment

There are mainly three ways for determining brucella illness: Serum serology testing, cultures, and molecular assays. Serological tests remain the routine screening methods. However, these

tests often provide nonspecific results that are difficult to interpret. The DNA assays such as polymerase chain reaction (PCR), is a very precise, quick, and accurate way to diagnose but it is relatively expensive (Qureshi et al., 2023). Despite the challenges associated with cultures, such as the slow growth of the bacteria and laboratory safety restrictions, cultures allow for accurate speciation during bacterial identification. Even though culture is considered the gold standard, serological assays are generally preferred due to the result's short turn-around-time which usually takes not more than one hour. Culture requires additional containment facilities because of the bacterium’s associated risk of environmental contamination and its complex growth requirements, (Yagupsky et al., 2019).

2.9.5 Serological Tests

Serological tests primarily use an indirect strategy to detect pathogens by measuring the rising titers of Brucella-specific antibodies. Despite potential drawbacks, these assays remain the main diagnostic methods for brucellosis. The antibody-mediated immune response to targets the polysaccharide portion of the Brucella S lipopolysaccharide (S-LPS), with a characteristic shift from IgM to IgG. Immunoglobulin M (IgM) antibodies are predominant during the active phase of disease and are produced immediately after infection, indicating an acute or recent infection (Yagupsky et al., 2019). Over time, IgM levels decline to background levels, and IgG becomes predominant in chronic infections. IgG antibodies suggest a long-term brucellosis infection before treatment (Qureshi et al., 2023). The presence of IgM without IgG can lead to the misdiagnosis of acute brucellosis. Commonly used serological assays include buffered agglutination tests such as the Rose Bengal test (RBT), enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT), and fluorescence polarization assay (FPA).

The Rose Bengal test (RBT) is a simple agglutination assay that determines the presence of Brucella antigens in a sample. It employs an 8% suspension of B. abortus antigens stained with Rose Bengal dye at pH 3.65 ± 0.05 (Yagupsky *et al.*, 2019). This test is straightforward and optimal for clinical laboratories with limited resources, it is user friendly and does not require specialized machines. Additionally, it is not prone to the prozone phenomenon observed in other serological tests such as the SAT. Studies have indicated that the RBT has high sensitivity (95%) and specificity (100%) (Qureshi et al., 2023). However, in endemic regions, studies have shown that the RBT has low sensitivity in cases of chronic infection (Díaz *et al*., 2011). Therefore, it is recommended that other tests such as PCR are utilized to confirm RBT results because of its low sensitivity.

The Serum Tube Agglutination Test (SAT) is considered the reference standard for serological tests (Al Dahouk *et al*., 2003; Yagupsky *et al*., 2019). It can also be used to detect *B. canis* because it identifies antibodies against Brucella S-LPS. (Yagupsky *et al*., 2019).

Enzyme-linked immunosorbent assay (ELISA) is an Antibody antigen based serological test for diagnosing brucella ssp and many other pathogens, thus is able to detect the presence of IgM antibodies and chacterize the titrs required for diagnosis. However, ELISA has limitations in that it is prone to false-negative results in the presence of excess IgG antibodies, in the contrary it leads to false positive reaction in the when the rheumatoid factors are present in the test serum. ELISA rapid diagnostic test kits are commercially available and can detect both IgM and IgG antibodies against Brucella antigens (Osoba *et al*., 2001; Xu *et al*., 2020). However, due to the general nonspecificity and sensitivity of serological tests, an alternative confirmatory will be required. With advances in technology, new non-S-LPS antigens, such as the outer membrane protein 19 kDa (OMP19), are being evaluated as potential antigens for diagnosing brucellosis (Golchin *et al*., 2023).

2.9.6 Polymerase chain reaction

Isolation of Brucella bacteria is considered the gold standard for brucellosis detection.
However, isolating this infectious pathogen is time-consuming, costly, and labor-intensive, It calls for a highly skilled technical team and a level 3 biocontainment lab for safe sample handling and processing including biotyping. Live cultures are highly hazardous and call for strict biosafety and biosecurity measures. To sidestep these grave dangers, more secure methods like polymerase chain reaction (PCR) have been embraced (Yu & Nelson, 2010).
The development of real time PCR technologies has further reduced the processing time as well as the risks of laboratory-based infections. PCR amplification is one of the most reliable methods for diagnosing brucellosis. It surpasses both serological and culture techniques in terms of sensitivity, specificity, and reproducibility (Wang et al., 2014). This method accurately detects both animal and human brucellosis (Yu &Nelson, 2010). Target genes typically include BCSP31, 16S rRNA, and the 23S ribosomal RNA operon (Chakravorty et al., 2007).

2.10 Conceptual Framework

A questionnaire was used to interview consenting patients and assenting minors on possible risk factors of exposure. Laboratory characterization confirmed Q-fever disease status in the sera of study participants.

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Data collection Methods
- Administration of questionnaires
- Laboratory characterization of samples and results

Variables

Dependent variables
-IFA Q fever results

Independent variables
-Brucella ssp results by ELISA, RBT, and FBAT methods -Sociodemographic variables
-Risk factors associated with Q fever disease
- Health seeking behavior of the study population
-Knowledge of Q fever disease by the study population
- Co-infection between C. burnetii and Brucella ssp. bacteria.

Outcomes

- Presence of C. burnetii IgG (phase I), and IgM (phase II) antibodies in sera of the study population in Meto health centre
- Significant Risk factors identified
- Co-infection of C. burnetii and Brucella ssp. among the study population

- Absence of C. burnetii antibodies

Figure 1: Conceptual Framework

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CHAPTER THREE

RESEARCH METHODOLOGY

3.1. Study Design

This was a prospective cross-sectional study that leveraged on human samples collected from patients presenting at Meto Hospitals with brucellosis like non-specific symptoms that included fever, joint and back aches and those with history of exposure

3.2. Study area

The study was carried out at Meto Health Centre located in Kajiado central sub county in Matapato South ward (Figure 2). The health center is situated at the border of Kenya and Tanzania, about 87 km from Kajiado town and 171 km from Nairobi city. It serves as a business hub for a myriad of cross-border activities. This facility is a referral site for three dispensaries in Matapato Southward, namely Oloimirimiri, CMF-Kumpa, and Ilpartimaro. It serves a catchment population of approximately 8700 persons spanning thirteen villages. Due to the free primary health care services in Kenya, communities from the neighboring country of Tanzania frequently cross the border and get treated at Meto Health center. The catchment area of this facility is largely occupied by nomadic pastoralist communities and who have close interaction with domestic animals and wildlife. The selection of the study area was mainly based on animal morbidity reports from the Veterinary department showing high burden of abortions by the domestic animals and reported cases of Animal Q fever disease in the Meto area.

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Figure 2: Map of Matapato South ward portraying Meto health centre location.

3.3. Sample size and sampling

3.3.1 Sample size determination

The formula n = Z[P(1- P)]/d2 was used to estimate the sample size (Naing et al., (2022). In order to determine the prevalence of acute and convalescent Q fever infections, 95% confidence level for a z score of 1.96 for a normal population distribution, and an absolute error of 5% (margin of error for ±5% i.e., 0.05), was taken into consideration.

P denoted prevalence or proportion and this was calculated from the mean of previous human Q fever studies done by (Nakeel et al., 2016), and (Mwololo et al., 2022) who reported seroprevalences of 26% and 24.44% (95% CI: 21.77–27.26) respectively. The average seropositivity of 25% from the two studies was adopted as the expected prevalence. A total of 147 paired serum samples was arrived at.

$$\text{Sample size} = \frac{Z_{1-\alpha/2}^{2}\, p(1-p)}{d^{2}}$$

where n = Sample size,

Z = Z statistic for a level of confidence (1.96 for 95% confidence interval),

P = Expected prevalence or proportion

d = Precision. (Margin of error for ±5% i.e., 0.05), (Naing *et al.,* 2022).

Substitution, Formula in simple form; n=Z[P(1- P)]/d2

$$\text{Sample size} = 1.96 \times \left[\frac{0.25(1 - 0.25)}{0.05^2}\right] = 147$$

3.4. Inclusion Criteria and Exclusion Criteria

3.4.1 Inclusion criteria

All consenting and assenting patients aged 5 years and above attending outpatient clinic presenting with fever (temperature ≥ 38 ^{0}C), acute or chronic complain of joint, back and calf muscle pains, and/or confirmed or suspected brucellosis. Patients who reported an exposure history such as consumption of raw milk or raw blood or those who reported to have assisted birthing animals were also considered.

3.4.2 Exclusion Criteria

Individuals excluded included those who did not agree to take part in the study, dissenting minors, patients below 5 years of age, and patients for which acute samples that were not paired.

3.5. Sample collection and processing

This study adopted and customized a structured pre-designed questionnaire from a Zoonotic study that was done in six semiarid areas of Kajiado county between the year 2020 and 2023.

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The questionnaire was administered to collect information on demographic data, knowledge of Q fever disease, Cultural practices that could lead to Q fever exposure and health-seeking behavior of the study population. An informed consent was obtained from all study participants, and informed assent from minors. Subsequently, 3 ml of venous blood was collected aseptically into labelled 4 ml vacutainer tubes with clot activators from the recruited study participants and were labelled as 'acute samples'. A second sample (labelled convalescent) was collected from the same study participants 14 days after the collection of the initial sample. Venous blood collection procedure was done as per the Safe-phlebotomy Standard operation procedure (SOP) that was developed in line with the National Infection Prevention and Control Guidelines for Health Care Services in Kenya, (2010). A total of 100 convalescent samples were collected at the second time point. Ninety-five patients did not turn up for convalescent sample collection hence, they were excluded from the study. The vacutainers were allowed to stand for 2 hours on the workbench to permit separation of serum from whole blood. The serum was then aliquoted into 2 ml cryovials labeled as per the initial label on the respective vacutainers. The serum samples in the cryovials were immediately refrigerated at 2 to 4 ^{0}C and later they were transported to Kajiado County Referral Hospital in batches for analysis. Cold chain condition was maintained all through until the samples arrived the testing laboratory where they were frozen at -20 ^{0}C. The management of the convalescent serum followed the same procedure as for the acute samples. Thawing was done during sample analysis sessions and after that the samples were returned to the freezer.

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3.6. Laboratory Analysis

3.6.1 Detection of *Coxiella burnetii* phase I and II antibodies

The patients' sera were analyzed using the immunofluorescence assay (IFA) test kit (Vircell *C. burnetii* I+II IFA IgG/IgM/IgA; Ref. PCOBU I+II - Abacus dx Spain). A Standard Operation Procedure (SOP) No: MMLS/2021/42471/004Version:1.0 was developed using the Vircell IFA kit insert. The kit contained a Vircell *Coxiella burnetii* phase I+II Slide: 10 slides with 10 pairs of wells each, coated with C. burnetii Nine Mile strain organisms (ATCC 616-VR). Each pair contained one well with phase I antigen and another with phase II antigen. Both antigens are formaldehyde inactivated and suspended in 0.5% normal chicken yolk sac, to improve the adhesion and avoid bacterial aggregation. The antigens are acetone fixed to the slide. Detection of phase I IgG antibody and phase II IgM antibody detection utilized two distinct procedures.

IgG determination procedure: Phosphate buffer saline (PBS), pH 7.2, was prepared in advance as directed by the manufacturer, and stored at 2-8ºC. A 1:64 dilution of each sample was prepared and 5 µl of the dilutions was added to the wells. The same was done for the positive and negative controls, and the slides were incubated in a humid chamber for 30 minutes at 37ºC. The slides were rinsed using PBS, and then were immersed for 10 minutes in a jar containing PBS, then rinsed with distilled water and allowed to air dry. The observation was done under the 400X objective using a Zeiss Primo Star (Olympus) fluorescent microscope.

