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Epidemiologic study of q fever in smallholder dairy cattle in selected regions of Tanzania

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EPIDEMIOLOGIC STUDY OF Q FEVER IN SMALLHOLDER DAIRY CATTLE IN SELECTED REGIONS OF TANZANIA

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

A cross-sectional study was conducted to determine prevalence and the risk factors associated with *Coxiella burnetii* seropositivity in smallholder dairy cattle in six regions from the southern highland and northern zones of Tanzania between July 2019 and October 2020. A total of 2049 blood samples and 1920 vaginal swabs were collected from dairy cattle. Serum was tested for antibodies against *C. burnetii* using an indirect enzyme-linked immunosorbent assay (ELISA). *C. burnetii* DNA was identified from vaginal swabs using quantitative polymerase chain reaction (qPCR) analyses. A questionnaire survey was designed and uploaded to the Open Data Kit (ODK) cloud platformsoftware version 1.22.4. At each household, the cattle owner aging equal or over 18 years was interviewed to understand the risk factors related to animal health, husbandry, and farmer socioeconomics, as well as environmental variables. *C. burnetii* prevalence was calculated at different epidemiological levels and maps were created to visualize Q fever spatial distribution. Logistic generalized linear mixed effects models were built to explorethe association between ELISA binomial results, and a set of risk factors and environmental variables important for *C. burnetii* occurrence. An overall animal seroprevalence of 3.86% (79, 2049) 95% CI 3.06 - 4.78 was estimated across the study regions. Among these regions, Tanga (northern zone) and Iringa (southern zone) showed the highest prevalence with 8.21% (95% CI 6.0 - 10.89%) and 4.63% (95% CI: 2.49 - 7.78%) respectively. On the other hand, molecular identification of *C. burnetii* from vaginal swabs of selected cows revealed an overall prevalence of 0.94% (18,1920) (95% CI 0.5-1.4%). Among the northern zone regions, Arusha had the highest of 2.3%(95%CI 0.9-4.7%) and on the southern highlands zone, Iringa emanated to have the highest prevalence of 0.73% (95%CI 0.08-2.5%). Animal age, extensive feeding system, and temperature were univariably significant risk factors to *C. burnetii* seropositivity. The final multivariable logistic regression model showed that old animal age, extensive feeding system, and low precipitation were strongly associated with Q fever seropositivity. These findings indicate that *C. burnetii* circulates among smallholder dairy cattle across regions in Tanzania. However, Tanga, Arusha and Iringa are the three regions with the highest seroprevalence and should be considered for the systematic and active surveillance. Furthermore, “One health” approaches are necessary to fully understand disease transmission and control of this zoonotic disease.

DECLARATION

I, **Shedrack Festo Bwatota**, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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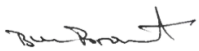
Name of Candidate	**Signature**	**Date**

The above declaration is confirmed by:

Prof. Gabriel Mkilema Shirima		
Name of Supervisor 1	**Signature**	**Date**

Prof. Barend Mark de Clare Bronsvoort		21.08.2021
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Dr. Elizabeth Anne Jessie Cook		21.08.2023
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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a thesis titled ***"Epidemiologic study of Q fever in smallholder dairy cattle in selected regions of Tanzania"*** in partial fulfilment of the requirements for the degree of Master's in Health and Biomedical Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

I would heartfully dedicate this work to my parents (Father and Mother) if they would be alive, may God rest their souls in eternal peace! I rather dedicate it to my beloved sisters, brothers, my wife and importantly to super and cheerful upcoming family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ADGG	African Dairy Genetic Gain
CDC	Centres for Disease Control and Prevention
CFSPH	Centre for Food Security and Public Health
COSTECH	Tanzania Commission for Science and Technology
DED	District Executive Directors
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
IFA	Immunofrolescence Assay
ILRI	International Livestock Research Institute
LCV	Large Cell variant
LMICs	Lower- and Middle-Income Countries
MLF	The Ministry of Livestock and Fisheries
NM-AIST	Nelson Mandela African Institution of Science and Technology
ODK	Open Data Kit
OIE	World Organization for Animal Health
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
SCV	Small Cell Variant
SLP	Spore-like Particles
SSA	Sub-Saharan African

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Q Fever (Query Fever) also known as Coxiellosis /Abattoir Fever, is a zoonotic disease distributed worldwide and has a diverse host range. Infection primarily causes reproductive losses in domestic ruminants and poses significant public health concerns due to the range of severity it can cause in people especially those who work in close contact with animals or handling animal products (Benaissa *et al.,* 2017; CFSPH, 2017; Esmaeili *et al.,* 2016; Rahaman *et al.,* 2019; Vanderburg *et al.,* 2014). The disease is caused by the taxonomical and phylogenetically unique gram-negative bacteria known as *Coxiella burnetii* which is an obligate intracellular bacterium and produces spore-like particles which are exceptionally resistant to physico-chemical factors resulting to environmental contamination when discharged from infected animals (Bielawska-drózd *et al.,* 2013; CFSPH, 2017; ECDC, 2010). Q fever/*C. burnetii* has respectively been considered as an emerging infectious disease by the WHO/FAO/OIE and EFSA/ECDC (Bielawska-drózd *et al.,* 2013; Deressa *et al.,* 2020; ILRI, 2012) and as a category B Critical Biological agent and potential weapon for bioterrorism by the Centers for Disease Control and Prevention and is listed as a notifiable disease by OIE. Furthermore, the disease is known to be poorly reported and its surveillance is frequently neglected especially in sub-Saharan African (SSA) countries including Tanzania (Czerwińska *et al.,* 2014; Gumi *et al.,* 2013; Porter *et al.,* 2011). In low and middle income countries (LMICs), Q fever is neglected in the differential diagnosis of conditions with similar clinical signs such as brucellosis, leptospirosis, Rift Valley Fever, Infectious Bovine Rhinotracheitis, listeriosis, and campylobacteriosis (Abu Dhabi Food Control Authority, 2011; Tagesu, 2019).

The main reservoirs for the pathogen (*C. burnetii*) are domestic ruminants including cattle, sheep, and goats. However, it can also infect a wide variety of other hosts including rodents, humans, dogs, cats, fish, reptiles, and birds (Bielawska-drózd *et al.,* 2013; ECDC, 2010; CFSPH, 2017; Khamesipour *et al.,* 2018; OBOGE, 2018; Prabhu *et al.,* 2011). In sheep and goats, Q fever is characterized clinically by abortions, stillbirths, premature deliveries, and weak offspring. However, the disease is reported to be asymptomatic in cattle, but infected cows may develop irregular repeat breeding, metritis, mastitis, and infertility (Porter *et al.,* 2011; Pozzo *et al.,* 2016). Similar clinical signs have been reported to be associated with

reproductive disorders in dairy cattle in Tanzania (Muhairwa *et al.,* 2005). The infected animals including natural reservoirs (cattle, sheep, and goats) shed the pathogen s through milk, urine, feces, semen, placentas, parturient fluids, and aborted fetuses (Czerwińska *et al.,* 2014; Khamesipour *et al.,* 2018; Vanderburg *et al.,* 2014).

Humans become infected directly through consumption of raw milk, raw milk products and indirectly by inhaling of infected aerosols generated from infected placentas, body fluids (e.g., when handling abortions and assist birth) or contaminated dust resulting from manure and desiccated infected placenta because of bacteria shedding from infected animals to the environment (Anchang *et al.,* 2014; CFSPH, 2017; Johnson *et al.,* 2019; Tagesu, 2019). The diseasehas been reported in abattoir workers (Wardrop *et al.,* 2016) and people practicing mixed crop farming where manure is used as fertilizer (Anchang *et al.*, 2014). Acute infection in humans can be debilitating and commonly presenting with high fevers, pneumonia, rash, headache, hepatitis, influenza like syndromes, myalgia, arthralgia, and unproductive coughing (Tagesu, 2019; Steffens & Wilson, 2012). Human fatalities are rare, its rate is around 1% of the untreated cases, however on outbreaks it causes widespread health problems including vascular aneurysms, endocarditis, associated heart failure, and chronic fatigue syndrome (Academies & Spring, 2004; Njeru *et al.,* 2016; Tagesu, 2019). Essentially, up to 60% of chronic infections in humans caused by *C. burnetii* are thought to be asymptomatic (Njeru *et al.,* 2016).

Since the first report of Q fever in Africa in 1947 (Vanderburg *et al.,* 2014b), studies have been conducted in different countries and reported a wide range of disease burdens in dairy cattle. Studies on Q fever from 11 African countries reported seroprevalences between 4 and 32% (Vanderburg *et al.,* 2014; Wardrop *et al.,* 2016; Hussien *et al.,* 2017; Gumi *et al.,* 2013). In addition, scholars within Africa reported the molecular identification of *C. burnetii* DNA from cattle vaginal swabs and blood (Knobel *et al.,* 2013; Qiu *et al.,* 2013; M. Rahal *et al.,* 2018; Rahal *et al.,* 2020). Furthermore, studies to understand the associated risk of Q fever in cattle such as sex (Abakar *et al.,* 2014; Ibrahim *et al.,* 2021; Mangena *et al.,* 2021; Scolamacchia *et al.,* 2010), age (Deressa *et al.,* 2020; Abakar *et al.,* 2014; Ibrahim *et al.,* 2021; Kelly *et al.,* 2021; Scolamacchia *et al.,* 2010), large herd size above 30 animals (Cadmus *et al.,* 2021; Cadmus *et al.,* 2020), new animals in the herd (Mangena *et al.,* 2021; Menadi *et al.,* 2020; Wardrop *et al.,* 2016), extensive feeding system (Deressa *et al.,* 2020), and nuisance animals like dogs, cats and rodents (Deressa *et al.,* 2020) have been reported. Apart from research that have been conducted in Africa, the lack of adequate laboratory facilities and non-specific

clinical presentation of Q fever have limited its diagnosis (Tagesu, 2019). This leads to misdiagnosis and underreporting of the disease both in animals and humans (URT, 2020). Despite the availability of a range of diagnostic approaches, the knowledge on prevalence and distribution of Q fever/*C. burnetii* in dairy settings in Tanzania is still limited and thus compromise any possible interventions.

Since 1961, Tanzania has been striving to improve the dairy sector and from 2010 the sector has made tremendous improvement in milk production and genetic gains through different development partners and Dairy Development Projects. The Ministry of Livestock and Fisheries (MLF) has improved access to financial services through increasing incentives for the dairy cattle keepers to engage in the production, processing, and marketing of livestock products (Nell *et al.,* 2014). The Dairy Development projects have contributed to genetic improvement of local breeds through the establishment of heifer breeding units known as Livestock Multiplication Units. In addition, the National Artificial Insemination Center in Arusha and livestock research centers conduct research on genetic improvements; and educating farmers on the new genetic technologies such as artificial insemination, multiple ovulations, and embryo transfer (Nell *et al.,* 2014; ILRI., 2014; ADGG, 2018). The genetic improvement of dairy cattle has resulted in rapid increase in milk production from 2.3-12 million liters per day (ADGG, 2018) in an interval of five years before the initiatives (ADGG, 2018). This has accelerated the development of small dairy support programmes and national stakeholders' organizations including Tanzania Dairy Board, Tanzania Milk Producers and Tanzania Milk Processors Associations (Nell *et al.,* 2014). The large milk processors are Tanga Fresh in Tanga and ASAS Dairies in Iringa, also microprocessors are found in cities of Arusha and Moshi (Nell *et al.,* 2014; ADGG, 2018). Furthermore, dairy products are highly needed for food availability/security, source of employment opportunities & income generation, as well as for improving overall nutrition and livelihood of the regional and global growth population. Thus, the dairy production strongly cements the local, regional, and national level economies (Grout *et al.,* 2020). At present, the wide range of dairy crosses in Tanzania has been concentrated in the cool highlands of Arusha and Kilimanjaro and in the Southern highlands in Iringa, Njombe, and Mbeya (ILRI, 2012; Nell *et al.,* 2014). The population of improved dairy cattle in these regions has been reported to be 161 984 in Kilimanjaro, 87 197 in Arusha, 72 724 in Mbeya, 41 639 in Tanga and 14 258 in Iringa. Q fever has been reported in dairy cattle in neighboring countries including Kenya, Sudan, and Ethiopia (Hussien *et al.,* 2017; Wardrop *et al.,* 2016; Gumi *et al.,* 2013) and movement of animals and animal products between

Tanzania and these countries are high due to porous nature of our borders. This suggests the possibility of transmitting the diseases to Tanzanian cattle (Vanderburg *et al.*,2014; Njeru *et al.*, 2016; Nusinovici *et al.*, 2015).

Despite the efforts of improving the dairy sector, livestock diseases are still challenging the subsector and calls for resources and strategic intervention to alleviate the burden. Studies conducted in northern Tanzania showed that Q fever was at a prevalence of 5% in humans presenting with febrile illnesses at health facilities (Prabhu *et al.*, 2011). A single study conducted around Serengeti documented a seroprevalence of 7-17% in animals with limited spatial distribution due to limited data (Vanderburg *et al.*, 2014). Therefore, animals could be the source of infection to humans via environmental contamination and consuming of raw milk (Prabhu *et al.*, 2011). However, little is known about the associated risk factors for the disease in the smallholder dairy cattle production system. Recent studies in Tanzania revealed little knowledge about the disease among pastoralists in Morogoro and Tanga (Alonso *et al.*, 2015). Although Q fever was not included in the six priority zoonotic diseases in Tanzania, its distribution and health impacts need to be assessed (CDC, 2017) for possible interventions. Therefore, the current study aims at determining the epidemiology of the disease in smallholder dairy cattle populations for future management.

1.2 Statement of the Problem

Although in Tanzania, there are other conditions such as brucellosis (Shirima *et al.*, 2018), leptospirosis (Schoonman & Swai, 2010), listeriosis (Msalya, 2017), and campylobacteriosis (Komba *et al.*, 2013) with similar clinical signs to Q fever, the information about *C. burnetii* in livestock data is lacking especially those kept under the smallholder dairy system. Since disease surveillance system is not a common practice cross borders then *C. burnetii* transmission between countries may be possible as Q fever has been reported in neighboring countries. For example, five recent cross-sectional studies studied Q fever in dairy cattle in other parts of Africa reported a seroprevalence of 1.7-29.0% (Ameur *et al.*, 2018; Dechicha *et al.*, 2010; Derdour *et al.*, 2017; Gumi *et al.*, 2013; Kelly *et al.*, 2021; Menadi *et al.*, 2020; Wardrop *et al.*, 2016). On the other hand, three studies reported the molecular identification of *C. burnetii* from blood (7%), vaginal swabs (2.1%) and placental tissues (19.1%) collected from dairy cattle (Knobel *et al.*, 2013; Menadi *et al.*, 2022; Rahal *et al.*, 2018). Furthermore, risk factors including age (adults) (Kelly *et al.*,2021), sex (females) (Mangena *et al.*, 2021), introducing new animals in the herd (Wardrop *et al.*, 2016), and feeding systems (extensive

system) (Deressa *et al.*, 2020). With that said, Tanzanian dairy cattle whose number has increased tremendously especially in the highlands may be at high risk of exposure to zoonotic pathogens as regular surveillance is limited.

1.3 Rationale of the Study

Detecting zoonotic disease in humans (Ali *et al.*, 2020) raised a concern to animals and animal products thus calls for concrete studies to establish and device control interventions at animal level. Although Q fever is considered as a neglected zoonoses, occurrence in humans and animals in developing countries such Tanzania warranty further studies. Therefore, this study will generate data to the scientific community, policy makers and implementers on the best intervention measures to control the disease in the smallholder dairy sector, to reduce transmission, and thus the incidence of the disease in dairy cattle and ultimately reduce or prevent human infections.

1.4 Research Objectives

1.4.1 General Objective

To understand the epidemiology of Q fever in the smallholder dairy cattle population in the six high producing regions of Tanzania for improved management and disease control strategies.

1.4.2 Specific Objectives

The study aimed to achieve the following specific objectives:

(i) To determine the seroprevalence and spatial distribution of Q fever in smallholder dairy cattle populations in the selected regions across Tanzania.

(ii) To determine the risk factors associated with Q fever seropositivity in smallholder dairy cattle populations in the selected regions across Tanzania.

(iii) To molecularly identify *C. burnetii* DNA from vaginal discharges collected from the smallholder dairy cattle populations in the selected regions across Tanzania.

1.5 Research Questions

(i) What are the seroprevalence and geographical distribution of Q fever in smallholder dairy cattle populations in selected regions of Tanzania?

(ii) What are the risk factors associated with Q fever seropositivity in smallholder dairy cattle populations in selected regions of Tanzania?

(iii) Is there shedding of *C. burnetii* from vaginal discharges in smallholder dairy cattle populations in selected regions of Tanzania?

1.6 Significance of the Study

The outcome of this study contributes to the clear understanding on the prevalence of Q fever in smallholder dairy cattle in six regions of Tanzania. Furthermore, this study focuses on predictors i.e., risk and protective factors associated with disease seroprevalence in Tanzania, adds to our understanding of transmission and sources of *C. burnetii* in the country. The understanding of the disease interlinks Tanzania to other countries because it is difficult for a single country alone to fight against the disease. Containing and controlling Q fever at the animal and animal product level will ultimately prevent infections to humans and thus improve the life standards of youths and women whose main source of income is smallholder dairy cattle in Tanzania.

1.7 Delineation of the Study

The study focused on the epidemiology of Q fever in six regions, three from southern highlands and the remaining from northern zones of Tanzania. During implementation, the study examined the seroprevalence of antibodies against *C. burnetii* in smallholder dairy cattle. Furtherly determined the active shedding of pathogen through vaginal discharges in similar animals sampled for antibody testing. It also examined the factors associated with the disease exposure in dairy cattle through interviewing the cattle owners at the study areas. Analysis of serological binomial results and the disease predictors were clearly stated in chapter three of this document. The results of this study are based on serology (exposure) and molecular determinations of the pathogen in cattle sera and vaginal swabs respectively and chapter five of this document summarizes about the findings and some gaps for the subsequent studies in Tanzania

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Qery (Q) Fever is a bacterial zoonotic disease distributed worldwide except for Antarctica and New Zealand (OIE, 2018; Tagesu, 2019). It affects a wide range of hosts including domestic animals, wildlife, humans, and pets. The main reservoirs of the disease among domestic animals are ruminants including cattle, sheep, and goats in which it causes reproductive losses. The zoonotic nature of the disease poses public health concerns due to the range and severity of conditions and also the potential for misdiagnosis as other febrile illnesses (WOAH, 2018, Rahaman *et al.,* 2019; CFSPH, 2017; Vanderburg *et al.,* 2014; deRooij *et al.,* 2012). It is included among the list of emerging infectious diseases and notifiable disease by the World-Organization for Animal Health (WOAH) (Porter *et al.,* 2011; Bielawska-drózd *et al.,* 2013).

2.2 Etiology of Q fever

Q fever is caused by *Coxiella burnetii*, a small pleomorphic rod measuring 0.2-0.4mm wide and 0.4-1.0 mm long, a taxonomical and phylogenetically unique gram-negative bacterium, proliferating intracellularly and producing spores which are exceptionally resistant to physico-chemical factors (Pexara *et al.,* 2018; CFSPH, 2017; Bielawska-drózd *et al.,* 2013). It is classified to the family Coxiellaceae in the genus *Coxiella* and species *Coxiella burnetii*. It has an alternating life cycle between a large cell variant (LCV) and a small cell variant (SCV) being differentiated by their virulence, replication, and resistance to physical and chemical factors (Bielawska-drózd *et al.*,2013; Tagesu, 2019). The LCV replicates within a cell while the SCV is non-replicating, infectious and form spore-like particles (SLPs) with highly condensed chromatin which makes it resistant to many environmental conditions (CFSPH, 2017). Furthermore, *C. burnetii* is characterized by two antigenic phases namely phase I and phase II based on its lipopolysaccharide membrane. Phase I is highly virulent and resistant to host cells attack compared to phase II (CFSPH, 2017; Blut *et al.*,2014).

2.3 Epidemiology

Prevalence of Q fever antibodies in Africa suggest the high level of exposure to *Coxiella* burnetii in different animal species, humans, and invertebrates such as ticks (Nusinovici *et al.*,

2010; Lacheheb & Raoult, 2009). An epidemiologic survey of Q fever in Egypt by Klemmer *et al.* (2018) reported the seroprevalence of 6.8-40.7% in goats, sheep, buffaloes, cattle, and camels. Moreover, a systematic review of *C. burnetii* epidemiology across Africa by Vanderburg *et al.* (2014) bared a seroprevalence range between 5 to 55% in domesticated ruminants and humans. The recent studies in Tanzania reported the seroprevalence of 5% in humans with febrile illness and little knowledge about the disease (Prabhu *et al.,* 2011; Alonso *et al.,* 2015). The wild animals (rodents, lagomorphs, and ruminants) and domestic ruminants (cattle, goats, and sheep) are claimed to be the natural reservoirs and the infected ones act as a source of infections to other susceptible hosts including pets, birds, reptiles and mammals including humans (Boroduske *et al.,* 2017). The pathogen is shed in aborted materials, birth materials, urine, faeces/manure, milk, and semen of infected animals (Tagesu, 2019; CFSPH, 2017, Anast & Universit, 2016). Despite the worldwide distribution of the disease, it is highly misdiagnosed and under reported, especially in developing countries including Tanzania (URT, 2020). Several studies have reported a wide disease distribution that compounded by; advancement of animal breeding technologies, movement of animals/animal products, changing of feeding habits and increased interactions between animals and humans (Vanderburg *et al.,* 2014; Bielawska-drózd *et al.,* 2013; Njeru *et al.,*2016). Other risk factors associated with Q fever infection to dairy cattle are age (adult) (Ibrahim *et al.,* 2021; Scolamacchia *et al.,* 2010), sex (female) (Fayiz Abakar *et al.,* 2014; Mangena *et al.,* 2021), Large herd size (Cadmus *et al.,* 2021), new animals in the herd (Mangena *et al.,* 2021; Menadi *et al.,* 2020; Wardrop *et al.,* 2016), extensive and semi-intensive feeding systems (Deressa *et al.,* 2020), herd to herd contact (Deressa *et al.,* 2020), and indigenous breeds (Cadmus *et al.,* 2021). On the other hand high rainfall (precipitation) has been reported as the protective factor to the Q fever transmission (Wardrop *et al.,* 2016)

2.4 Survival of *C. burnetii*

The spore-like particles of *C. burnetii* are impervious to physical factors (heat and drying) and common chemical factors like disinfectants hence survive longer in the environment (Abu Dhabi Food Control Authority, 2011). The bacteria can survive long in animal products such as 42 months in milk, more than a year in wool at 4-6°C or 7-10 months at ambient temperature, fresh meat for 1 month and 40 months in skimmed milk (Group & Countries, 2011). In addition, the pathogen can survive in dust for 4 months, dry state on surfaces at 15-20℃ for 7-10 months and at least 19 months in tick feces (CFSPH, 2017). Furthermore, these particles are resistant

to temperature (62°C for 30 min), UV light and pressure up to 300 000 kPa, acids up to pH of 4.5 and for more than 6 months in 10% salt solutions (Walter *et al.*, 2014). Despite its resistance to physico-chemical factors, *C. burnetii* is killed when exposed to 5% H_2O_2, 0.5% hypochlorite and 70% ethanol for 30 minutes and for less than 30 minutes to 5% chloroform or formaldehyde gas and milk pasteurization at 71.66°C for fifteen seconds.

2.5 Pathogenesis

The pathogen enters via the oropharyngeal route into the intestine and lungs of both animals and humans (Tagesu, 2019). It is phagocytosed by monocytes and macrophages of the host cells and transported to phagolysosome. Within the phagolysosome, the *Coxiella* organism creates a low pH level for its metabolic activation, survival, and replication. Small cell variants (SCV) undergo vegetative growth to form large cell variants (LCV) (Tagesu, 2019). The LCV then actively divides and stay within the phagolysosome whereby finally undergoes saprogenic differentiation to form resistant, spore-like particles (SLPs) namely small cell variants and small dense cells (Tagesu, 2019; Pexara *et al.*, 2018). The SLPs can undergo further development to become metabolically inactive and exorcised from the infected host cells by either exocytosis or cell lysis. The metabolic inactive particles resist the environmental factors hence stay in the soil and dust over many years and can spread far distance about 18 km (Pexara *et al.*, 2018).

2.6 Clinical Manifestations of the Disease in Domestic Ruminants

Clinical manifestations of Q fever are non-specific and exposure history to animals might be not helpful. The organism (*C. burnetii*) has been acutely found in liver, blood, and spleen of experimental animals, whereas persistent shedding of bacteria in feces and urine from chronically infected animals has been documented (Chitanga *et al.*, 2018). Although the disease is said to be asymptomatic in domestic ruminants, the significant clinical signs of Q fever in infected ruminants (cattle, sheep, and goats) are claimed to be associated with pregnancy including stillbirths, birth of small or weak offspring and abortions. Most abortions occur without significant premonitory signs and are reported to occur in the third trimester, sporadic reproductive losses, and other uncommon clinical signs like anorexia, agalactia, retained placenta and infection of subsequent pregnancies have been reported in domestic ruminants (Tagesu, 2019; CFSPH, 2017; Chitanga *et al.*, 2018). Abortion rate is relatively higher in ewes and goats than in cows and abortion is usually observed in late pregnancy in both ewes and

goats (Chitanga *et al.,* 2018). Normally the aborted fetus appears normal while discolored exudate and intracotyledonary fibrous thickening may be observed in an infected placenta (Chitanga *et al.,* 2018). In addition, severe myometrial inflammation and metritis are frequently observed in goats and cows, respectively (Chitanga *et al.,* 2018; CFSPH, 2017).