IgM determination procedure: 25 µl of sample was added to 25 µl of PBS pH 7.2, making a 1:2 dilution. The diluted samples were treated with anti-human IgG sorbent. This was done by adding 10 µl of diluted sample to 50 µl of sorbent and mixing thoroughly using a vortex mixer. However, the control sera were not diluted and were not treated as well. Five µl of diluted sorbent-treated sample was added in every slide well of the IFA 10 welled slide negative and

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positive controls were added to each well and the slides were placed in a water bath set at 37ºC for 90 minutes. After that incubation, the slides were rinsed with PBS and then immersed for 10 minutes in a jar containing PBS after which we rinsed them using distilled water. The slides were allowed to dry in a drying chamber. Next, 5 µl of anti-human IgM FITC conjugate solution was added to each well including the control wells and incubated at 37ºC for 30 minutes. After the incubation the PBS and distilled water rinsing steps were repeated, followed by air-drying and examination of the florescent smears at 400X magnification by Zeiss Primo Star (Olympus) fluorescent microscope.

Preparation of phosphate buffer saline (PBS) at a pH of 7.2 was done beforehand as directed by the manufacturer, and stored at 2-8ºC. A 1:64 dilution of each sample was prepared and 5 µl of the wells were then diluted. Proceeding in the same manner, the positive and negative controls were placed in a humid chamber at 37ºC for 30 minutes to incubate their slides. The slides were rinsed using PBS, and then were immersed for 10 minutes in a jar containing PBS, I let it air dry after rinsing it with distilled water. The observation was done under the 400X objective using a Zeiss Primo Star (Olympus) fluorescent microscope.

Quality Control: Positive and negative controls were included in each test run to allow validation of the assay and kit.

Results: Positive results showed apple green fluorescence of cocco-bacillary morphology reflecting antibody staining against a dark background, while negative results did not show any fluorescence.

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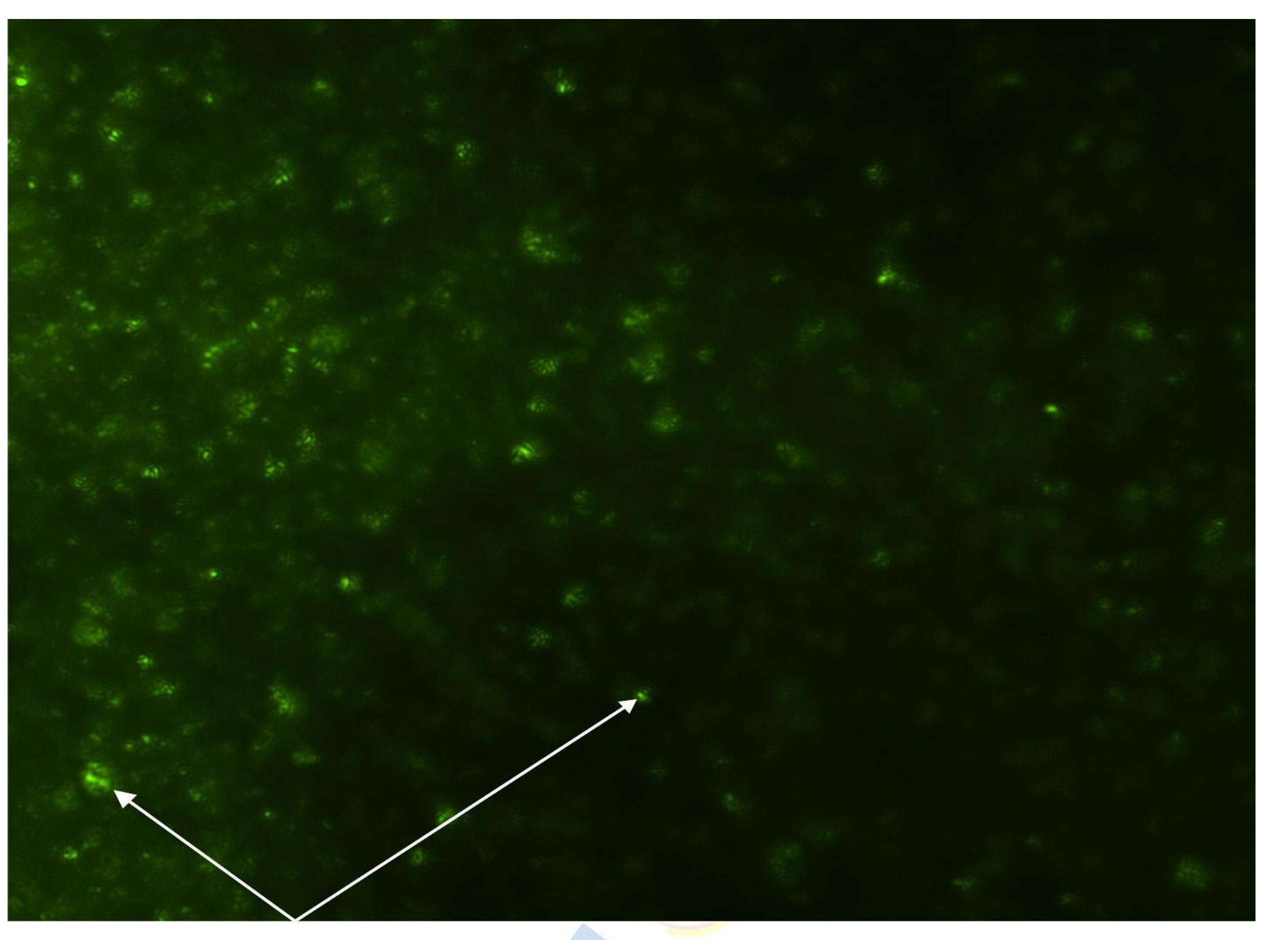

Figure 3: Photomicrograph of *Coxiella burnetii* IFA positive control slide *showing the* apple green fluorescence of cocco-bacillar morphology *(Vircell IgM PC), Image by Zeiss-ApoTome 195-041769, Imager Colibri 7- 2D*

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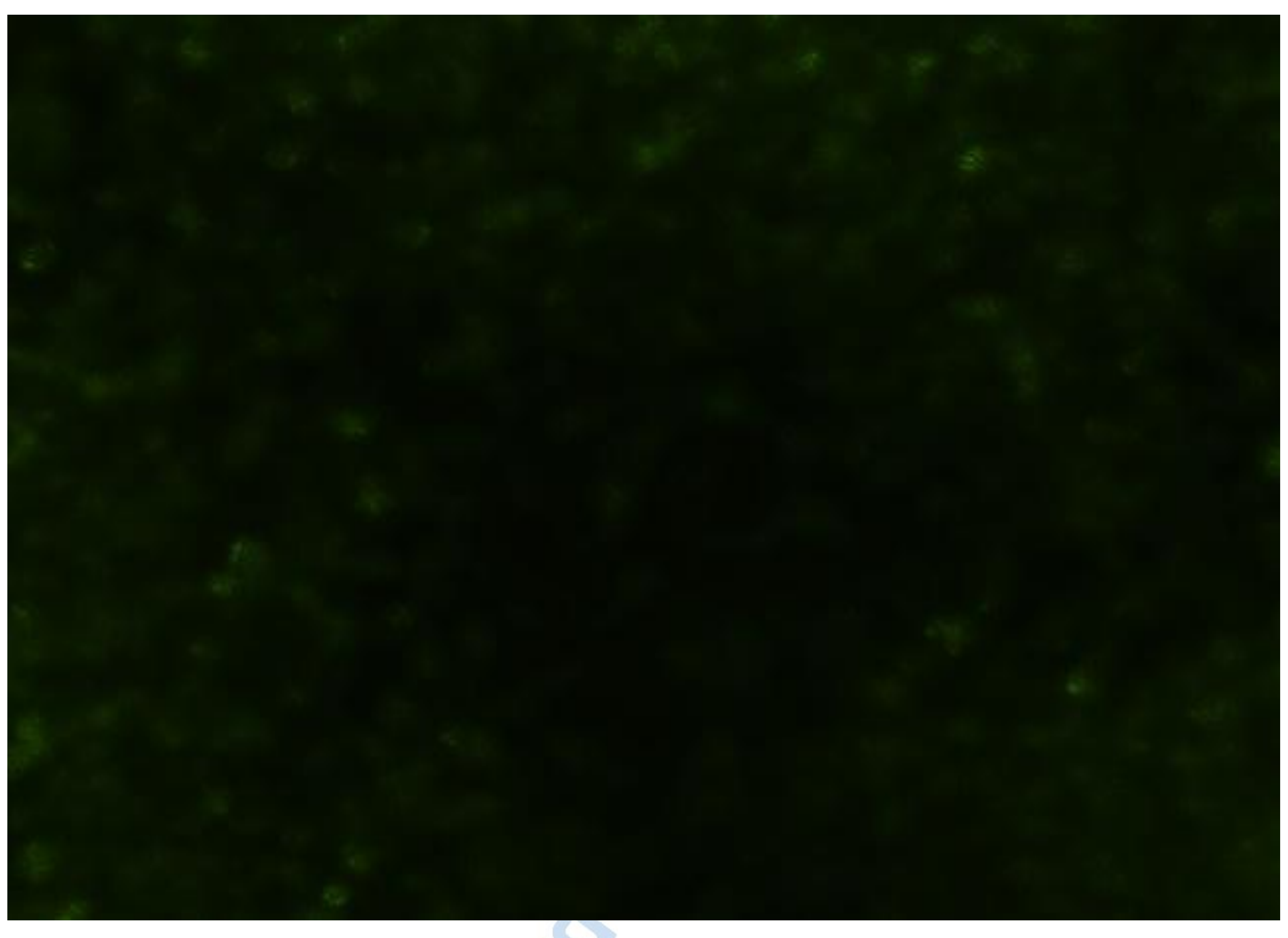

Figure 4*: Photomicrograph of Coxiella burnetii IFA negative control slide (Vircell IgM NC), Image by Zeiss-ApoTome 195-041769, Imager Colibri 7- 2D. Apple green fluorescence particles are absent***.**

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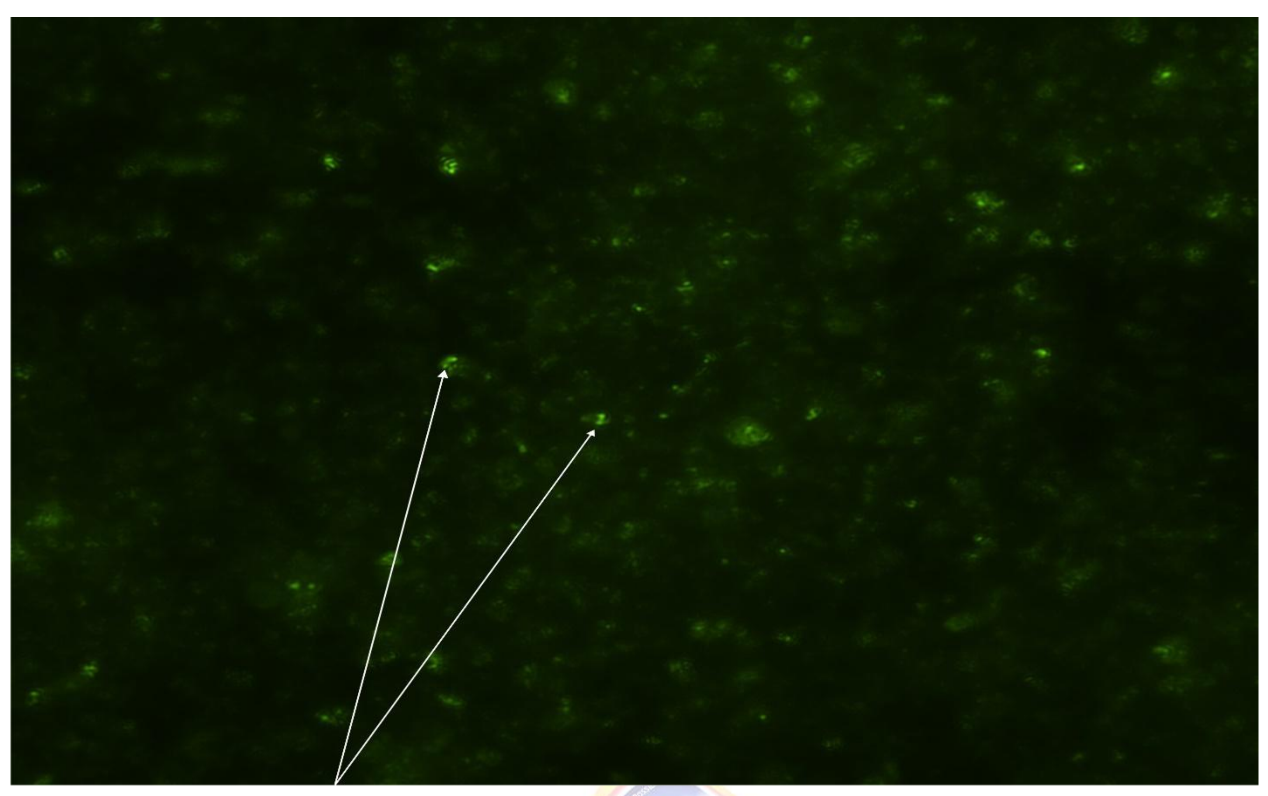