2.7 Clinical Manifestations of the Disease in Humans

In humans, the symptoms of Q fever have been categorized into asymptomatic, acute, and chronic forms. The mild symptoms include coughing, fever, and minimal auscultatory abnormalities, pneumonia, and neurologic manifestations. The acute symptoms whose incubation period ranges from 2-4 weeks include sudden high fever (up to 400℃), chills, sweat, vomiting, headache, myalgia, arthralgia, and coughing while the chronic form is characterized by abortion, stillbirth, or premature delivery in pregnant women, osteo-articular infections, chronic hepatitis, and chronic fatigue syndrome (Johnson, 2013; Tagesu, 2019; Khamesipour *et al.,* 2018). In addition, Tagesu (2019), Cook *et al.* (2015) and Wardrop *et al.* (2016) reported that chronic Q fever occurs in individuals under high risk including those with immunosuppression, vascular abnormalities, heart valve lesions, pregnant women, and abattoir workers.

2.8 Laboratory Diagnosis

Culturing of *C. burnetii* obliges a bio-safety level 3 laboratory since the bacterium is highly infective and dangerous to laboratory workers (Aphl, 2016). In addition, growing a bacterium takes at least 72 hours leading to delay of both diagnosis and initiation of treatment, thereby increasing the chronicity of the disease (El-Kholy *et al.,* 2015). Similar study El-Kholy*et al.* (2015), reported the positive impact of alternative diagnostic methods like serology and molecular methods which was used in patient suffering from chronic endocarditis, after *C. burnetii* failed to grow upon blood culture. Therefore, diagnosis of Q fever in humans and animals is normally based on serology and molecular methods.

2.8.1 Serology

The Enzyme Linked Immunosorbent Assays (ELISA), compliment fixation tests (CFT), immunofluorescence assay (IFA) are presently the serological tests used for diagnosis of *C. burnetii* in animals. The CFT has been reported to be less sensitive compared to the other two test (Tagesu, 2019). The immunofluorescence assay (IFA) and ELISA tests are said to

have high sensitivity and specificity to phase I and phase II antigens of the bacterium (Tagesu, 2019; Blut *et al.,* 2014). The direct and indirect IFA assays use a fluorescent marker joined with a specific antibody to detect the antibody-antigen reaction (Tagesu, 2019). The positive results are considered when the fluorescent marker in the Ab-Ag complexes emit green light color, detected under the fluorescent microscope. The indirect ELISA test was speculated to be the most highly specific and sensitive to the antibodies against the bacterium (Blut *et al.,* 2014). The ELISA has been used as an indirect test for screening *C. burnetii* infections in ruminants by utilizing a horseradish peroxidase-labeled monoclonal ant ruminant IgG (Blut *et al.,* 2014). The T-cell dependent immune system controls the *C. burnetii* in humans and results into production of specific antibodies. For example, Immunoglobulin G (Ig G) is specific to Phase II antigens while immunoglobulin M (IgM) is specific to both Phase I and Phase II antigens (de Rooij*et al.,* 2012). Furthermore, the level of IgM is considered to be a marker of active infection because it increases shortly after infection and persist for months, while IgG levels surges few weeks after infection, but remain detectable for many years or even lifetime (deRooij *et al.,* 2012). Although commercial IFAs and ELISAs are available and predominately used, there is still a wide interlaboratory variability due to absence of consensus about Ig G and IgM cut-off levels. Therefore, the assay used depended on its ability to detect antigen in a population study (de Rooij *et al.,* 2012).

2.8.2 Molecular Methods

Polymerase Chain Reaction is the rapid diagnostic test for the direct detection of *C. burnetii.* It has been used to detect the bacteria in a wide range of samples such as milk, blood, sera, vaginal swabs, fetal fluids, faeces, semen and urine from animals in many laboratories (Centers for Epidemiology and Animal Health, 2013; Diseases, 2019). The biochemical markers of the bacteria such as plasmid genes, transposase-encoding genes and chromosomal genes found in the insertion elements of the bacteria (IS1111, IS30 and ISA1) increase the sensitivity of the molecular detection of the bacteria to the extent of single cell (Bielawska-drózd *et al.,* 2013). These can be identified by nested PCR assay, real-time PCR, touch-down PCR, and trans-PCR (Selim *et al.,* 2018). Also strain typing techniques are used including multi-locus variable number of tandem repeats analysis (MLVA) and multispacer sequence typing (MST) that allow the typing of *C. burnetii* without necessity of culturing the bacteria (Tagesu, 2019; Porter *et al.,* 2011). In addition, it helps in gaining insights on the pathogenicity and epidemiology of the pathogen and evaluating the control measures (Plummer *et al.,* 2018; Selim *et al.,* 2018).

2.9 Differential Diagnosis

In ruminants especially cattle, Q fever has been reported to be asymptomatic although the diseased animals present reproductive disorders similar to brucellosis, leptospirosis, campylobacteriosis, listeriosis, chlamydiosis, trichomoniasis, Infectious Bovine Rhinotracheitis (IBR) and Rift Valley Fever (RVF). The other diseases are Blue Tongue, Tick borne fever and toxoplasmosis (Tagesu, 2019; Abu Dhabi Food Control Authority, 2011). Furthermore, diagnosis of Q fever especially in LMICs is a challenge due to lack of adequate facilities and non-specific clinical presentation (Tagesu, 2019). In humans, the disease has been misdiagnosed and confused with other diseases such as malaria, influenza, rotavirus, typhoid, and pneumonia as they all have similar symptoms as Q fever (Njeru *et al.,* 2016; Porter *et al.,* 2011; URT, 2020).

2.10 Treatment

2.10.1 Treatment of Q fever in Animals

Studies have reported the use of antibiotics such as tetracycline, macrolides, or quinolones in the treatment of the disease in animals (Alemneh & Ayelign., 2018). Despite the use of antibiotics, mostly tetracyclines, for treatment of the disease and reduction of its severity, the clear evidence of their efficacy has not yet been established and some are reported to promote drug resistance in humans and animals (Tagesu, 2019). In addition, Alemneh and Ayelign (2018) reported that, the highly acidic environment within the phagolysosome where the bacterium replicates lower the antimicrobial activity to fight against the pathogen; alternatively, the use of chloroquine for alkalizing the cells has been suggested to increase the efficacy of some drugs like chloramphenicol, enrofloxacin, and trimethoprim (Alemneh & Ayelign, 2018).

2.10.2 Treatment of Q fever and its Effects in Humans

Treatment of the acute form has been achieved by using tetracyclines in non-pregnant patients and trimethoprim/sulfamethoxazole (cotrimoxazole) is usually used in pregnant women in order to avoid the side effects from other drugs (Porter *et al.*, 2011; Tagesu, 2019; Eldin *et al.*, 2017).

Treatment of chronic Q fever was reported to be difficult to the extent that a single antibiotic

is not generally effective, therefore a synergistic combination of tetracycline and hydroxychloroquine has been suggested (Tagesu, 2019; Kersh, 2015). A combination of tetracycline with quinolones has been reported to be used successfully (Tagesu, 2019). Also, Tagesu (2019) suggested surgical replacement for damaged heart valves and for treatment of aneurysms.

2.11 Control and Prevention

2.11.1 Control and Prevention of Q fever in Animals

It is expensive, difficult, and impractical to identify and cleanse infected areas and to vaccinate the domestic animals (Alemneh & Ayelign., 2018.). Despite the challenges, the use of inactivated whole or extracts of *C. burnetii* phase II vaccine in animals has been done to improve their immunity against the disease (Alemneh & Ayelign, 2018; Bewley, 2013). In addition, a Phase I formalin inactivated Q fever vaccine has been published to provide effective immunization in dairy cattle and it was proven to eliminate shedding of organisms in milk (Alemneh & Ayelign., 2018). Other preventive measures include isolation of aborted animals, burning or burying of aborted foetus, vaginal discharges, and placentas as well as composting of manure for about six months before application to agricultural fields and segregating parturient ruminants (Alemneh &Ayelign, 2018; CFSPH, 2017; Guatteo *et al.,* 2006). Tick control, covering of manure and animal beddings when being stored or transported as well as disposing them where water-run off and pollution are minimal help to reduce environmental contamination (Tagesu, 2019; Sobsey *et al.,* 2011).

2.11.2 Control and Prevention of Q fever in Humans

Proper diagnosis and detection of infections in livestock, reduction of contact with animals or contaminated dust and preventing the consumption of raw milk and products (Gale *et al.,* 2015; Anderson *et al.,* 2013; Signs *et al.,* 2012) were suggested as preventive measures. The personnel under high risk of contracting the pathogen such as animal attendants, abattoir workers, veterinarians and laboratory technicians are advised to use personal protective equipment such as coverall, lab coats, and surgical masks when performing activities involving genital tissues, secretions, excretions, or aborted animals (deRooij *et al.,* 2012; Alemneh & Ayelign, 2018). Also, studies suggest vaccination to be done in endemic areas (Alemneh & Ayelign, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The study was conducted in six regions (Fig. 1) with high population of dairy cattle (Nell *et al.*,2014). Three regions (Arusha, Kilimanjaro and Tanga) were from northern zone and the remaining three regions from southern highland zone (Iringa, Njombe ad Mbeya). These zones receive relatively heavy rains with a cool climate and temperatures range between 14°C and 32°C (URT, 2018). The current statistics show a high dairy cattle population in these regions numbering 161 984 in Kilimanjaro, 78 638 in Arusha, 72 724 in Mbeya, 21 132 in Njombe, 41 639 in Tanga and 14 258 in Iringa (Nell *et al.,* 2014) whereas dominant breeds were Shorthorn-Zebu cross Friesian and Shorthorn-Zebu cross Ayrshire.

Map shows the location (black dots) of sampled sites across the Mbeya, Njombe, Iringa, Kilimanjaro, Arusha and Tanga regions of Tanzania.

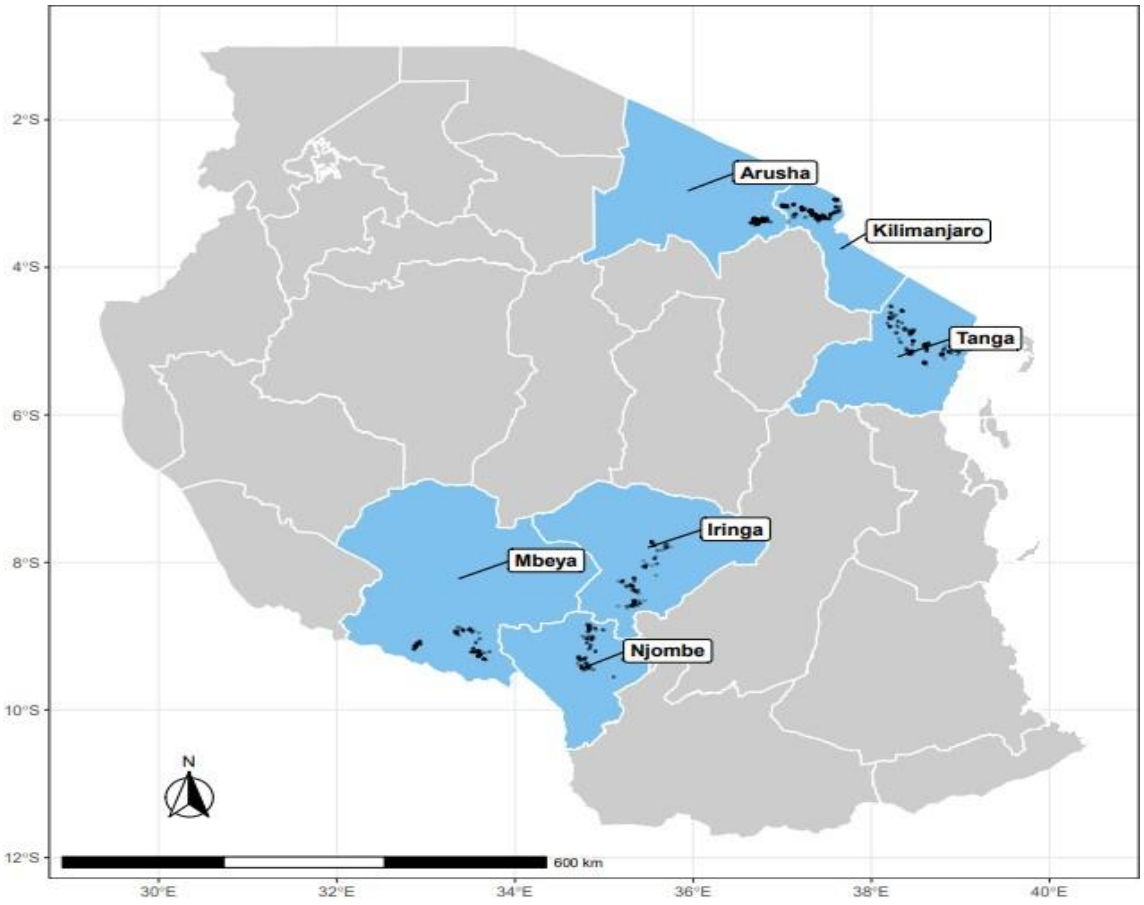

Figure 1: Geographic location of samples across six regions of Tanzania

3.2 Climate of the Selected Regions

Kilimanjaro and Arusha are found in the North-eastern highlands and Tanga in the Northern Coast parts of Tanzania. The places receive long rains (masika) which normally occur from March to May and short rains (vuli) occurring in October to December. Mbeya, Songwe, Iringa and Njombe are found in the South-western Highlands of Tanzania with prolonged rainfall occurring from November to April (URT, 2018). Temperature ranges from 16.5-32°C in Northern parts whereas in Southern highlands range from 14.4-27°C (URT, 2018).

3.3 Study Design

A cross-sectional epidemiological study design for determining the seroprevalence of *C. burnetii* in dairy cattle was used. Six (6) regions (Arusha, Tanga, Kilimanjaro, Iringa, Njombe, and Mbeya) and then 23 districts (n=11 from southern highland zone and n=12 from northern zone) were previously identified and selected by the African Dairy Genetic Gains (ADGG) project based on the high number of dairy cattle. The project aimed to enhance animal production and increase profitability to smallholder dairy farmers through genetic selection. A total of 52500 animals were registered in the project and ~4000 was randomly selected and genotyped to understand their genetic make-up and could be identified by their preliminary information such as an ear tag number, age, and sex from the ADGG database. Enumerators (livestock extension workers) were selected and trained from each district for administering questionnaires and samples collection.

3.4 Sample Size and Data Collection

The sample size was calculated based on Arya and Antonisamy (2015) formula:

$$n = \frac{1.96^2 p(1-p)}{d^2} \qquad (1)$$

where n = required sample size, p = estimated prevalence of Q fever, and d = precision. Since the prevalence of Q fever in dairy cattle was unknown, a prevalence of 50% was estimated at 95% confidence and a precision of 5% giving a required minimum size of 385 per region making a grand total of 2310 for six regions to be sampled. However, only 2049 were sampled in all six regions between July 2019 and October 2020. The rest were not found during sampling due to the different reasons such as death, slaughter and selling. During sampling animals were identified by their unique ear tag numbers, sex, age, as well as the owners of the

farm.

3.4.1 First Objective

To determine the seroprevalence and distribution of Q fever in smallholder dairy cattle in selected regions of Tanzania.

Blood samples were collected as described by Noden *et al.* (2020). Cattle was approached, restrained by using a rope, and through venipuncture of jugular vein the blood sample was collected into 10 ml plain vacutainer tubes (Noden *et al.*, 2020) (Fig. 2 ii). Tubes were labelled with date, animal identification number and barcoded and the barcode was scanned into the Open Data Kit (ODK) survey form to link the animal's biodata and the farm/herd owners. The tubes containing blood were placed vertically in a cool box packed with freezing pads and were allowed to clot before shipped to the local regional laboratory for further processing. At the laboratory, clotted blood was centrifuged at 3000 revolution per minute (rpm) for 15 minutes to get clear serum separation (Emery *et al.*, 2014). Then serum was aliquoted out (Fig. 2 iii) and preserved into a 1.8ml transparent self-standing screw-capped cryogenic vials with gasket, well labelled with a barcode to link to the animal biodata. Sample storage details were captured in a Microsoft® Access® 2013 database (i.e., sample identification number, date of sample collection, field barcode and laboratory barcode on the cryovials storage box) and kept in a freezer at -20°C before shipment. The serum samples were then shipped to the Nelson Mandela African Institution of Science and Technology Laboratory, Arusha Tanzania and stored at -20°C before analysis. Figure 2 details on (ii) Collecting blood from the jugular vein (iii) Sera aliquoting.

ii iii

Figure 2: Sample processing

3.4.2 Second Objective

To determine the risk factors associated with Q fever seropositivity in smallholder dairy cattle populations in selected regions of Tanzania.

3.5 Questionnaire Data

A questionnaire survey was designed and uploaded to the Open Data Kit (ODK) cloud platform software, version 1.22.4. Before finalizing, the questionnaire was downloaded to an android device which had ODK app installed via Google Play. The face-to-face interview was done in the field immediately after cattle sampling, targeting the farm owners/animal caretakers whose animals were blood sampled for serology and the answers were recorded onto the ODK form. Specifically, the farm owners/animal caretakers who was an adult (18 years and above) and familiar with cattle rearing was interviewed (Fig. 3 i). The information captured included demographics of the owner, animal age, animal sex, animal breed, reproduction history (e.g., previous pregnancies, abortion), herd management (e.g., number of animals in the herd, distance between next herd, water and feeding management, milking, presence of other animals within the household and placenta disposal). Finally, geographical coordinates for each farmers' household where animals were sampled, and questionnaires were administered. Coordinates were recorded to allowing mapping and to obtain environmental variables from publicly available databases. Environmental data such as population density and solar radiation were sourced from the open.africa, elevation map from USGS, land cover from CCI Land Cover LC, and the mean annual temperature and precipitation from worldclim.org. After each day of fieldwork, finalized forms were transmitted securely over the internet and aggregated on the server at ILRI, Nairobi, Kenya, prior to analysis. Figure 3 details on (i) the interview and farmers' consent.

i

Figure 3: Questionnaire data collection

3.5.1 Third Objective

To determine the pathogen shedding through vaginal discharges in smallholder dairy cattle populations in selected regions of Tanzania.

The vaginal swab samples were collected from female cattle sampled for serum. The animal was restrained manually by using a rope and a dry sterile flexible shaft cotton wool swab (10 cm) was introduced into the vagina and rotated within to absorb the mucus after vulva disinfection by using 10% chlorhexidine solution and dried up by using a tissue paper. The vaginal swab sample was immersed into an individual 1.8 ml transparent self-standing screw-capped cryogenic vial with gasket containing 1 ml of phosphate buffered saline (PBS). Once saturated, the vaginal swab sample was agitated and pressed against the edges of the cryogenic vial to release the sample. Then the cryogenic vial containing the vaginal swab suspension was labelled with date, animal identification number and barcoded and the barcode was scanned into the ODK survey form to link the animal's biodata and the farm/herd owners. The cryogenic vials containing the sample were placed vertically in a cool box packed with freezing pads, shipped to a local regional laboratory. At the laboratory, the sample storage details were captured in a Microsoft® Access® 2013 database (i.e., sample identification number, date of sample collection, field barcode and laboratory barcode on the cryovials storage box) and kept in a freezer at -20°C before shipment. Finally, the cryovials storage boxes with samples in a cool box with ice packs were shipped to Nelson Mandela African Institution of Science and Technology laboratory where were stored at -20°C before analysis.

3.6 Laboratory Analysis

3.6.1 Serological Analysis

Anti-*Coxiella burnetii* antibodies in serum were detected using a commercially available indirect ELISA kit (PrioCHECKIT™ Ruminant Q fever Ab Plate Kit– ELISACOXLS2, ELISACOXLS5) following manufacturer's instructions (Fig. 4 iv-vi). Before performing the test, sera were allowed to thaw, and the testing reagents were brought at room temperature (25°C). When testing, pre-dilution of 5 µl serum samples and controls (positive and negative) with 95 µl of Q fever sample dilution buffer was done in 96 wells-flat bottom uncoated plate and incubated for five minutes at room temperature. Then, 5 µl of pre-diluted serum samples and controls (positive and negative) were added in the wells of the Q fever-coated microplate and 95 µl of Q fever sample dilution buffer added to each well containing the controls or

samples. The plate was gently shaken, covered with an adhesive cover plate, and incubated for 1 hour at 37°C. The plate was manually washed three times using diluted wash solution (1:100) and tapped on an absorbent tissue paper to eliminate any traces of unbounded antibodies before adding 100 µl of diluted conjugate Q fever solution, covered the plate with an adhesive cover plate and incubated for 1 hour at 37°C. The plate was again manually washed three times and tapped on an absorbent tissue paper and 100 µl of substrate added to each well and incubated for 10 minutes at room temperature in darkness followed by 100 µl of stop solution to each well. The results were read within 30 minutes after stopping the reaction at 450 nm in a microplate reader (Bio Tek S1LFTA - USA). The results were expressed as S/P (sample/positive) ratio and the percentage positivity (PP) were calculated as follow:

$$\frac{\boldsymbol{S}}{\boldsymbol{P}} = \frac{\boldsymbol{ODsample - meanOD\ negative\ control}}{\boldsymbol{meanOD\ positive\ control - meanOD\ negative\ control}} \qquad (\mathbf{1})$$

$$\boldsymbol{PP} = \frac{\boldsymbol{S}}{\boldsymbol{p}}\boldsymbol{x}\mathbf{100}\% \qquad (\mathbf{2})$$

The results were interpreted as follows: PP ≤ 40 was defined as negative, 40< PP ≤ 100 was defined as weak positive +, 100< PP ≤ 200 was defined as moderate positive ++, 200< PP≤ 300 was defined as strong positive +++ and PP >300 was defined as the strongest positive ++++.

Figure 4 details on (iv) loading serology plate (v) Mounting plate in ELISA reader (vi) Color change after adding stop solution i.e., yellow color represents positive and colorless represent negative results.

iv v vi

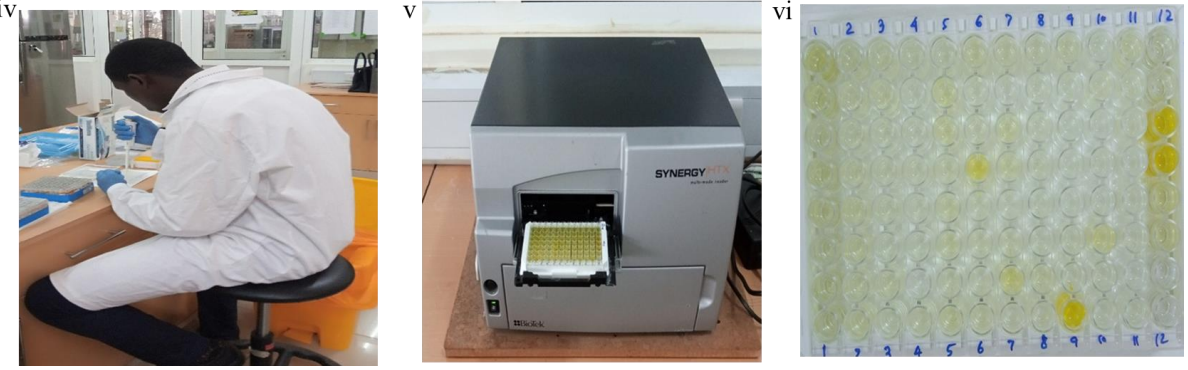

Figure 4: Serological analysis

3.6.2 Molecular Analysis

(i) The DNA extraction

DNA extraction followed the instructions given in the Quick-StartProtocol QIAGEN® DNeasy ® (QIAGEN Group). 1066955 01/2011 © 2011 QIAGEN). Procedurally, vaginal swab samples previously kept in PBS were thawed (from -20°C to 25°C). 200 µl of vaginal swab sample previously kept in PBS were mixed with 20 µl Proteinase K in a 2ml Eppendorf tube, vortexed for 15 seconds and spined down. The mixture was incubated at 56°C for 10 minutes. Thereafter, 200 µl of absolute alcohol was added followed by vortexing and centrifugation for 15 seconds. The content in Eppendorf tubes was transferred to QIAamp min spin column (in a 2ml collecting tube) without wetting the rim. The cap was closed, spinning down and centrifugation at 6000 x g (8000 rpm) for 1 minute. The QIAamp Min spin column was placed in a clean 2ml collection tube and closed to avoid aerosol formation, and discard the tube containing the filtrate. The QIAamp Mini spin column was opened, 500 µl of Buffer AW1 was added without wetting the rim then cap was closed. Centrifugation was performed at 6000 x g (8000 rpm) for 1min followed by placing the QIAamp Mini spin column in a clean 2ml collection tube and discarding the tube containing the filtrate. Next the QIAamp Mini spin column was carefully opened added with 500 µl Buffer AW2 without wetting the rim. The cap was closed, and centrifugation was performed at full speed of (20 000 x g; 14 000 rpm) for 3 min. Lastly, the QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. This was followed by carefully opening the QIAamp Mini spin column for 30minutes to let the alcohol evaporate, then 100µl Buffer AE (elution) were added and incubated at room temperature (25°C) for 1 min, and then centrifugation at 6000 x g (8000 rpm) for 1 min to yield the DNA. The yielded DNA were stored at -20°C freezer before subsequent analysis. These procedures were performed at the International Livestock Research Institute (ILRI) Nairobi, Kenya.

(ii) Developing a Standard Working Positive Control

To obtain the standard working protocol for positive control, serial dilution of concentrated control by TAE buffer at 10:90 was performed. The lowest limiting detection point of positive control was determined based on standard curve (Fig. 5) and the inherent amplification plot (Fig. 3). From the standard curve measured the efficiency of 97.5% and error of 0.05 (Fig. 2) was attained and the chosen standard amplification working control

was the standard number 8 on the amplification plot as shown in Fig. 6 (the lowest detection point).