Figure 5: Photomicrograph sample showing positive IgM *Coxiella burnetii* antibodies

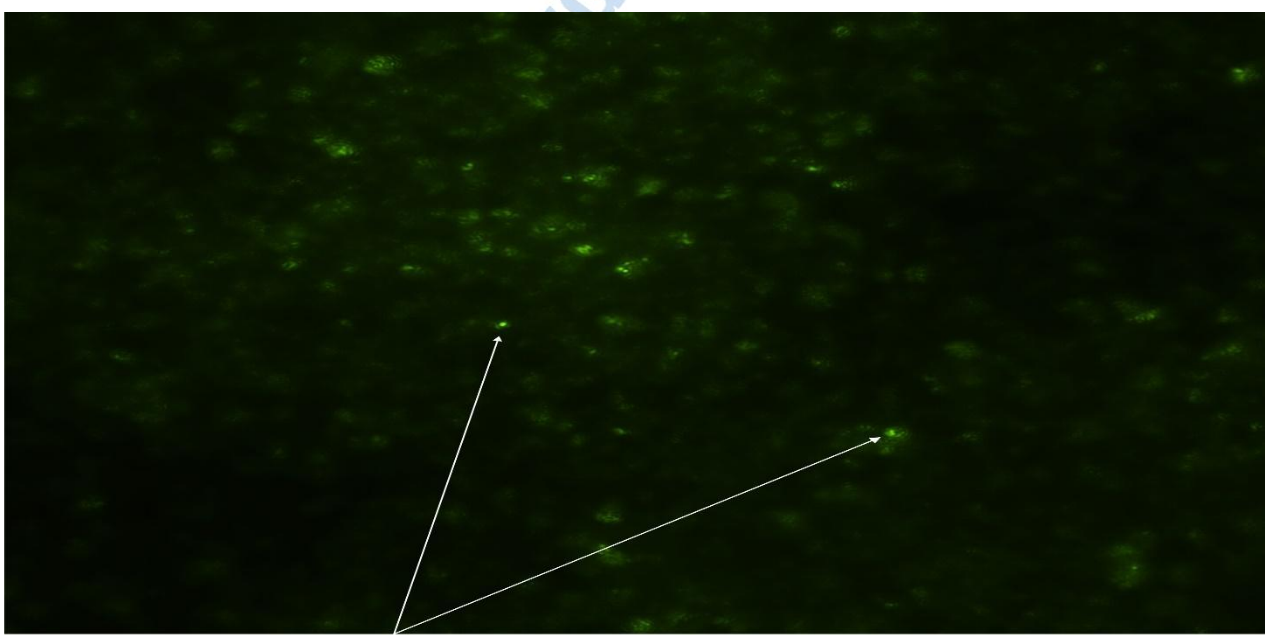


Figure 6: Photomicrograph sample showing positive IgG *Coxiella burnetii* antibodies

3.6.2. Indirect Enzyme-Link Immunosorbent Assay (i-ELISA) for Brucella spp. IgG.

The iELISA kit ((Brucella IgA/IgG/IgM ELISA, Immuno-Biologic Laboratories Inc. IBL - *America-Nr:V 116.15 –Lot no. EM0045*) was utilized to detect Brucella IgG antibodies in patient sera using the method specified by the manufacturer. All samples had their optical densities (OD) evaluated using a Biobase Elisa Microplate Reader (Bk-EL10c, Shandong, China) at wavelengths ranging from 450 nm to 630 nm. Duplicate runs of sera and controls were performed, and the OD measurements were compared. According to the manufacturer's guidelines, before assessment, the average optical density (OD) of the control substrate was subtracted from each sample's OD. The mean OD value of the standards was then multiplied by the quality control certificate's numerical data provided by the manufacturer in an insert, to find the threshold values, both high and low. For the specific kit used, the quality control certificate indicated a Lot specific corrective factor of 0.666, a reference OD value of 0.93, a validity ranges of 0.47 to 1.58, and a gray zone of 14%. The lower cut-off and the upper cut-off were therefore calculated as follows; lower cut-off: (0.93 *0.666) – 14% = **OD** 0.533, and upper cut-off: (0.93 * 0.666 + 14% = **OD** 0.706. Assuming an optical density (OD) of 0.706 or above, a test sample was deemed positive and a sample result was considered negative if the optical density was equal or lower than OD 0.533. The range between the upper and the lower ODs was termed as gray zone, the ODs in the gray zone were considered indeterminate. In this study, these values were categorized as negative.

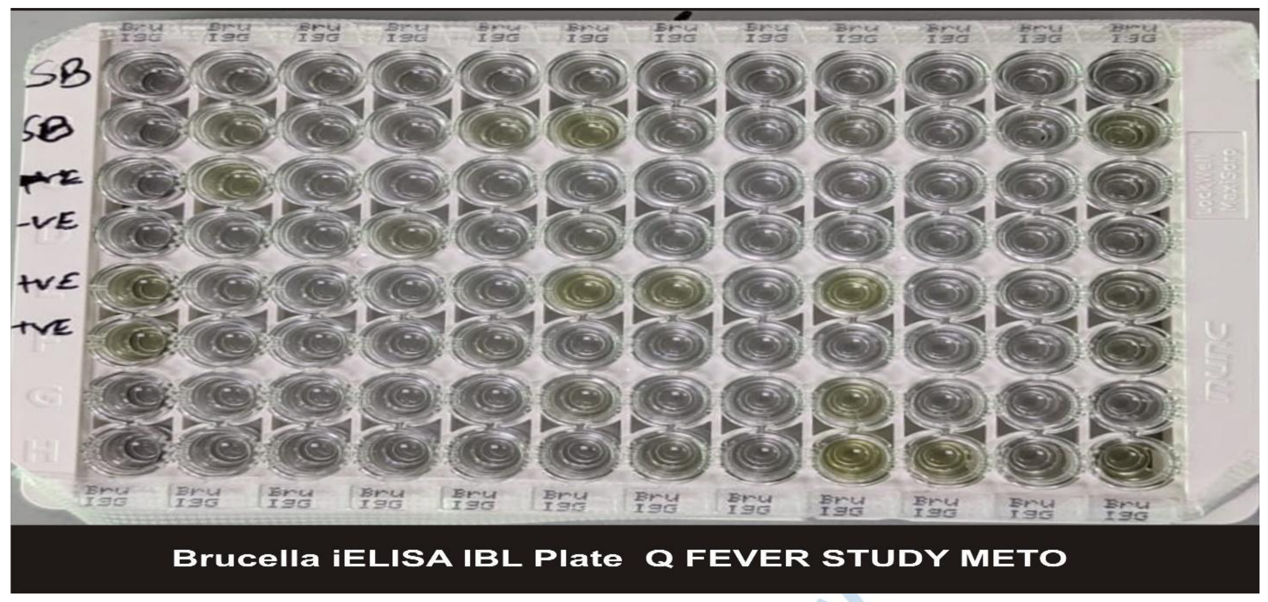
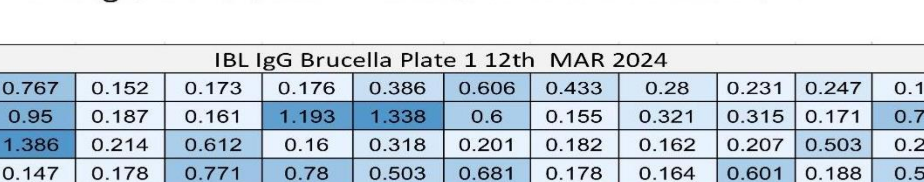


Figure 7: (a) Brucella Indirect ELISA microtiter plate showing color change immediately after adding the stop solution.



IBL IgG BRUCELLA iELISA PLATE AMERICA

	IBL IgG Brucella Plate 1 12th MAR 2024											
SB A	0.169	0.767	0.152	0.173	0.176	0.386	0.606	0.433	0.28	0.231	0.247	0.164
SB B	0.218	0.95	0.187	0.161	1.193	1.338	0.6	0.155	0.321	0.315	0.171	0.787
NC C	0.176	1.386	0.214	0.612	0.16	0.318	0.201	0.182	0.162	0.207	0.503	0.213
NC D	0.177	0.147	0.178	0.771	0.78	0.503	0.681	0.178	0.164	0.601	0.188	0.504
PC E	0.979	0.158	0.189	0.42	0.255	1.734	1.479	0.57	0.938	0.199	0.308	0.198
PC F	0.97	0.564	0.44	0.421	0.693	0.349	0.186	0.168	0.225	0.182	0.184	0.318
Pt G	0.25	0.51	0.277	0.634	0.39	0.187	0.177	0.203	0.993	0.516	0.416	0.159
Pt H	0.166	0.249	0.157	0.207	0.157	0.185	0.654	0.25	1.598	0.885	0.821	0.721

Figure 8: ELISA reader print out showing the Optical Densities of Brucella results

3.6.3 Detection of Brucella spp. IgG antibodies using the Rose Bengal Test (RBT)

Using the Atlas Medical-PPI1503A01- Rev A (02.09.2019), Blankenfelde-Mahlow, Germany Rose Bengal slide agglutination test, Sera from the research subjects were quantitatively analyzed for antibodies to Brucella ssp. The suspension of strain S99 of *Brucella abortus*, preserved at pH

3.6 with 1 mol/L of lactate buffer and 5 g/L of phenol, was the source of the Rose Bengal Brucella antigens. Every batch of slide reactions carried out during sample processing was accompanied with a positive control animal serum containing an anti-*B. abortus* at a concentration of 50 IU/ml and a negative control animal serum that both contained preservatives. After the reagents and the samples have attained room temperature, equal drops of reagent and patient sample (50 μl) were placed on a reaction tile and mixed using a different stirrer for each sample. The next step was to shake the response tile for four minutes to initiate the reaction. The results were examined macroscopically for presence or absence of agglutination. Any agglutination that occurred between one and four minutes was scored positive (+), After four minutes of rocking, the lack of agglutination was considered negative (−). Every plate that did not exhibit agglutination was given a negative score. The presence of agglutination indicated an anti-Brucella concentration equal or greater than 25 IU/ml, this was calculated using the formula 25 x anti-Brucella Titer = IU/Ml as provided in the kit insert.