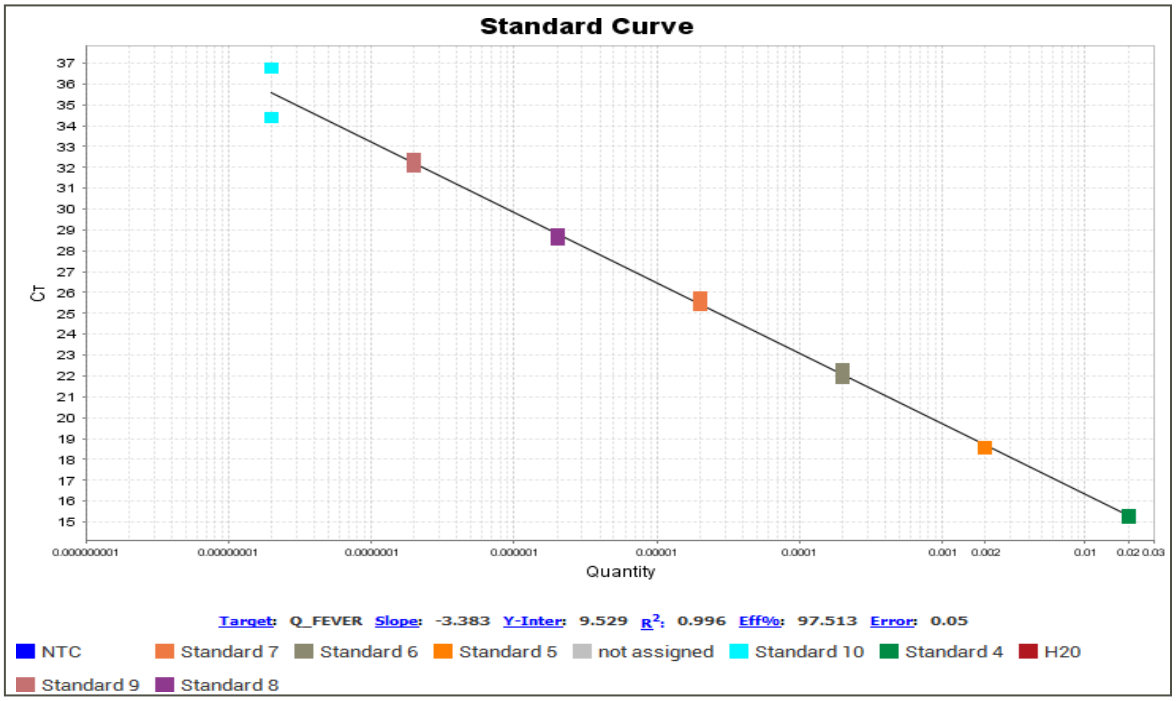

Figure 5: Standard curve for the positive control 10:90 TAE buffer dilution

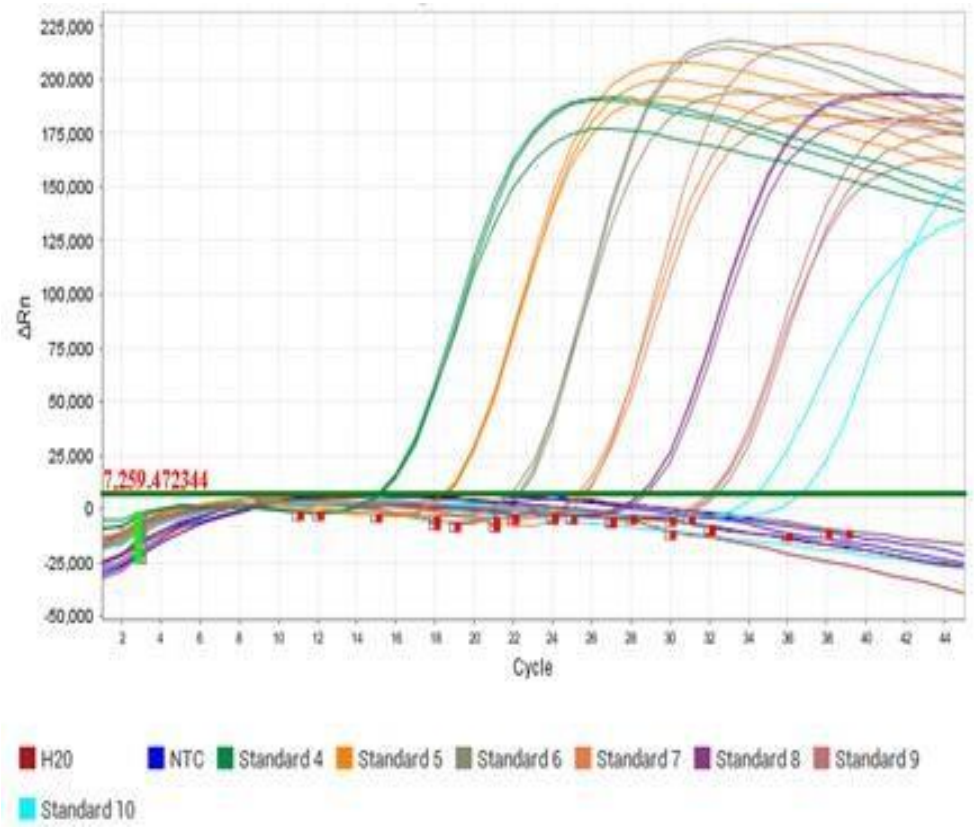

Figure 6: The amplification plot to determine the lowest detection limit of positive control

(iii) The DNA Amplification/qPCR Procedures

The Luna® Universal Probe qPCR Master Mix (M3004L) from New England Biolabs® inc. 6-carboxyfluorescein FAM-labelled probe was used. A total mixture of Luna® Universal Probe qPCR Master Mix (7.5µl) plus 0.75µl of reverse 5'-GGTTGGTCCCTCGACAACAT-3 and forward primers 5'CATCACATTGCCGCGTTTAC-3' and TE buffer (low salt) plus free water (4.75 µl) making a total of 13 µl of the mixture. Then 13µl were pipetted into a 96 PCR plate well in the hood cabinet (Fi. 7 a). Then 2 µl of the thawed vaginal swab DNA sample was added to each well containing the master mix except the two wells for positive and negative controls (Nuclease-free water) in which the 2µl of each was added (Fig. 7 b). The overall mixture measured 15 µl in each well. The PCR plate was covered with an adhesive cover, spined down to remove any bubbles (Fig. 7 c) and finally placed in the QuantStudio™ Design & Analysis Software (PCR reader) (Fig. 7 d) and the following setting were set as per Luna® Universal Probe qPCR Master Mix protocol instructions: initial denaturation at 95°C for 60 seconds, denaturation at 95°C for 15 seconds and extension at 60°C for 30minutes. The process run at 45 cycles and finally the results were read. Any amplification above the by-default threshold line was interpreted as the positive test (Fig. 7 e**)**.

The qPCR procedures such as (a) Loading primers and probe (b) Loading DNA samples (c) Spinning (d) Mounting a PCR plate in Quant-studio machine (e) Reading results were shown in Fig. 7.

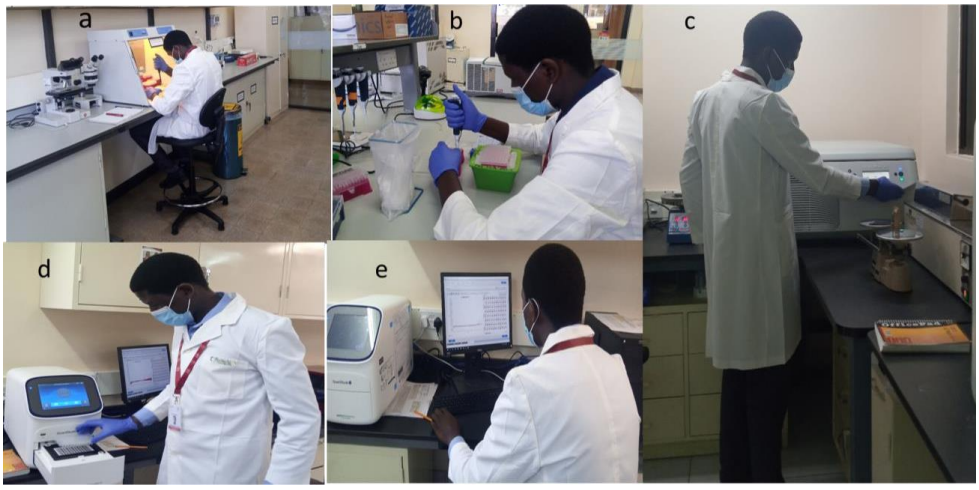

Figure 7: qPCR procedures for *C. burnetii* DNA determination

3.7 Statistical Analysis

Data stored in the database was accessed, entered in Microsoft Excel spreadsheet, saved as

CSV (Comma delimited) (*.csv) and all statistical analyses were performed using RStudio version 4.2.0 (2022-04-22 ucrt) and Platform: x86_64-w64-mingw32/x64 (64-bit). Individual animal and farm seroprevalence were calculated as:

$$p = \frac{x}{n} \qquad (4)$$

where x= total number of animals/farms tested positive for *C. burnetii* antibodies, and n= total number of animals/farms tested for *C. burnetii* antibodies. The formula was also used to compute the overall seroprevalence i.e., all six regions and in particular region. Further, confidence intervals (CI) for binomial proportions of seropositive was implemented in *binom.test* function. To measure associations between all variables and the binary response, odds ratios were estimatedand confidence intervals (0.95 confidence level) using conditional maximum likelihood and normal approximation, respectively, which were implemented in the *epitools* R package (Aragon*et al.,* 2020). Further, confidence intervals (C. I) for binomial proportions of seropositive was implemented in *binom.test* function. Variables with p-value < 0.2 in association analyses and hypothesized Q fever risks were chosen for multivariable analyses. To model the relationship between the ELISA binomial results and a set of covariates, binomial (logistic) was built and generalized linear mixed effects models (GLMM) with a log link function implemented in the template model builder *glmmTMB* R package (Bolker, 2017) and assuming a linear relationship.

$$Yij \sim \text{Bin}\ (1,\ pij)$$

$$E(Yij) = \sim (\ pij)$$

$$\text{logit}(pij) = a + \beta_1 x_1 + \beta_2 x_2 + \ldots \beta_{ij} x_{ij} + a_i$$

$$a_i \sim N\ (0,\ \sigma^2_a)$$

Where, conditional on a random intercept, *district*, and 44 of covariates, Yij is the *j*th ELISA result binomially distributed with a conditional probability, pij, in *district i,* and $i = 1\ldots. 23$, and *district*, a_i, is the random intercept, which is assumed to be normally distributed with mean 0 andvariance σ^2. Continuous fixed effects variables were mean-centered and scaled to standard deviation using the *scale* function. To avoid multicollinearity, Spearman's rank correlation coefficient (rho) test was run on continuous variables pairs to ensure they were uncorrelated (rho < 0.29 based on Cohen (1992). A backward stepwise model selection approach was carried

out to eliminate one variable at a time based on the model best fit criteria. For instance, keeping nested models with the lowest Akaike Information Criterion (AIC) and significant (p-value < 0.05) χ^2 statistics from likelihood ratio tests. In parallel, marginal, and conditional R^2 calculated using the *rsquaredGLMM* functionimplemented in the *MuMIn* R package (Barton, 2022) was used to select the model explaining most of the data variance. The best model was validated by simulating residuals using the *simulateResiduals* function from the *DHARMa* R package (Dharm, 2020). The model was valid if residuals were plotted versus fitted values and each fixed effect showed no clear clustering patterns.

3.8 Ethical Clearance

Ethics of the study for animal subjects was reviewed and approved by the International Livestock Research Institute Institutional Animal Care and Use Committee (ILRI-IACUC2018-27) and the research permit was granted by the Tanzania Commission for Science and Technology (COSTECH), Ref. (2019-207-NA-2019-95). Written consent forms were signed by cattle owners before the interview and sample collection. A qualified Livestock Field Officer (LFO) was engaged to restrain the animals during sampling. Local approval was sought from regional, district and village government authorities (LGAs) under the President's Office, Regional Administration and Local Government Authorities (PO-RALGA).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Social Demographic Characteristics of the Respondents

Of the interviewed respondents, 780 (56.8%) were males and 593 (43.2%) were females. 77.6% of all respondents had primary education level, 2.2% no formal education, 11.5% secondary level, and 8.7% had tertiary education level. Regarding looking at the animals, 72.3% of the respondents were primarily looking at the animals and depended on these animals as the main source of income whereas 27.7% were rarely looking at the animals and regarded them as a secondary source of income. Furthermore, 1226 (89.3%) compared to 147 (10.7%) had up to thirty years' experience in keeping cattle. Most of the respondents (72.6%) had no regular livestock trainings and almost 99% unaware about placenta and milk as the sources of Q fever infection to humans.

4.1.2 Information Related to Dairy Cattle

For the present study, 2310 dairy cattle were to be sampled, however due to logistic constraints and decreased number of previously enumerated (due to death, selling and slaughter) a total of 2049 blood samples were collected from dairy cattle in 1374 herds with a median of two dairy cattle per herd/farm (Fig. 8 A) and processed to obtain sera for serological analysis. Among dairy cattle sampled, high proportion were females (97.2%) with SHZ-Friesian cross being predominant (68.4%) followed by SHZ-Ayrshire (20.6%) and the rest (11%) were SHZ-Jersey and other indigenous breeds (Fig. 8 B). Among these breeds abortion cases with unknown cause were reported by the respondents and their prevalence presented in (Table 1). Furthermore, 57% of the sampled population had less than five years old and averagely 62% intermingled with other animals like goats, sheep, pigs, carnivores (dogs and cats) within and/or neighboring compound. In addition, about 90% of the interviewed farmers reported rodents in their household vicinities including loitering in cattle farms.

Figure 8 shows the plots showing (A) the number of sampled animals per farm/herd and (B) showing the distribution of dairy cattle in six regions of Tanzania where blood samples were collected.

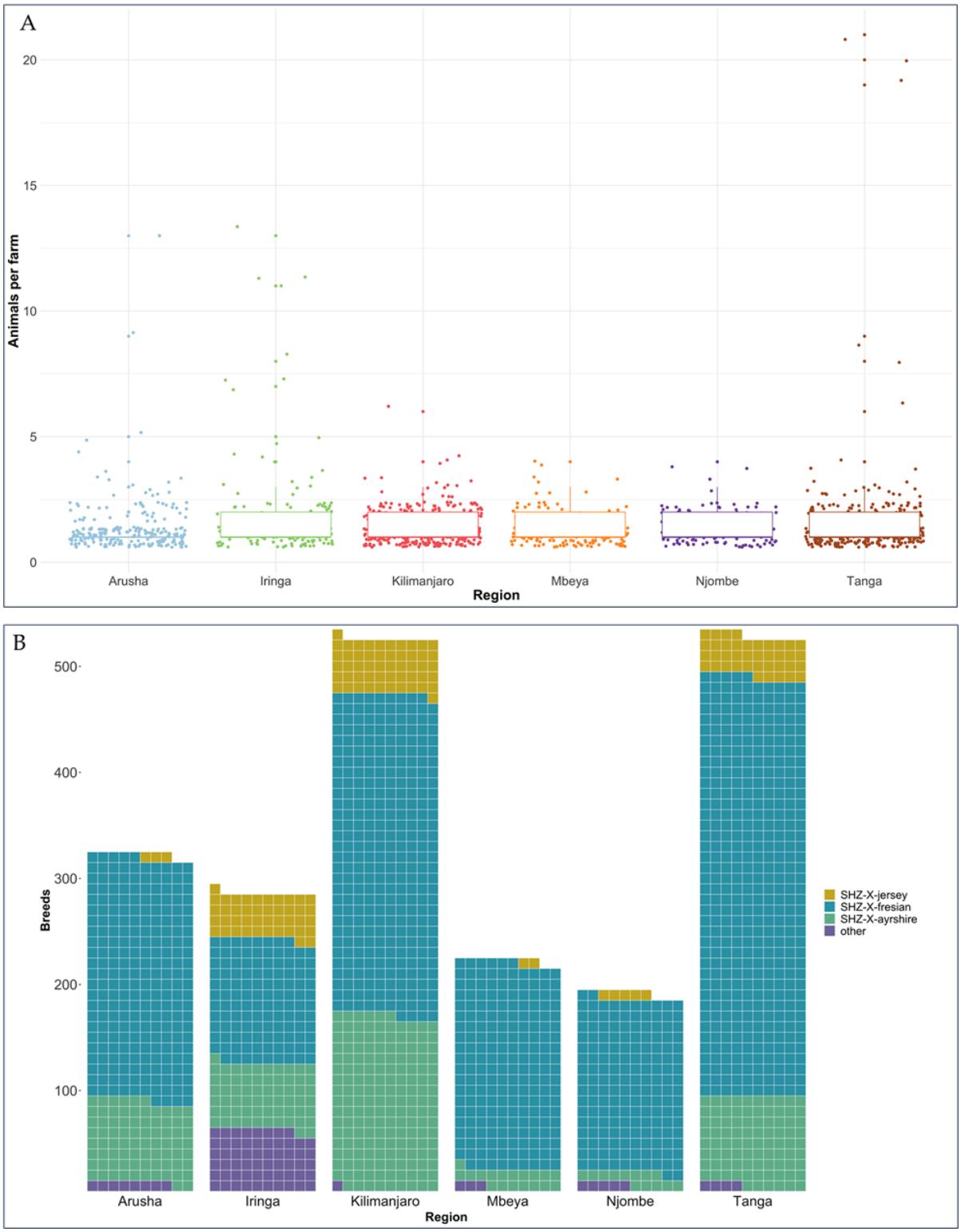

Figure 8: Plots showing information related to dairy cattle

Table 1: Summary of history of abortion in a farm/herd in the last 12 months before data collection

Region	Other	Ayrshire	Friesian	Jersey	Abortion	No abortion	Total number of animals	% of abortion
Arusha	1	6	22	0	29	271	300	9.7
Iringa	21	11	32	7	71	210	281	25.3
Kilimanjaro	0	6	8	0	14	503	517	2.7
Mbeya	0	1	23	1	25	193	218	11.5
Njombe	0	1	7	0	8	179	187	4.3
Tanga	0	9	24	2	35	382	417	8.4
Total	**22**	**34**	**116**	**10**	**182**	**1738**	**1920**	**9.5**

For each region, number of cases reported in dairy cattle breeds indigenous (Other), Cross-bred of short horn zebu and Ayrshire (Ayrshire), Cross-bred of short horn zebu and Friesian (Friesian), Cross-bred of short horn zebu and Jersey (Jersey), total number of abortion cases (Abortion), no reports of abortion (No abortion), total number of female animals (total number of animals) and Prevalence per region (% of abortion) were provided.

4.1.3 Results for Serological Analysis

Out of 2049 tested sera using ELISA, 79 were seropositive making an overall seroprevalence of Q fever being 3.86% (Table 2). Regional wise Tanga region had the highest seropositivity (8.21%) followed by Iringa (4.63%) and none in Mbeya region (Table 2). The summarized results in Tables 3 and 4 detail on the farms and individual animal prevalence to anti-*C. burnetii* antibodies at the district level. In northern zone, the farm prevalence ranged between 0-41.17% and 0-15.8% at individual level (Table 3). In the southern highlands zone, farm prevalence ranged from 0 to 15.8% whereas at individual animal level was 0 to 4.4% (Table 4). By using a Purely Spatial analysis scanning for cluster with high rates using the Bernoulli model, one highly significant cluster **(***p* < 0.01) was detected in Tanga region located at (5.164720 S, 38.895229 E,). The cluster composed of 362 animals of which 42 were seropositive making a seroprevalence of 11.6% (95% CI: 8.5–15.4) and a relative risk of 5.29. Additionally, Fig. 7 depicts the spatial distribution of the disease at the district level found in the respective regions.

Table 2: Q fever seroprevalence in smallholder dairy cattle across six economical important regions in Tanzania

Region(s)	-	+	Total	seroprevalence%	95% CI	Pops	Weights
Arusha	314	4	318	1.26	0.34 - 3.12	78637	247
Tanga	481	43	524	8.21	6.0 - 10.89	41639	79
Kilimanjaro	505	16	521	3.07	1.77 - 4.94	161984	311
Mbeya	218	0	218	0	0.0 - 1.68	72724	334
Njombe	184	3	187	1.6	0.33 - 4.62	7177	38
Iringa	268	13	281	4.63	2.49 - 7.78	7081	25
Total	**1970**	**79**	**2049**	**3.86**	**3.06 - 4.78**	**369242**	

For each region, the number of seronegative (-) and seropositive (+) from the total sampled animals, as well as seroprevalences (%) with 95% confidence intervals, population size (Pops) and weight proportion (%) are provided.

Table 3: Seroprevalence of Q fever in smallholder dairy cattle at a district level in northern zone of Tanzania

Region(s)	District	No. of farms	+ve farms	Prevalence %	No. of animals	+ve cattle	Prevalence %
Arusha	ACC	83	3	3.6	140	3	2.1
Arusha	ADC	65	0	0	79	0	0.0
Arusha	MDC	76	1	1.3	98	1	1.0
Kilimanjaro	HDC	51	7	13.7	102	7	6.9
Kilimanjaro	MRDC	220	9	4.1	277	9	3.2
Kilimanjaro	RDC	38	0	0	45	0	0.0
Kilimanjaro	SDC	66	0	0	97	0	0.0
Tanga	TCC	51	21	41.2	133	21	15.8
Tanga	KDC	61	6	9.9	101	6	5.9
Tanga	KTC	31	2	6.5	44	2	4.5
Tanga	MuDC	91	14	15.4	139	14	10.1
Tanga	LDC	88	0	0	107	0	0.0

ACC= Arush City Council, **ADC**= Arusha District Council, **MDC**= Meru District Council, **HDC**=Hai District Council, **MRDC**= Moshi Rural District Council, **RDC**= Rombo District Council, **SDC**= Siha District Council, **TCC**= Tanga City Council, **KDC**=Korogwe District Council,**KTC**=Korogwe Town Council, **MuDC**=Muheza District Council and **LDC**=Lushoto District Council.

Table 4: Seroprevalence of Q fever in smallholder dairy cattle at a district level in southern zone of Tanzania

Region	District	No. of farms	+ve farms	Prevalence %	No. of animals	+ve cattle	Prevalence%
Iringa	IDC	41	3	7.32	74	3	4.1
Iringa	IMC	13	2	15.38	45	2	4.4
Njombe	MTC	37	4	10.81	94	4	4.3
Njombe	MkTC	36	1	2.78	55	1	1.8
Njombe	MuDC	57	4	7.01	98	4	4.1
Njombe	NDC	38	2	5.26	51	2	3.9
Njombe	NTC	62	0	0	81	0	0.0
Mbeya	MCC	23	0	0	27	0	0.0
Mbeya	MDC	21	0	0	27	0	0.0
Mbeya	MbDC	41	0	0	66	0	0.0
Mbeya	RDC	80	0	0	99	0	0.0

IDC= Iringa District Council, **IMC**= Iringa Municipal Council, **MTC**= Mafinga Town Council, MkTC= Makambako Town Council, **MuDC**= Mufindi District Council, **NDC**= Njombe District Council, **NTC**= Njombe Town Council, **MCC**= Mbeya City Council, **MDC**= Mbeya District Council, **MbDC**= Mbozi District Council, **RDC**= Rungwe District Council.

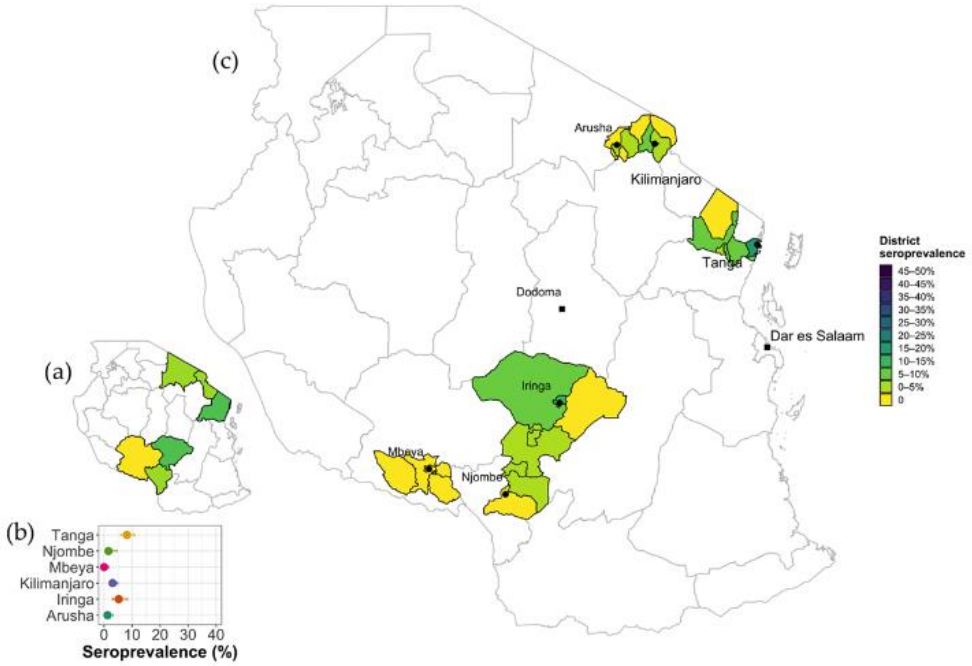

Figure 9: Q fever seroprevalence spatial variation across sampled Regions and Districts in Tanzania

(**a**) Inset map showing regional seroprevalence across six study Regions. Colored areas were sampled, and white areas were not sampled (**b**). Forest plot with Q fever seroprevalence (%) point estimate and 95% CI for each Region. (**c**) Choropleth map showing the seroprevalence at District level including; Arusha District Council, Arusha City Council, Meru District Council, Siha District Council, Rombo District Council, Hai District Council, Moshi Rural District Council, Lushoto District Council, Korogwe District Council, Korogwe Town Council, Muheza District Council, Tanga City Council, Mbozi District Council, Mbeya City Council, Mbeya District Council, Rungwe District Council, Makambako Town Council, Njombe District Council, Njombe Town Council, Iringa District Council, Iringa Municipal Council, Mafinga Town Council, and Mufindi District Council.