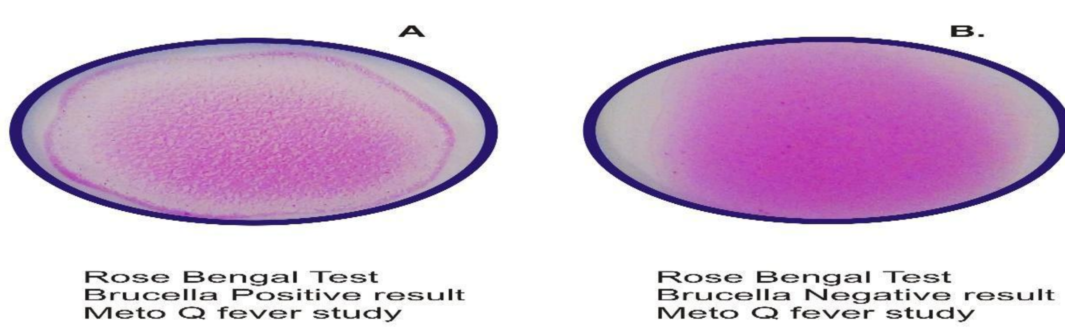


Figure 9: Rose Bengal positive antibody antigen reaction showing visible agglutination, B) Rose Bengal negative reaction. The sample is homogeneous, there is no agglutination.

3.6.4 Detection of brucellosis using rapid diagnostic tests (FBAT)

Brucella abortus and *Brucella melitensis* antibodies were characterized by use of Biomax Maxwin Health-PVT Febrile Brucella Agglutination Test from Tamilnadu, India. When the supplies and reagents became equilibrated to room temperature, two separate drops of neat serum were set on a white reaction tile. As per the guidelines for performing slide agglutination, one drop of *B. abortus* antigen was added to the first drop of patient serum on the reaction tile, subsequently, a drop of *B. melitensis* antigen was put on the other serum drop on the second reaction area on the tile. The serum and antigens were mixed with separate stirring sticks until the mixtures were homogenous. The tile was gently rocked for up to 2 minutes whilst looking for clumping. Positive and negative control tests were also done alongside the patient samples. When clumping was evident, the result was recorded as positive reaction and when there was no clumping, it was considered to be a negative reaction.

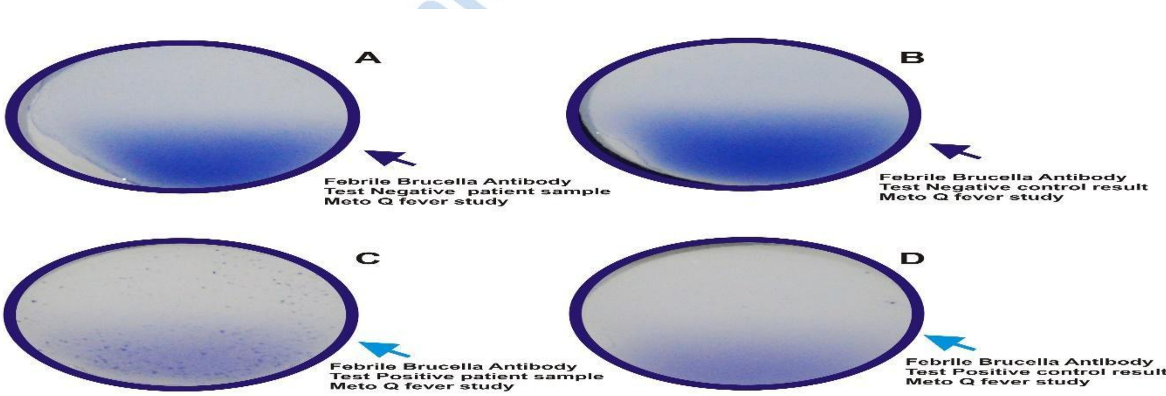


Figure 10: Febrile Brucella antibody test (FBAT)

A – negative sample, B – negative control, C – Positive sample, D – Positive control.

3.7. Data management and analysis

. The biographic data of patients was collected by a questionnaire uploaded in the Google Forms. The answers the participants provided in the Google form was downloaded and transformed into an Excel file in Microsoft Excel® 2013. Analyzing this data was done under the Statistical Package for the Social Sciences (SPSS for Windows version 17). 0 (SPSS, 1999: Sociological Plans Statistical, SPSS Inc., Chicago, Illinois). The findings were presented in tabular forms and in pie charts on frequencies or percentage of each variable. In assessing whether the collected information was qualitatively significant, the Chi square test was performed. Average prevalence estimates and their Wilson score interval was computed on the prevalence values for each survey. Using the binary logistic regression analysis, the hypothesis that Q fever seroprevalence in the general population was associated with risk factors was tested. A p value < 0.001 was deemed statistically significant.

3.8. Ethical Approvals

Ethical approval was acquired from the Mount Kenya University Ethical Review Committee (MKUERC). evidenced by the Ethical clearance letter reference number MKU/ISERC/2896. The research permit license number NACOSTI/P/23/2774 was provided by the National Commission for Science Technology and Innovation (NACOSTI). The authorization to carry out the study at Meto Health Centre was granted by the office of the County Director of Medical Services, Kajiado County, in a letter dated 31st July, 2023, REF: CGK/MEDICALSERVICES/01/VOL.111/141. The final approval being the informed Consents and Assents given by the study participants to participate in the study.

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CHAPTER FOUR

RESEARCH RESULTS AND DISCUSSION

4.0 Introduction

The results section illustrated the study findings of *Coxiella burnetii* infections in Meto Health Centre in Matapato South, Kajiado County, Kenya. The study was aimed at establishing the seroprevalence among febrile patients in Meto Health Centre, Kajiado County, Kenya. Based on an evaluation of the serological data and assessment of risk factors, the research raises awareness about the current state of Q fever in that region. The results provide overall prevalence rates of *C. burnetii* phase I (IgG) and phase II (IgM) antibodies, and the associated sociodemographic risks factors among the study participants, thus focusing on the relevance of the problem in the framework of population health and the need for the enhancement of diagnostic and intervention.

4.1 The seroprevalence of *Coxiella burnetii* IgG (phase I) and IgM (phase II) antibodies among patients presenting with Brucella-like symptoms at Meto Health Centre.

Table 1 presents chi square test of the seroprevalence of human anti *Coxiella burnetii* IgG and IgM antibodies as well as Brucella IgG antibodies that were characterized using three serological tests namely, iELISA, RBT, and FBAT.

Table 1: Seroprevalence of human anti-*Coxiella burnetii* IgG and IgM antibodies and antibodies of *Brucella spp*. by iELISA, RBT and FBAT methods among patients presenting with brucella-like symptoms seeking treatment in Meto Health centre in Kajiado, Kenya.

Serological test N=100	**Number of positives**	**Prevalence % (95% CI)**	**Significance (2 tailed p value)**
Acute/Phase II Q fever (IgM IFA)	27	27.0 (19.27-36.43)	<0.001
Chronic/convalescent/Phase I Q fever (IgG IFA)	49	49.0 (39.42-58.65)	0.920
Brucellosis (iELISA)	13	13.0 (7.76-20.98)	<0.001
Brucellosis (RBT)	1	1.0 (0.18-5.45)	<0.001
Brucellosis (FBAT)	6	6.0 (2.78-12.48)	<0.001
Q fever and Brucellosis coinfection	**11**	**11 (8.25-18.63)**	**<0.001**

RBT - Rose Bengal test; iELISA Indirect Enzyme linked immunosorbent assay; FBAT- Febrile Brucella agglutination test; IFA- Immunofluorescence assay; CI-Confidence intep-valuerval; p value – probability value

4.2 Seroprevalence of human brucellosis by age and gender.

Table 2 Presents seroprevalence of brucellosis by age category and gender. This information was of crucial importance as a differential for Q fever diagnosis. The two diseases have similar characteristics; they are both zoonotic infections that may be asymptomatic or may present with

non-specific febrile illnesses that are either organ-specific or multi-organ disease. Likewise, the mode of transmission for the two zoonosis is largely similar.

Table 2: Seroprevalence of human brucellosis by age category and gender (2x2 cross-tabulation of dependent factor and independent factors p<0.05)

Variables	N	Positive	Sero-prevalence (%)	Chi sq.	*p-value*
Demographic factors					
Age Category					
5 -12 years	4 (4.0%)	1 (0.0)	25.0	1.830	0.608
13 – 24 years	4 (4.0%)	0 (0.0)	0.0		
25 – 59 years	67 (67.0)	14 (11.0)	20.9		
≥ 60 years	25 (25.0)	4 (5.0)	16.0		
Gender					
Male	34 (34.0)	9 (6.0)	26.5	1.74	0.676
Female	66 (66.0)	11 (10.0)	16.7		

Note: N = 100 Prevalence was calculated as (infected/examined) *100

Statistically significant variables at ($p < 0.05$)

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4.3. Seroprevalence of *Coxiella burnetii* and *Brucella spp*. co-infection among the study population at Meto Health Centre.

Q-fever and Brucellosis co-infections.

This section investigated the seroprevalence of Q-fever and Brucellosis co-infections among the study population at Meto Health Centre, focusing on how these infections were distributed by age and gender. Table 3, presents the co-infection rates across different age groups and between males and females, using univariate analysis (chi square) to determine the significance of these sociodemographic factors. Understanding these trends is critical to identifying risk groups and developing treatments to limit the zoonotic sickness spread among the population.

Table 3: *Coxiella burnetii* and *Brucella spp*. co-infection distribution among the study population of the Q fever study at Meto health Centre, Kajiado County, ($p<0.05$)

	N	**Number co-infected**	**Co-infection prevalence % (95% CI)**	**Univariate analysis *P* value**
Age category				
5-12	4	0	0.0 (-)	0.132
13-24	4	0	0.0 (-)	
25-59	67	8	11.9 (6.18-21.83)	
> 60	25	3	12.0 (4.17-29.96)	
Gender				
Male	34	5	15.2 (6.65-30.92)	0.763
Female	66	6	8.7 (4.17-18.19)	

* Statistical significance difference between different groups of univariates ($P < 0.001$) by Chi-square test

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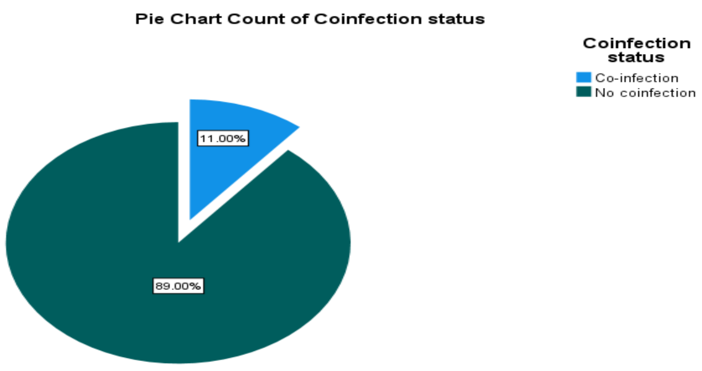

Figure 11: Co-infection status of *C. burnetii* and *Brucella spp*.

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4.4. Objective 3: Sociodemographic risk factors associated with Q-fever infection among the study population at Meto health centre

Table 4, 5 and 6 outlines the sociodemographic behavior and health seeking behavior variables that put people at risk of contracting Q fever disease from infected animal products or from contaminated environment. The tables present an overview of the sociodemographic factors and exposure risks such as occupation, and cultural practices, and how they influenced the outcome of the study results. In statistical analysis, the chi-square test was used to evaluate the correlation between these sociodemographic risks and the seropositivity of Q-fever disease among the study participants.

Table 3: Objective 3: Sociodemographic predictors of Q-fever infection among study participants in Meto Health centre, Kajiado Kenya, by Gender , Age and Occupation(n=100)

variable	category	Total (n=100)	positive	Positive (%)	X^2	P value
Gender	Female	66	40	60.6	0.160	0.689
	male	34	22	64.7		
Age	13-24	4	3	75	1.365	0.714
	25-59	67	39	58.2		
	5-12	4	3	75		
	60+	25	17	68		
occupation	Formal employment	1	1	100	3.037	0.219
	Livestock farming	76	50	65.7		
	Mixed farming	23	11	47.8		

* Statistically significant difference between different groups of univariates ($P < 0.05$) by Chi-square test

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Table 4: Objective 3: Sociocultural risks factors associated with Q-fever infection among study participants in Meto Health centre, Kajiado, Kenya, by exposure.

variable	category	Total (n=100)	positive	Positive (%)	X^2	P value
Do you boil milk at home?	Yes	91	54	59.3	3.035	0.081
	No	9	8	88.9		
Assisting animal in birthing	1 month ago	20	17	85	28.92	<0.001
	1 week ago	28	24	85.7		
	1 year ago	5	2	40		
	2 weeks ago	26	13	50		
	3 months ago	6	4	66.7		
	6 months ago	16	2	12.5		
Taking row blood in ceremonies	Yes	52	41	78.8	13.04	<0.001
	No	48	21	43.8		

* Statistically significant difference between different groups of univariates ($P < 0.05$) by Chi-square test

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Table 5: Objective 3: The health-seeking behavior of the study participants in Meto Health centre, Kajiado, Kenya.

variable	category	Total (n=100)	positive	Positive (%)	X^2	P value
Number of times the study participant visited a health facility for the same illness	2 times	32	24	75	11.46	0.075
	3 times	26	15	57.7		
	More than 4 times	24	16	66.7		
	Today alone	19	7	36.8		
Duration from onset of illness to the day of interview	1-3 days ago	8	5	62.5	102.7	<0.001
	1 month	30	16	53.3		
	1 week ago	31	21	67.7		
	1 year	18	12	66.7		
	3 months	7	4	57.1		
	6 months	6	4	66.7		

* Statistically significant difference between different groups of univariates ($P < 0.05$) by Chi-square test

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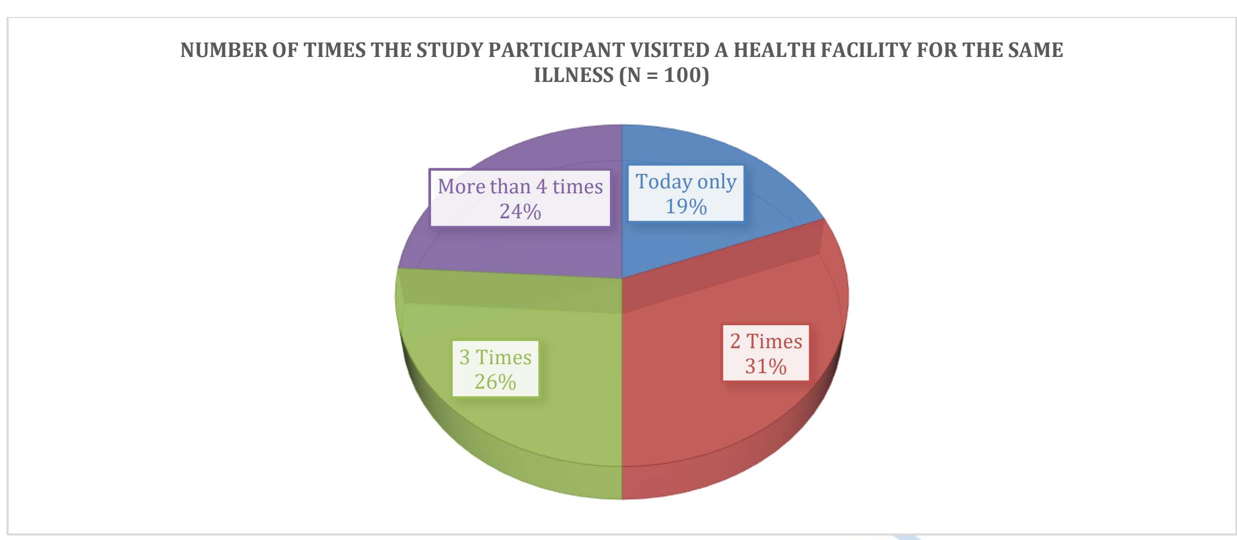

Figure 12: Multiple hospital visits by the study population for the same illness

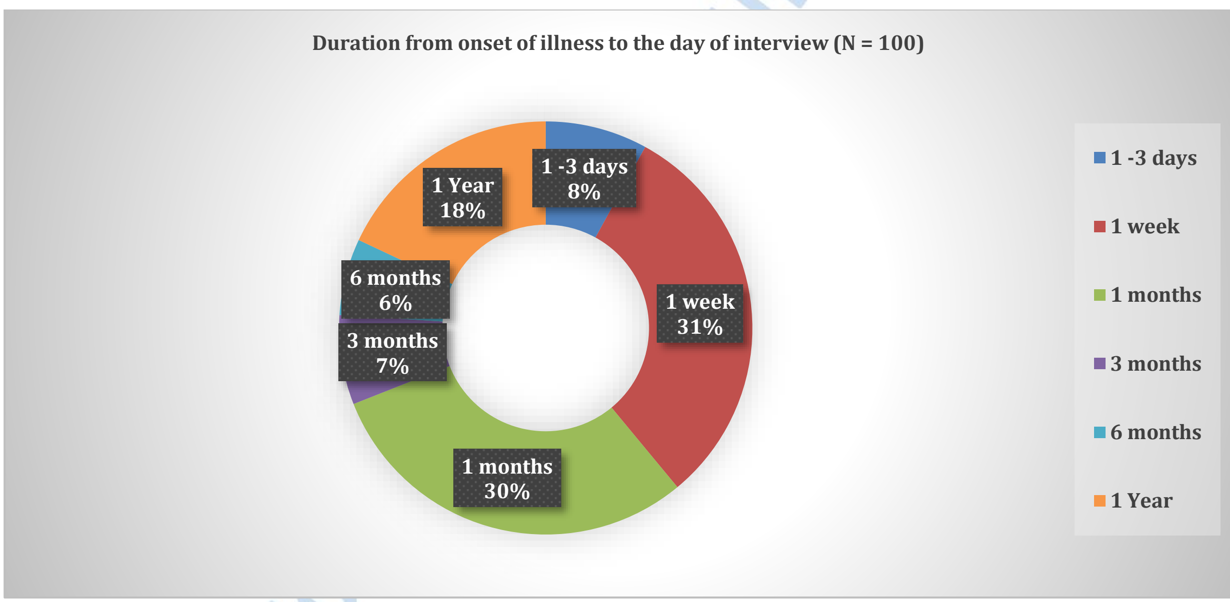

Figure 13: Duration from onset of illness to seeking health services in a health facility by the study population

4.5. Knowledge of Q fever disease by the study population from Meto area of Kajiado county

Figure 12, displays knowledge level of the study population on Q fever disease. This variable was important to inform whether or not the community was aware of zoonotic diseases, specifically Q fever disease. The findings from this variable was also aimed at guiding in the improvement of health fluency among the populations living in Meto. Inadequate health fluency has proved to result in limited healthy choices, risky behavior and frequent sicknesses, and this needed to be understood, for effective interventions.

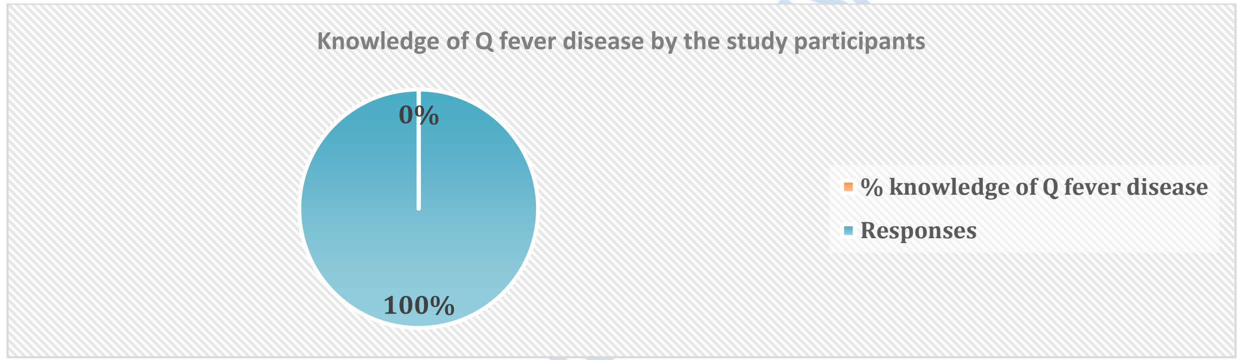

Figure 14: knowledge level of the study population on Q fever disease, (N=100).

4.6. Discussion of results.

4.6.1 Seroprevalence of *Coxiella burnetii* IgG (phase I) and IgM (phase II) antibodies.

Table 1 used binomial test to analyze the apparent seropositivity of Querry fever and brucellosis per serological method. The phase II (IgM) antibodies and the phase I (IgG) antibodies of *C. burnetii* pathogen were characterized using Indirect Immunofluorescence assay (IFA).

The presence of phase I antibodies to *C. burnetii* suggested active Q fever disease (acute Q fever) among the study population. Presence of phase II IgG, antibodies suggested chronic infection and that the population has exposure to *Coxiella burnetii* infection. The seropositivity of Phase

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II antibodies was 27.0% (95% CI: 19.27-36.43%) and was higher **than** findings by (Lemtudo et al., 2021 who reported acute phase II, IgM antibodies seropositivity of 11.0%. Subsiquently, the IgG antibodies were reported at 49.0% (95% CI: 39.42-58.65%), which was comparable to findings by (Knobel et al., 2013) who reported *C. burnetii* IgG antibody seropositivity of 30.9%, **but the IgG seropositivity** was much higher than reports by (Lemtudo et al., 2021) and (Nakeel et al., 2016) who both recorded seropositivity of 26%, while (Mwololo et al., 2022) reported a much low**er positivity** of 18%.

4.6.2 Seroprevalence of *Coxiella burnetii* and *Brucella spp.* co-infection among the study population at Meto Health Centre.

The characterization of antibodies against *C. burnetii* and *Brucella ssp.* bacteria was done using the 100 acute samples, (Table 2). 11 samples were positive for both diseases with 11.0% (95% CI: 8.25-18.63). The coinfection was mainly reported among the age categories of 25 to 59 years that had 8/67 positives and a coinfection rate of 12.0% (95% CI: 6.18 – 21.83) This was followed by the older population, (>60) where 3/25 positive cases were positive, translating to a coinfection rate of 12.0% (95% CI: 4.17– 29.96). The 11.0% Q fever and Brucellosis coinfection rate recorded in this study was much elevated than the 1.13% reported by (Ta et al., 2022), in a prevalence survey of Coxiellosis and Brucellosis among humans in Northeastern Mongolia. Ages 25 -69 and >60 were the most affected and this finding also agreed with (Ta et al., 2022), which pointed the same age category being the most affected. This may be supported by the fact that the two zoonoses are occupational-related. This middle-aged group in many population setups are mostly employed in abattoirs, they attend to sick animals or assist in birthing and in the process become more exposed to contaminated animal materials. The older population also interact with animals coupled by the physiology factors that tend to lowered immunity.

The 5-12 and 13 -24 age categories did not exhibit coinfection and were reported at 0.0% coinfection. Additionally, the univariate analysis ($P < 0.001$) by Chi-square test showed that the sociodemographic factor of age was not statistically significant to influence Q fever and Brucellosis coinfection.