4.1.4 Molecular Detection of *C. burnetii* in Vaginal Swabs

A total of 1920 vaginal swabs were subjected to the quantitative PCR (qPCR) analyses. Out of these, 18 samples tested positive making an overall prevalence of 0.94% (Table 4). Region-wise, Arusha had the highest prevalence (2.3%) followed by Tanga (1.4%) and least Kilimanjaro with 0.39% (Fig. 8). On southern highland zone Iringa had higher prevalence (0.71%) than Njombe (0.53%) and none from Mbeya region (Table 5).

For each region, positive samples from the total sampled animals, as well as prevalence (%) with 95% confidence intervals are provided.

Table 5: qPCR prevalence in smallholder dairy cattle (Females) across the study areas

Region	Samples collected	Positive samples	Prevalence %	95%CI
Arusha	300	7	2.3	0.9-4.7
Iringa	281	2	0.71	0.08-2.5
Kilimanjaro	517	2	0.39	0.04-1.4
Mbeya	218	0	0	0.0-1.6
Njombe	187	1	0.53	0.01-2.9
Tanga	417	6	1.44	0.5-3.1
Total	**1920**	**18**	**0.94**	**0.6-1.5**

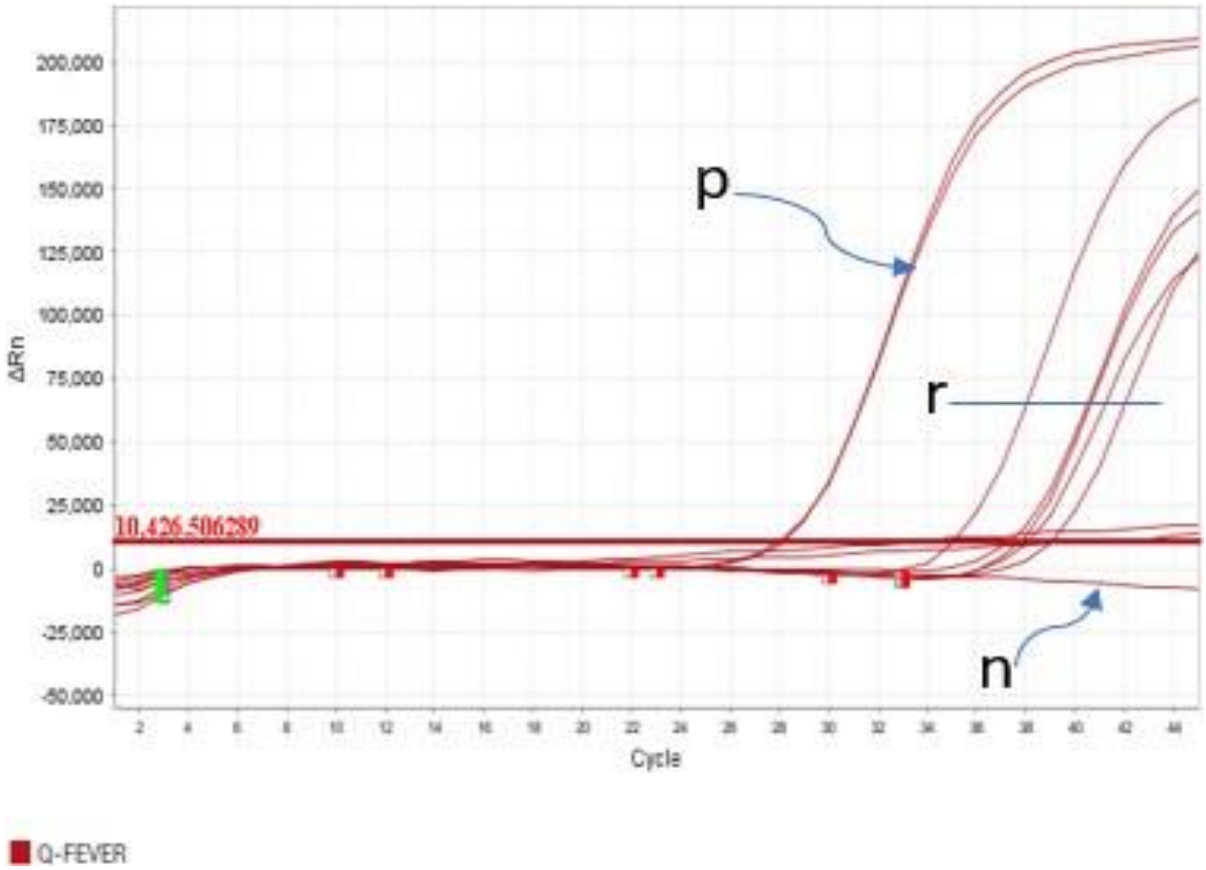

Figure 10: Plot presenting the qPCR Q fever results

p=shows the positive control, n=negative control, and r= positive samples

4.1.5 Univariable Analysis for *C. burnetii* Seropositivity

The Univariable analysis involved the animal, herd, farm management, location, and environmental related risk/protective factors for *C. burnetii* seropositivity as summarized in Table 5. Of three variables presented under animal category, all had increased odds but not statistically significant associated with *C. burnetii* seropositivity. At the herd level, presence of rodents increased the odds of *C. burnetii* seropositivity (OR 5.44, 95% CI 0.75-39.43). Interestingly, keeping pigs appeared to be protective factor with decreased odds of dairy cattle being seropositive (OR 0.62, 95% CI 0.39-0.98). Factors categorized under farm management such as herd size (more than three cattle per herd) (OR 2.00, 95% CI 1.28-3.14) and feeding management (extensive system) (OR 2.43, 95% CI 1.54-3.83) were significantly associated with *C. burnetii* seropositivity. Furthermore, environmental factors such as annual average ambient temperature over 20°C (OR 3.63, 95% CI 2.28-5.78) and solar radiation over 5W/m^2 (OR 2.34, 95% CI 1.16-4.71) were significantly associated with *C. burnetii* seropositivity. Finally, comparing the two dairy cattle keeping zones from which the samples were collected, animals from the northern zone were two times more likely to be seropositive compared to those originated from southern highland zone (OR 2.03, 95% CI 1.17-3.55).

Table 6: Summary of univariable analysis results for *C. burnetii* seropositivity in smallholder dairy cattle

Variables	Levels	Negative	Positive	OR	95% CI	*p Value*
Animal related variables						
Age	0–4 Years old	742	24	1		
	>4 Years old	1228	55	1.38	0.85–2.25	0.24
Animal sex	Male	57	1	1		
	Female	1913	78	2.31	0.31–16.89	0.72
Breed type	Cross-bred	1895	75	1		
	Indigenous	75	4	1.33	0.47–3.74	0.55
Herd related variables						
Presence of rodents	No	127	1	1		
	Yes	1843	78	5.44	0.75–39.43	0.05
Keeping dogs	No	229	6	1		
	Yes	1741	73	1.62	0.7–3.77	0.37
Keeping cats	No	172	10	1		
	Yes	1798	69	0.67	0.34–1.32	0.23
Keeping goats	No	648	24	1		
	Yes	1322	55	1.14	0.7–1.86	0.63
Keeping sheep	No	1482	65	1		
	Yes	488	14	0.65	0.36–1.17	0.18
Keeping pigs	No	905	46	1	0.39–0.98	0.04
	Yes	1065	33	0.62		
Farm management related variables						
Herd size	1–3	1280	38	1		
	>3	690	41	2.00	1.28–3.14	<0.01
Own bull for breeding	Yes	522	17	1		
	No	1448	62	1.32	0.76–2.27	0.36

Variables	Levels	Negative	Positive	OR	95% CI	*p Value*
Water source	Tap	1253	55	1		
	Ground	717	24	0.76	0.47–1.24	0.34
Feeding management	Intensive system	1489	44	1		
	Extensive system	481	35	2.43	1.54–3.83	<0.01
Placenta disposal		5	0	1		
	Destroy Environment	1975	79	0.45	0.02–8.21	1
Location related variables						
Region	Southern Highlands	671	16	1		
	Northern Zone	1299	63	2.03	1.17–3.55	0.01
Distance to next farm	>100 M	509	23	1		
	<100 M	1461	56	0.86	0.52–1.41	0.60
Environmental related variables						
Temperature	≤20 °C on average annually	1359	30	1		
	>20 °C on average annually	611	49	3.63	2.28–5.78	<0.01
Precipitation	>1000 mm on average annually	1637	60	1	0.92–2.64	0.13
	≤1000 mm on average annually	333	19	1.56		
Wind speed	≤7 Km/h	1092	35	1		
	>7 Km/h	876	44	1.54	0.98–2.42	0.07
Solar radiation	≤5 W/m^2	450	9	1	1.16–4.71	0.01
	>5 W/m^2	1520	70	2.34		

4.1.6 Multivariable Logistic Regression of Independent Variables

Twelve independent variables (animal age, animal sex, breed, herd size, feeding management, presence of rodents, keeping pigs, region, district, temperature, precipitation, and wind speed)

which were included and backward elimination of one variable to obtain the best fit model (Table 6). Three variables (animal age, feeding management, and precipitation) had the lowest Akaike Information Criterion (AIC) of 613.09, conditional and marginal R^2 of 0.34 and, 0.08, respectively. On validating the best fit model, it showed no obvious clustering patterns of simulated residuals and over dispersion, zero-inflation and outlier tests were not significant (*p*-value > 0.05). Therefore, animal age over four years old, extensive grazing system and precipitations >1000 mm was the risk factors of *C. burnetii* seropositivity in smallholder dairy cattle (Fig. 9).

Table 7: Comparison of mixed-effect logistic regression risk factor model for Q fever seropositivity

Model	Model Formula	AIC
1.	Q fever elisa~Age + Sex + Keeping cats + Keeping sheep + Wind speed + Keeping pigs + Herd size + Feeding management + Breed + Temperature + Precipitation + Solar radiation + (1|district)	625.41
2.	Wind speed + Keeping pigs + Herd size + Feeding management+ Temperature + Precipitation + Solar radiation + (1|district)	623.54
3.	Wind speed + Herd size + Feeding management + Temperature+ Precipitation + Solar radiation + (1|district)	621.73
4.	Q fever elisa~Age + Sex + Keeping cats + Keeping sheep + Wind speed + Herd size + Feeding management + Temperature+ Precipitation + (1 | district)	619.85
5.	Q fever elisa ~ Age + Sex + Keeping cats + Keeping sheep + Herd size + Feeding management + Temperature + Precipitation+ (1 | district)	617.96
6.	Q fever elisa~Age + Sex + Keeping cats + Herd size + Feeding management + Temperature + Precipitation + (1|district)	616.4
7.	Q fever elisa~Age + Sex + Keeping cats + Feeding management+ Temperature + Precipitation + (1|district)	614.76
8.	Q fever elisa~Age + Sex + Feeding management+ Temperature + Precipitation + (1|district)	613.63
9.	Q fever elisa~Age + Feeding management + Temperature+ Precipitation + (1|district)	613.52
10.	Q fever elisa~Age + Feeding management + Precipitation + (1|district)	613.09

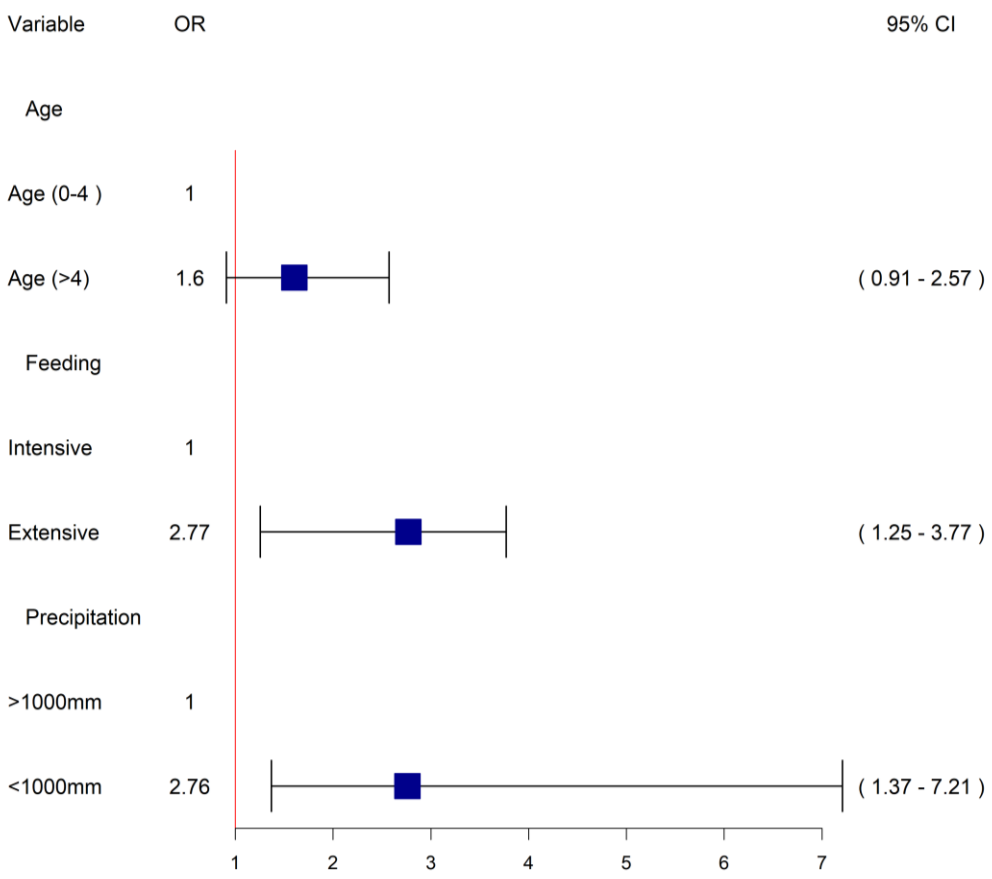

Figure 11: Forest plot showing the final multivariable logistic model

For each category the variables, levels, odds ratios (OR) with 95% confidence interval (95% CI), are provided. Horizontal lines in black and boxes in dark blue colors are, respectively, depicting the 95% confidence intervals and odds ratio of each variable. Precipitation data is measured as average annually.