4.6.3 Seroprevalence of human brucellosis among the study participants at Meto Health centre

Human brucellosis was determined in this study as a differential for Q fever (Table 3). Three serological methods were utilized to determine Brucella seropositivity. The Rose Bengal test yielded seropositivity of 1.0% (CI:0.18-5.45; p<0.001) according to Table 3, whereas the febrile Brucella antigen test (FBAT) and the iELISA test yielded a seropositivity of 6% (CI:2.78-12.48, p<0.001) and 13% (CI:7.76-20.98, p<0.001) respectively, giving an almost similar trend of seropositivity of Brucella antibodies by varying serological methods in other studies. Munyua and colleagues (2021) reported 16.5% positivity for Brucella antibodies by IgG ELISA, 2.1% by IgM ELISA and 1.7% by RBT and Nakeel et al., 2016) reported human brucellosis seropositivity of 1.3% by RBT method.

Another study conducted in Marigat and Koibatek areas in Baringo County utilized the c-ELISA method, revealing a low seropositivity of 0.7% and 0.5% respectively (Lokamar, et al., 2022). Individual and household study conducted in Garbatula, Isiolo County, Kenya reported high seroprevalences of brucellosis at 54.0% (95% CI: 50.2–58.0) and 86.4% (95% CI: 84.0–89.0), respectively (Mwatondo et al., 2023). Marsabit and Narok counties have reported relatively high seropositivity of human brucellosis **by** at 40.1% (95% CI 32.5–44.8) **by ELISA method**, and 19.3% (95% CI 14.5–24.9, n = 238) for the same samples by RBT (Akoko, et al., 2021). The prevalence reported in this study was lower to previous findings in Nigeria, Bauchi State, South West Uganda, and the Southern District of Zambia, where prevalence rates were reported at

33.5%. 14.9%, and 20.3% by (Ukwueze et al., 2022, Bushoborozi et al., 2023, and Mubanga et al., 2021), respectively.

The varying brucellosis seroprevalence findings over time could be because of the priority that has been given to brucellosis in Kenya where strategies to decrease the disease in animals, mostly through animal immunization and community sensitization on preventive strategies, have been given significant attention. The techniques used to determine the seroprevalence of Brucella has consistently shown variations. Rose Bengal test maintained a low positivity of 1% as is reported in this study which agreed with 1.7% seropositivity as revealed in a study done in Marigat, Baringo county (Lokamar et al., 2022). The Meto study reported a higher seropositivity of 13% by ELISA method. Other studies that utilized the ELISA technique reported a high seropositivity as well of up to 54% (Lokamar et al., 2022; Mwatondo et al., 2023).

Sociodemographic risk factors associated with Brucellosis in Matapato South Ward of Kajiado Sub County were found to be non-significant for age and gender, these results align with cross-sectional studies on Q fever sero-epidemiology among pastoralists in northern Kenya (Muema et al., 2022; Muturi et al., 2018), although some studies have registered considerable correlation between age, gender and brucellosis.

Brucellosis in children aged 5-12 years was high at 75% (table 3). This was similar to earlier findings in Uganda and Tanzania (Hildegalda et al., 2022; Tumwine et al., 2015). This is explained by the customary role that young males play in pastoral communities' management of cattle. Early pastoralism upbringing and everyday livestock activities exposes adolescent males, thus increasing their risk of contracting brucellosis as earlier argued elsewhere (Hildegalda et al., (2022).

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In a separate study on risk factors for brucellosis, it was also noted that gender was not significantly linked to Q fever occurrence (Mwololo, et al., 2022). Similarly, studies in Uganda among agro-pastoralist communities found that gender was not a significant risk factor for brucellosis (Nguna et al., 2019; Tumwine et al., 2015). Several other studies in Kenya have reported similar findings.

4.6.4. Sociodemographic risk factors associated with Q-fever infection and their correlation with study results.

The sociodemographic exposure to Q fever infection was put in three categories under specific objective 3 variables. The first category was the sociodemographic factors of Age, Gender, and occupation, (Table 4), and the second category was the Sociocultural risk factors associated with Q fever infection (Table 5). Lastly, the variables addressing the health-seeking behavior of the study participants (Table 6), and lastly, knowledge of Q fever disease by the study population, figure 14. Cross-tabulation test was executed to evaluate the correlation between age category and gender on the outcome of Q fever study results. It was evidenced that, age and gender did not influence *C. burnetii* infection. ($p>0.714$) among the study population.

4.6.4.1. Sociodemographic risk factor variable by Gender

Univariate analyses within the demographic variables by Chi-square evaluation did not reveal any consequential variation of Q fever prevalence by gender (P = 0.69). Males (22/34, 64.7%, CI 49.61-80.25) and females 40/66 (60.6%, CI 49.22-71.95) had similar prevalences, with the females depicting slightly lower seropositivity. The majority of the study population were female (N=66); (66%). This was in agreement with the Kajiado County Outpatient services utilization data, reflected in the district health information software, version 2, (DHIS2) - MOH 705B (Revised 2020), which includes outpatient summery aggregate data for five years. These reports

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have consistently and continuously shown that women utilized outpatient (OP) services more than men. This can be supported by the fact that women have other scheduled services such as the antenatal clinics, the child welfare clinics and the family planning appointments that they ride on and seek for other medical consultations at the outpatient.

Although gender was not associated as a risk factor for Q fever infection in our study, men depicted a high frequency 64.7% of Q fever suggesting that they were more susceptible or exposed to the disease compared to the female. Previous findings (Eldin et al., 2017; Rodriguez-Alonso et al., 2020) also supported this possibility. This lends further credence to the prior argument (Eldin et al., 2017; Loenhout et al., 2015) that men are more likely than women to appear with symptoms of Q fever. This can be further explained by the phenomenon of hormonal changes following puberty, in particular the release of 17-ß-estradiol, a hormone synthesized primarily in the ovaries that is the most abundant estrogen in non-pregnant females of reproductive age and reported to have immunoregulatory effects against pathogens (Collins et al., 2022: Estrada et al., 2018).

4.6.4.2. Sociodemographic risk factor variable by Age

The apparent seroprevalence of Q fever was high across all age categories, however, this demographic variable was not a statistically significant factor and thus, did not influence seropositivity of Q fever disease among the study population. Q fever prevalence at 95% CI, by age category presented as follows: children aged 5 – 12 years, 3/4 (75.0%, CI 30.06-95.44), adolescents/youths aged 13 – 24years, 2/4 (50.0%, CI 15.00-85.00), adults, 25 -59years, 41/67 (61.2%, CI 49.22-71.95), and the elderly, 60 years and above had a seroprevalence of 17/25 (68.0%, CI 48.41-82.79), (P=0.714) (Table 4), Children aged 5-12 years depicted a high prevalence of Q fever (25%) in the study population, implying that this group of individuals is

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more exposed to the disease compared to the adolescents, adults, and the elderly. Also, a previous study conducted in Kajiado (Nakeel et al., 2016), showed individuals aged 5-18 years were more exposed to Q fever and supported by a study on pediatric Q fever (Cherry et al., 2020), that reported positive cases among children.

4.6.4.3. Sociodemographic risk factor variable by Occupation

Q fever prevalence varied significantly based on occupation of the individuals presenting for treatment at the health centre. A larger fraction of livestock farmers (51/76, 67.1%, CI 55.94-76.62) had been exposed to the disease compared to those practicing mixed farming (11/23, 47.8%, CI 29.24-67.04) (p=0.219, Table 4. This could be because the community living in Meto and the entire Matapato South ward of Kajiado county are pastoralists who depend on the animal products as their staple food, hence close interaction and exposure to zoonotic diseases. Previous studies have associated occupation with Q fever transmission (Groten *et al*., 2020).

4.6.5. The sociocultural risk factors associated with Q fever infection in relation to study results by exposure variables.

Boiling milk at home, assisting animals in birthing and taking raw milk during community ceremonies were assessed in this study. (Table 5). Consuming unpasteurized milk has been evidenced to be a potential mode of transmitting *C. burnetii* to the exposed population (Winter, 2021; Ghugey et al., 2021). Out of the 92 study participants in Meto, who boiled milk, 50 of them were positive for Q fever antibodies (seroprevalence of 54.3%). However, out of the 100 study participants interviewed, only 8 individuals did not boil milk, with one testing positive with a corresponding seroprevalence percentage of (12.5%). As such, the variable resulted as not statistically significant, (p=0.139) and could suggest that the high seroprevalence of Q fever

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among the individuals who boiled milk in their homes would be associated with other risk factors other than boiling milk.

On the other hand, assisting birthing animals was clearly was substantial in the occurrence of Q fever among the study subjects. The univariate analysis denoted statistical significance ($p<0.001$). The variables for this risk factor were assessing the duration from exposure by assisting a birthing animal to the day the study participant visited the health facility with brucellosis-like symptoms. The respondents that had durations of 1 week, 2 weeks, 1 month and 2 months depicted high Q fever seroprevalence: 24/28=85.7%, 13/26=50%, 17/20=85%, and 4/6=66.7%, respectively. Respondents with a duration longer than 6 months exhibited a lower Q fever seropositivity (2/15=13. 3%). Thus, the result of the analysis showed that the independent variable that assessed exposure associated with assisting birthing animals revealed evidence of the zoonoses among the study participants. Consequently, these findings agreed with the findings of a survey on the associations of occupational risk factors with Q fever regarding different occupations (Groten et al., 2020), that reported that exposure to birth fluids and foetal tissues increased the risk of *Coxiella burnetii* infection, hence the associations of occupational risk factors with Q fever and the need to use protective attires as a control measure. Depending on the type of animal and the shedding route, it has been reported that infected ruminants may continue to discharge pathogens into the environment for prolonged periods without showing any signs (Muema et al., 2022).

Another variable examined in this study was the consumption of raw blood, especially during cultural ceremonies. The univariate test depicted a p-value of less than 0.001, indicating a direct correlation between raw blood consumption and the risk of contracting Q-fever. Out of 52 respondents who consumed raw blood, 41 contracted the Q fever disease and the seroprevalence ratio was 78.8%. Consequently, out of a total of 48 respondents who did not consume raw blood,

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21 of them tested positive for the disease which gave a seroprevalence of 43.7%. This proportion is enough to express that the population is at risk of exposure to the two zoonoses, Q fever, and Brucellosis.

Like the majority of *C burnetii* studies, this study was limited to the determination of only three social demographic risk factors that have been discussed herein, they include exposure by taking raw milk, the exposure by consumption of raw blood during community celebrations and the exposure by assisting animals in birthing. However, exposure by inhalation which has been reported as the major mode of transmission, was not characterized.

4.6.6. The sociocultural risk factors associated with Q fever infection in relation to study results by health-seeking behavior and Knowledge of Q fever disease variables.

The variable on health-seeking behavior looked at the number of times the study participant visited a health facility for the same illness and duration from onset of illness to the day of interview.

The variable that looked at the number of times the study participant visited a health facility for the same illness, showed that the population visited health facilities multiple times. Another concern of this variable was the possible treatment the respondents received the many times they visited the different health facilities. There could be the possibility of them being given antibiotics, and this may pose issues of occurrence of Antimicrobial resistance (AMR). Other than the genetic science of gene transfer that may result in AMR, practices such as incorrect diagnosis, exposure to second-line generic antibiotics, and inadequate or lack of point-of-care diagnosis play a role in development of resistance by the microorganisms (Aljelda et al., 2022) A study estimated that 5% to 10% of patients who attended to a primary health facility for lower backache with time developed chronic backaches and further revealed that the patients with

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chronic back pains will make repeated visits to healthcare facilities, which also is associated with suboptimal medication (Neplash et al., 2023; McPhillips et al.,1998). Subsequently, this results to catastrophic costs of transport and medicine, hence economic depletion.

The variable of duration from onset of illness to seeking health services in a health facility by the study population was determined. The univariate analysis was statistically significant with p-value <-0.001. There was so much delay on the side of the patient, spanning from 3 days, 1 week, one, three, six months, and others up to 1 year This delay could have allowed Q fever disease to progress from the Acute phase to the Chronic phase further causing more health and economic suffering for the population. Additionally, this variable posed as a pointer to the lack of differential testing in the health facilities and possible misdiagnosis of the disease (Neplash et al., 2023).

The knowledge of Q fever disease by the study participants was non-existent and none of the respondents were aware of the disease. This matched the results of another study conducted in Kajiado County (Nakeel et al. (2016) furthermore, it coincided with one conducted in Nigeria, (Cadmus et al., 2021). This further implies that Q fever is still ignored and neglected despite the disease having been reported in a number of studies locally and internationally. Kenya reported its first case 69 years ago in Nakuru, in a study conducted by Craddock and Gear, in 1955, (Lemtudo et al., 2021). Livestock farming being the main occupation of the Meto population coupled by suboptimal knowledge of the disease allows the persistence of acute and chronic infections in Meto community. It has been indicated in past studies that people who are exposed to zoonotic diseases at work have poor knowledge of the disease (Tan, *et al*., 2022).

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CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The seroprevalence of Q-fever disease in Meto is high and the majority of the population is already infected, *Coxiella burnetti* and *Brucella spp* co-infection among the study population was demonstrated. The sociodemographic risks of exposure to *Coxiella burnetii* infection by assisting birthing animals and consumption of raw blood were statistically significant and thus the population will continuously be exposed unless effective interventions are put in place.

The likelihood of being infected by *C burnetii* bacterium is heightened by the lack of knowledge of Q fever disease by study population.

The catastrophic costs incurred by Meto population due to the many times they visited health facilities for the same illness and the long duration of the symptoms will negatively impact to the livelihood of the Meto population.

The Study has proven that IFA which is the gold standard for the diagnosis of acute and chronic Q fever disease does not require any additional infrastructure or equipment, thus the diagnosis of Q fever disease is implementable at peripheral Laboratories.

5.2. Recommendations

- This study recommends the diagnosis of Q fever disease using the Indirect Immunofluorescent Assay (IFA) as a point-of-care test at Hospital and health center laboratories.
- This Study also recommends the utilization of the already existing Fluorescent microscopes, Water bath machines, and Laboratory refrigerators to support Q fever IFA testing since this method does not require any additional infrastructure.
- Initiation of Q fever disease notification and reporting is highly recommended by this study.
- This study pointed to considerable disparity in the epidemiology of Human Q fever in Meto, Kajiado County thus, recommends the need to strengthen the County's One Health approach strategies.
- Knowledge of Q fever disease was picked as a major gap amidst the Healthcare workers and Meto community, as such, public awareness strategies at community level, Health facilities level, the County as well as the National levels was highly recommended.

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APPENDICES

Appendix I: Informed Research Consent Form

SEROPREVALENCE OF COXIELLA BURNETII PHASE I AND PHASE II ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH BRUCELLA-LIKE ILLNESS IN METO HEALTH CENTRE, KAJIADO, KENYA
Principal Investigator: Esther S. Lemarkoko : Phone number: 0721418663
Subject Population: Patients ≥ 5 years of age attending outpatient clinic presenting with:Fever of ≥ 38 ^{0}C, Acute or chronic joint, back and Calf muscle pains as well as those with history of close contact with animals or exposure to animal products or animal waste.
Consent form version: MMLS/2021/42471/001/A

INFORMED RESEARCH CONSENT FORM: ADULT PARTICIPANT

Purpose of study

This is to request you to participate in this research study. Before you decide to take part, please allow me to debrief you about the research and why it is being done and what it will entail. This study as the title suggests, will seek to determine if patients who visit Meto health centre with fever or those with joint pains have Q fever disease. Two samples are required for Q fever testing, the first one will be taken today and the other will be taken two weeks from now as per the requirements of the study.

Permission has been granted to undertake this study by the Mount Kenya University, Ethics and Research Committee

Mount Kenya University

Risks

I want to confirm to you that there are no risks anticipated as a result of this study. We will remove a small amount of blood and you will only feel a little pain during blood sample collection and that is all.

I will take you through a questionnaire, you will kindly answer the questions. However, you may decline to answer any or all questions and you may terminate your participation at any time and we will respect your decision.

Benefits

The study will benefit you directly because if the disease is diagnosed then you will get correct treatment. More so, the information obtained from your participation and that of other participants will inform policy on prevalence of Q fever for prompt prevention and control. Treatment is also available.

Confidentiality

Ethics and confidentiality will be upheld throughout this study. Your identity and responses will strictly remain anonymous.

Contact Information

Whenever you have a question, or if you want to understand more about your rights as a study participant, or if any sort of misunderstanding arises at the health centre, please do not hesitate to call the number provided at the top of this form.

Voluntary participation

Mount Kenya University

Your participation in this study is absolutely voluntary. You will choose to participate or not to participate. If you agree to take part in the study you will be requested to sign this consent form at a space provided on the last page. Even after signing the consent you can still feel free to withdraw without necessarily giving any explanation. And this will not affect any other health services you will require from Meto health centre.

CONSENT

I hereby confirm that I have read and understood the information provided in this consent. I have also gotten a chance to ask questions. My participation is voluntary and I can choose to withdraw at any stage without giving any explanation and without cost. So, I have decided to take part in this study.

Participants's initials**:** **Sample Barcode Number:** **Gender:** **Age:**
Date**'Acute' sample** collected: / /2023
Date**'Convalescent' sample** collected: / /2023
Participant's middle name: ID.No.
Signature/thumb stamp : Date:
Investigator signature : Date:
Consent Form Valid Date: 01/5/2023 : **Study Expiration Date:** 30/9/2023

Mount Kenya University

Appendix II: Research Minor Assent Form

RESEARCH MINOR ASSENT FORM

The purpose of this form is to provide you with information about you taking part in this research study. You are required to read and understand this form. You do meet the inclusion criteria to take part in this research study that intend to demonstrate Q fever disease among febrile patients who come for health services in Meto Health centre. The study subjects are patients with fever who are equal to or above ≥5 years of age. Children in this bracket who are below 15 years will sign the Assent with permission of their parents or guardians.

Your parent/guardian knows that you are taking part in this study.

I also confirm to you that permission has been granted to undertake this study by the Mount Kenya University, Ethics and Research Committee

Mount Kenya University

STUDY TITLE: **SEROPREVALENCE OF COXIELLA BURNETII PHASE I AND PHASE II ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH BRUCELLA-LIKE ILLNESS IN METO HEALTH CENTRE, KAJIADO, KENYA**
Principal Investigator: Esther S. Lemarkoko Phone number: 0721418663
Subject Population: Patients ≥ 5 years of age attending outpatient clinic presenting with: Fever of ≥ 38 ^{0}C, Acute or chronic joint, back and Calf muscle pains as well as those with history of close contact with animals or exposure to animal products or animal waste.
Minor Assent form version: MMLS/2021/42471/001/C

Confidentiality

Ethics and confidentiality will be upheld throughout this study. Your identity and responses will strictly remain anonymous.

Please feel free to ask any question about this study. We will ask you to sign this Assent form, if you agree to participate in this research with your child. You can visit Meto health centre whenever you have a need.

With the above information, I believe you now understand the purpose of the study.

I therefore, request you to take part in this study because you are in the age category of 5 and 15 years, and you are presenting with fever, joint/back/muscle pains. Two blood samples will be required. One will be taken today and another blood sample will be taken in two weeks' time. In case you are diagnosed with Q fever, you will be given free treatment.

Minor's initials: **Sample Barcode Number:** **Age:**
Date**'Acute' sample** collected: / /2023
Date**'Convalescent' sample** collected: / /2023
Consent Form Valid Date: 01/5/2023 : **Study Expiration Date:** 30/9/2023
Parent/Guardian middle name: **ID.No.**
Minor Signature or thumb stamp

Mount Kenya University

STUDY TITLE: SEROPREVALENCE OF COXIELLA BURNETII PHASE I AND PHASE II ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH BRUCELLA-LIKE ILLNESS IN METO HEALTH CENTRE, KAJIADO, KENYA
Q fever case definition: Acute and Chronic phase
Acute Q fever disease: Acute fever usually accompanied by rigors, myalgia, malaise, and a severe retrobulbar headache. fatigue, night-sweats, dyspnea, confusion, nausea, diarrhea, abdominal pain, vomiting, non-productive cough, and chest pain have also been reported. ***(See definition of terms in the last page).***
Chronic Q fever disease: Severe disease can include acute hepatitis, atypical pneumonia with abnormal radiograph, and meningoencephalitis. Pregnant women are at risk for abortion and death.

Questionnaire

MMLS STUDENT – RESEARCH STUDY QUESTIONNAIRE

Questionnaire **form version: MMLS/2021/42471/MAY - SEPT 2023**

1.0. Demographic and Socioeconomic data (Please tick as appropriate)	
Name of the patient - Surname/first name/ middle name	□Name: ……………………………………………………………………….. □Age □Gender □Others(***specify***)

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Residence	☐ Sub county ☐ Ward ☐Location ☐ Village
Contact information	☐Phone No. ☐next of kin phone no. ☐Residence landmark
Level of education (if applicable) if not tick here. ☐**N/A**	☐ Primary ☐ Secondary ☐ College ☐ University
2.0. Social support	
Are you employed?	☐ Yes ☐ No
If yes, what's the name of your employer?	☐ Name(s)
If no, what do you do for a living?	☐
3.0. Health seeking behavior	
Sorry you (your child) is/ are not feeling well, When did the illness start?	☐ 1 - 3 days ago ☐ 1 week ago ☐ 1 month ☐ 3 months ☐ 6 months ☐ 1 year
What are the signs you are experiencing or you experienced?	☐ Chills and fever ☐Muscle pain ☐Backache ☐Joint pains ☐Chest pains ☐ Non productive cough ☐Headache ☐Night sweats ☐ Vomiting ☐Stiff neck ☐Jaundice ☐ Fatigue
How many times have you gone to hospital because of this illness	☐ Today only ☐ 2 times ☐ 3 times ☐ more than 4 times
4.0. Knowledge of Q fever disease	
Have you ever heard of Q fever disease?	☐ Yes ☐ No

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If yes, how did you get to hear about this disease?	☐ Radio ☐ Friend ☐ Health worker ☐ Television ☐ Article ☐ Google ☐ Others, specify..
Could you remember how it spreads?	☐ Yes ☐ No
If yes, please mention 4 modes of human infection?	(List them)
5.0. Exposure to infection	
Among the following animals, which one(s) do you keep?	☐ Cows ☐ Goats ☐ Sheep ☐ Camels ☐ Pigs ☐ Chicken
Is boiling of milk a practice in your village?	☐ Yes ☐ No
Is it a practice in your home?	☐ Yes ☐ No
In your home who can be allowed to assist a birthing animal?	☐ Father ☐ Moran ☐ Daughter ☐ Mother ☐ Herdsman ☐ Others
When was the last time you assisted a birthing animal?	☐ 1 week ago ☐ 2 weeks ago ☐ 1 month ago ☐ 3 months ago ☐ 6 months ago ☐ 1 year ago
Apart from drought, like the one experienced recently, what are other causes of death of livestock including calves?	☐ Abortion ☐ Anthrax ☐ Foot and mouth disease ☐ East coast fever ☐ Death of calves ☐ Rift valley fever ☐ Others….

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During community celebrations eg. the famous Emanyatta, Enkipaata, Eunoto and Olng'esherr and other celebrations do you take raw blood?	☐ Yes	☐ No	
When was the last time you participated in these ceremonies?	☐ 3 months ago	☐ 6 months ago	☐ 1 year ago
Did you take blood?	☐ Yes	☐ No	
Thank you very much for participating in this interview. Thank you for your time.			
Venous blood collected	☐ Yes	☐ Date:	☐ No
If no, state reasons for not collecting the blood	☐ ………………………………………………………………………………… …………………………………………………………………………………		
Interviewer/Clinician	Name: ……………………………………………………………………………… Date: ……………………………… Signature ………………………………… *Please stamp here*		

Mount Kenya University

Definition of terms

- **Acute fever** - sudden onset of fever
- **Rigors** - a sudden feeling of cold with shivering accompanied by a rise in temperature often with copious sweating, especially at the onset or height of a fever.
- **Myalgia** - muscle aches and pain
- **Malaise** - a general feeling of discomfort, illness, or unease whose exact cause is difficult to identify.
- **Retrobullar** - is inflammation of the part of the optic nerve lying immediately behind the eyeball.
- **Fatigue - a feeling of being tired, weak, or exhausted**.
- **Dyspnea** - is difficult or labored respiration
- **Abnormal radiograph** - It can indicate an underlying health problem or a litany of problems
- **Meningoencephalitis** - is a condition that causes a stiff neck, fever, headache, and sensitivity to light along with other more serious symptoms such as confusion and seizures. It is considered a medical emergency.
- **Morans** - Young men or Youths.
- **Landmark** - an object or feature of a landscape or town that is easily seen and recognized from a distance, especially one that enables someone to establish their location. Examples include, a School, a church, a cattle deep, water tank, watering point for animals, Chief's home, a vegetable farm etc.

Appendix IV: ERC Clarance Certificate

REF: **MKU/ISERC/2896** Date: 05 July 2023
TO: **ESTHER SOINTA LEMARKOKO**
REG: **MMLS/2021/42471**

Dear Sir/Madam,

RE: SEROLOGICAL EVIDENCE OF Q FEVER AMONG PATIENTS PRESENTING WITH NON-SPECIFIC FEBRILE ILLNESS IN METO HEALTH CENTRE, KAJIADO COUNTY, KENYA

This is to inform you that **Mount Kenya University** has reviewed and approved your above research proposal. Your application approval number is **1940.** The approval period is **05/07/2023 - 04/07/2024.**

This approval is subject to compliance with the following requirements:

i. Only approved documents including informed consents, study instruments, MTA will be used
ii. All changes including amendments, deviations and violations are submitted for review and approval by **Mount Kenya University**
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **Mount Kenya University** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affect the safety or welfare of study participants and others or affect the integrity of the research must be reported to **Mount Kenya University** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal
vii. Submission of an executive summary report within 90 days upon completion of the study to **Mount Kenya University**

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

The Chairman
Mount Kenya
P. O. Box 342 - 0100, Thika

Dr. Alfred Owino, PhD
Chairman, Mount Kenya University ISERC

Appendix V: Introduction Letter from Derectorate of Graduate Studies

DIRECTORATE OF GRADUATE STUDIES

MMLS/2021/42471

5th July, 2023

National Commission for Science Technology & Innovation (NACOSTI)
Off Waiyaki Way, Upper Kabete,
P.O Box 30623- 00100
NAIROBI, KENYA

Dear Sir/Madam,

RE: ESTHER SOINTA LEMARKOKO– REGISTRATION NO. MMLS/2021/42471

The purpose of this letter is to introduce the above named student who is pursuing **Master of Science in Medical Laboratory Sciences** in the Department of **Medical Laboratory Sciences** in the **Medical School.**

The title of the research is **"Serological Evidence of Q Fever among Patients Presenting with Non-Specific Febrile Illness in Meto Health Centre, Kajiado County, Kenya."** It has been cleared by the University's Ethics Review Committee (Certificate attached) and now has to proceed to the field to collect data between **July, 2023 and September, 2023.**

Any assistance accorded to the student will be highly appreciated.

Thank you.

Dr. Samuel M. Karenga, Ph.D
Director, Graduate Studies
Enc.

Main Campus, General Kago Road, P.O. Box 342-01000 Thika.
Tel: 020-2878 000, Cell: +254 709 153 000
Email: info@mku.ac.ke, Web: www.mku.ac.ke
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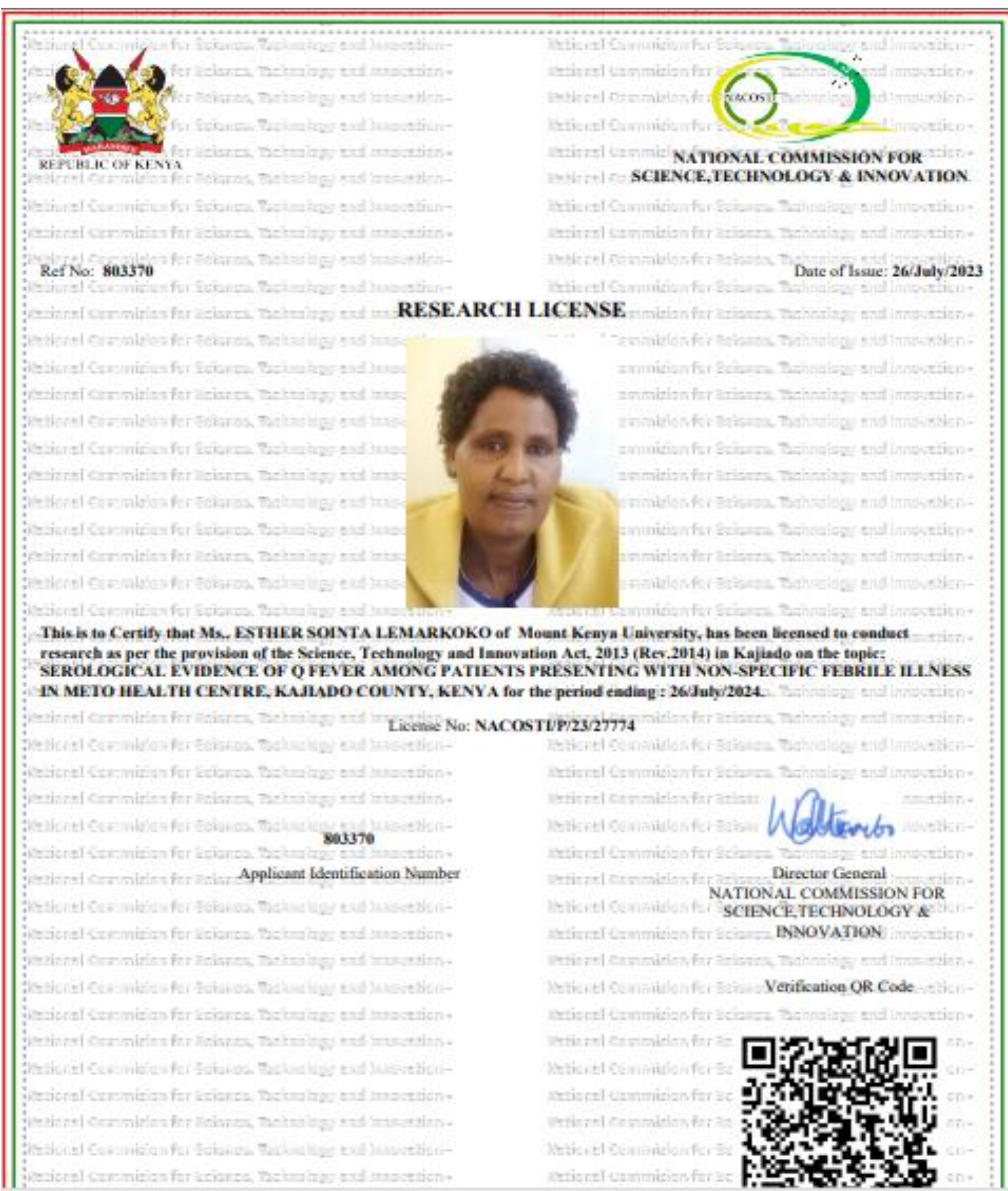
Appendix VI: Research Permit from NACOSTI



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 803370

Date of Issue: 26/July/2023

RESEARCH LICENSE

This is to Certify that Ms.. ESTHER SOINTA LEMARKOKO of Mount Kenya University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kajiado on the topic: SEROLOGICAL EVIDENCE OF Q FEVER AMONG PATIENTS PRESENTING WITH NON-SPECIFIC FEBRILE ILLNESS IN METO HEALTH CENTRE, KAJIADO COUNTY, KENYA for the period ending : 26/July/2024.

License No: NACOSTI/P/23/27774

803370

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

Appendix VII: Research Approval Letter from County Government of Kajiando

COUNTY GOVERNMENT OF KAJIADO

DEPARTMENT OF MEDICAL SERVICES & PUBLIC HEALTH
OFFICE OF THE COUNTY DIRECTOR OF MEDICAL SERVICES
P. O. BOX 31, KAJIADO

REF: CGK/MEDICAL SERVICES/01/VOL.111/141 — 31st July, 2023

ESTHER SOINTA LEMARKOKO
MSC STUDENT MOUNT KENYA UNIVERSITY
ADMISSION NO.MMLS/021/42471
P.O. BOX 31-01100 KAJIADO

RE: RESEARCH AUTHORIZATION

Reference is made to your request and your research license from the National Commission for Science, Technology and innovation reference No. NACOSTI/P/23/27774 for the period ending, 26th July 2024.

The Department has no objection in you carrying out research on '**Serological Evidence of Q fever among patients presenting with non-specific febrile illness in Meto Health centre, in Kajiado County.**

You are however required to share findings of your research with this office.

Thank you.

County Government of Kajiado
County Director of Medical Services
31 JUL 2023
Signature:...........
P. O. Box 31 - 01100, KAJIADO

DR. LYDIA MUNTEYIAN
COUNTY DIRECTOR MEDICAL SERVICES

CC: THE CHIEF OFFICER MEDICAL SERVICES AND PUBLIC HEALTH

Appendix VIII: Similarity Report

Esther Sointa Lemarkoko

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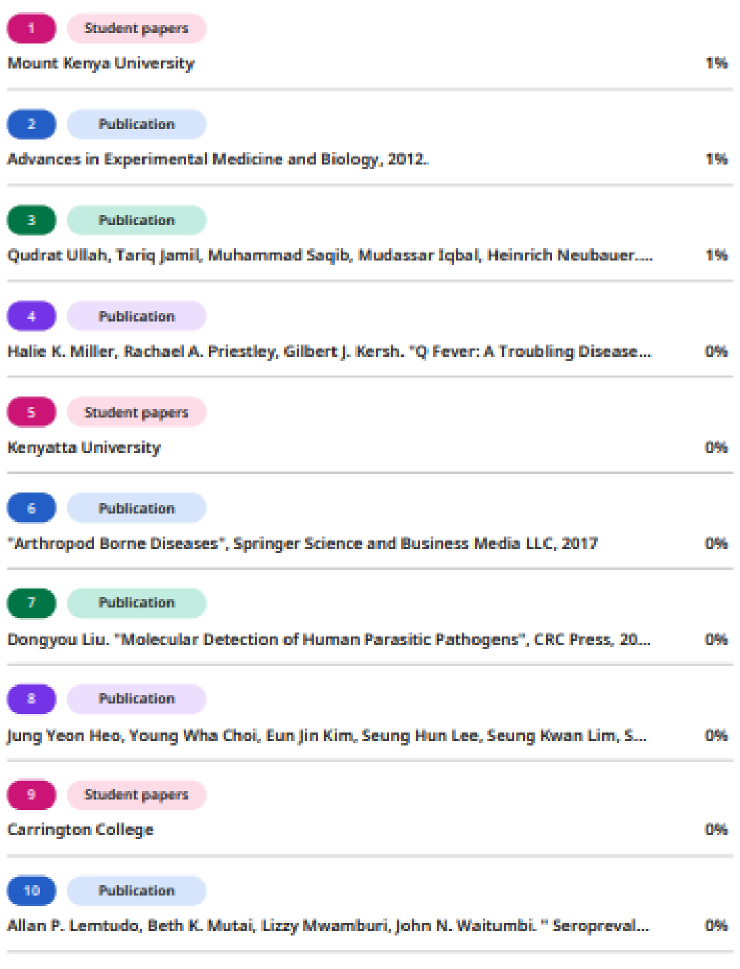

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