4.2 Discussion

A cross-sectional study survey was conducted in six regions within two zones of Tanzania to determine the sero/prevalence of Q fever in serum and vaginal swab samples collected from dairy cattle. Information regarding Q fever in dairy cattle is missing in Tanzania, therefore the current study will provide an insight on the epidemiology of Q fever in areas where dairy cattle production is a major enterprise. The overall seropositivity of *C. burnetii* in the two zones was 3.81% albeit varied between regions with highest in Tanga (8.21%) and least in Mbeya where none of the screened animals were seropositive. Moreover, to the district level, the highest seroprevalence (15.8%) was found in Tanga City council. This study was conducted in the study areas where the animals (dairy cattle) had never been vaccinated against Q fever, thus

the seropositivity obtained signifies the natural exposure. This study presents large overall seroprevalence compared to other two studies conducted in northern Africa by Horton *et al.* (2014) and Derdour *et al.* (2017). It aligns with the findings by Depuy *et al.* (2014) in Kenya and reports lower seroprevalence compared to those reported in other three African countries (Adamu *et al.,* 2018; Deressa *et al.,* 2020; Kazwala 2016; Larson *et al.,* 2019; Mwololo *et al.,* 2015; Kelly *et al.,* 2021; Ameur *et al.,* 2018; Menadi *et al.,* 2020). Additionally a recent systematic review of Q fever in domestic ruminants in Africa reported a seroprevalence range estimates of 3-89.7% in East Africa both in dairy and local breeds of cattle (Bwatota *et al.,* 2022). Molecular detection of *C. burnetii* DNA in vaginal swabs in female animals revealed the overall prevalence of 0.94% which signifies the current infections among the female animals. Three molecular studies in other countries reported higher prevalence of Q fever in dairy cattle ranging between 2.1 to 22.5% (Knobel *et al.,* 2013; Rahal *et al.,* 2018; Thomas *et al.,* 2021) than 0.94% of the current study. Generally, serological, and molecular prevalence variations observed between studies are associated with different sample sizes, study designs, geography and climatic conditions, management systems and different diagnostic techniques used (Vanderburg *et al.,* 2014; Bwatota *et al.,* 2022). Molecular detection of the pathogen in vaginal swabs signifies that healthy looking animals (asymptomatic) could be shedding pathogens via vaginal discharge and therefore, contribute to environmental contamination and thus spread of the pathogens to distant farms and/or other hosts including humans (Nusinovici *et al.,* 2015; Theonest *et al.,* 2020). This study gives some clues that smallholder dairy cattle in Tanzania could be among the reservoirs of *C. burnetii*, contribute to human infections, and fills some gaps to Q fever studies previously done in Tanzania with unknown source of infections (Prabhu *et al.,* 2011; Ali *et al.,* 2020; Anstey *et al.,* 1997). Therefore, the One Health approach is strictly warranted to understand the transmission dynamics and prevent or control the disease under low cost effectively.

Multivariable analysis revealed that animal age above four years had increased odds of *C. burnetii* seropositivity compared to animals with less than or equal to four years. This is comparable with studies conducted elsewhere in dairy cattle (Deressa *et al.,* 2020; Ibrahim *et al.,* 2021; Kelly *et al.,* 2021). The reason for the high exposure in adult cattle could be due to increased time of exposure as the animal grows in the same herd (Mazeri *et al.,* 2012; Troupin *et al.,* 2022). Other animal-based variables such as animal sex and animal breed were positively associated with the disease exposure but not statistically significant. Despite the insignificance of these results other studies have reported the statistical significance of these variables in

relation to Q fever exposure in dairy cattle (Agerholm, 2013; Abakar *et al.*, 2014; Hwang *et al.*, 2020; Mangena *et al.*, 2021; Mazeri *et al.*, 2012; Troupin *et al.*, 2022). The increased odds of exposure in females could be due to the high affinity of the pathogen (*C. burnetii*) in the reproductive system (Agerholm, 2013), and high priority of farmers keeping female animals for milk production compared to male animals (González-Barrio*et al.*, 2015).

Regarding to indigenous breed being at high risk compared to cross-bred could be due to the reason that indigenous breeds are extensively kept in large herds and move freely seeking for pastures and water (Hwang *et al.*, 2020). Therefore, could be easier for them to contract the disease during mingling with other herds than those kept indoors.

Presence of rodents appeared to be a significant risk factor to Q fever exposure in dairy cattle. This finding coincides with a cross-sectional study in Ethiopia which also reported the presence of mice to be a risk factor to Q fever in dairy cattle (Deressa *et al.*, 2020). In addition, other scholars have elucidated that rats qualify to be true reservoirs of *C. burnetii* (Gonz *et al.*, 2021; Reusken *et al.*, 2011), therefore could equally contribute to environmental contamination and spread the infections to both animals and humans (Reusken *et al.*, 2011). Similarly, a review article by Meerburg and Reusken (2011) in Netherlands potentiated the role of rodents in Q fever transmissions to other animals and humans. Moreover, a recent study conducted in Kilimanjaro region identified *C. burnetii* in small mammals (rodents) (Theonest *et al.*, 2020). Therefore, we recommend more studies in all hotspot areas of this study to identify the role of rodents on *C. burnetii* transmission dynamics.

The current study found animals over three in a herd had increased odds of *C. burnetii* seropositivity. This is comparable to Cadmus *et al.* (2020) and Boroduske *et al.* (2017) who found high risk of exposure in relatively big herds. Additionally, Gwida *et al.* (2014) elucidated that big herds facilitate animal interactions which in turn hasten the spread of the disease. Moreover, extensive feeding management systems had a significantly increased odds of exposure compared to intensively managed. These findings were supported by Capuano (2001), Deressa *et al.* (2020) and Elelu *et al.* (2020) who found high infection rate in extensive settings and reasoned that animals mingle during grazing and could also acquire the pathogen from the environment. Interestingly keeping both pigs and cattle in the same household had decreased odds of exposure to dairy cattle. These finding were contrary to a study in Netherlands which found an increased odds of disease exposure in dairy cattle kept in contact with pigs (Schimmer, 2014). More studies are needed to ascertain these findings especially in

areas where pigs are kept large number. The current study presents that keeping dairy cattle with dogs, and goats had increased odds of exposure but not statistically significant. The findings were contrary to two studies in Tunisia by Barkallah *et al.* (2018) and Ethiopia by Deressa *et al.* (2020). The two studies reported a significantly increased odds of exposure in cattle kept in the same compound with carnivores (dogs and cats). Similarly, to goats, an outbreak report of acute Q fever in a mixed farm of dairy goats and cattle revealed an exposure to cattle (Honarmand, 2012; Bauer *et al.*, 2021). With this reason, it is recommended to conduct further studies in especially in areas where goats are kept with cattle to ascertain the findings for the current study as goats are among the natural reservoirs of the pathogen (Bauer *et al.*, 2021).

Environmental factors including temperature, precipitation, and wind speed and solar radiation were all positively associated with *C. burnetii* seropositivity in dairy cattle. Areas with temperature over 20°C were statistically significant and favored the existence of the bacteria thus, increase the disease exposure. Many scholars have reported the positive correlation of high temperature with *C. burnetii* exposure in dairy cattle (Nusinovici *et al.*, 2015). Others have reported that *C. burnetii* spores like particles survive in harsh environmental conditions including high temperature (Leuken *et al.*, 2016). Therefore, temperature is a very crucial factor to consider in Q fever control program. The multivariable analysis revealed that precipitation less than or equal to 1000 mm is a risk factor to *C. burnetii* seropositivity in dairy cattle. This finding coincides with the previous studies that reported the positive impact of low precipitation in correlation to Q fever exposure in cattle (Nusinovici *et al.*, 2015; Wardrop *et al.*, 2016). Solar radiation was univariably significantly associated with Q fever exposure in dairy cattle. *C. burnetii* is inactivated by the radiation having a very short wavelength for example gamma radiation (Gürtler *et al.*, 2014; Scott *et al.*, 1989). This study found that the increase in solar radiation proclaim long wavelengths which have less impact to the bacteria, hence bacteria survival on the environment. In this case the bacteria can be easily carried out to long distance via wind and/or infect other susceptible hosts in place (Clark & Soares Magalhães, 2018; Pandit *et al.*, 2016a; Tissot-Dupont *et al.*, 1999).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The current study established for the first time the presence of Q fever in the smallholder dairy cattle in the selected regions of Tanzania with relatively high production of dairy cattle with variable magnitudes between regions attributed probably to managemental and environmental factors. Additionally, this study reports an active shedding of the *C. burnetii* via the vaginal swabs of the healthy-looking female animals with variable magnitude across the study areas. The multivariable analysis of different potential factors revealed that older age of the cattle under extensive feeding system in low precipitation areas (≤ 1000 mm) were significant risk factors associated with Q fever transmission.

5.2 Recommendations

The exposure and detection of *C. burnetii* DNA in smallholder dairy cattle in Tanzania coupled with the fact that is a zoonotic disease, placed under category B as critical biological agent and bioterrorism potential calls for concerted efforts to control the infection by instituting appropriate mitigation measures. A systematic and active surveillance should be considered to analyze all possible risk factors to manage the disease cost-effectively. Further investigations are recommended in areas with hot climate and large number of animals including domestic ruminants, and carnivores. To understand more about the transmission dynamics and to prevent further infections, studies in humans, environmental, and animals under One health umbrella are recommenced in all hotspot areas. Finally, interconnectedness between countries is highly recommended for no country alone can fight to combat the diseases. Also, the current study recommends creating awareness to the communities on possible ways to prevent infection stressing more on environmental factors.

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APPENDICES

Appendix 1: Semi-structured Questionnaire

Section	Question	Choices	Label	hint
Registration	District	Select/filter	District	
	Ward	Select/filter	Ward	
	Village	Select/filter	Village	
	Farmer Name	Select/filter	Farmer_Name	
	Animal ID	Select/filter	Animal_ID	
	Signed consent toallow sampling	Yes; no	Consent	If no terminate interview
	Interviewer	Mengele; Shabani; Shedrack; PhD2; MSc2	Interviewer	
	Date	Dd/mm/yyyy	Date	Today's date
Interviewee	Name	Free txt	Interviewee_name	
	Role in cattle manageme nt	Principle person looking after cattle; owner; occasionally look after cattle;do not look after the cattle	Role	Multiple options
	Gender	Male; female	Gender	
	Level of education	None; primary; secondary, tertiary	Education	Mark highest
	How many years' experience keeping cattle?	Number/integ er	Experience (years)	
	Have you ever been on a livestock training	Yes; No	Training	
	course for dairycattle			

Section	Question	Choices	Label	hint
If yes to above	What year did youhave your training	Integer (4 digits) drop down?	Training year	Enter year
	Are you aware of any diseases you could catch from your cow's milk?	Bovine TB, brucellosis , Qfever; RVF, other, none, Don't know	Milk_zoonoses	Tick all listed
	Are you aware of any diseases you could catch froman aborted calf?	Brucellosis, Q fever, leptospirosis, rift valley fever, other, none, Don't know	Abortion_zoonoses	Tick all listed
	Which of the following statements best describes this herds role for theowner?	A primary income sourceto owner; secondary income sourceto the owner; just for home consumptio n and sale to neighbours; Only home consumption	Reason_own_cattle	

Section	Question	Choices	Label	hint
Herd management	How many heifersand cows do you currently have in the herd?	integer	Herd_size	
	Do you keep yourown bull for breeding	Yes; No	Bull	
If yes above	Do you hire outthe bull to neighbours?	Yes; No	Bull_hire	
	In the last 12 months have youbrought new animals into thisherd?	Market; neighbour; none	New_animals	
If yes?	Did you do any pretesting?	Yes; No	Pretest	
	Do you keep sheep at the same household as thesecattle?	Yes; No	Sheep	
	Do you keep goatsat the same household as thesecattle?	Yes; No	Goats	
	Do you keep pigs at the same household as thesecattle?	Yes; No	Pigs	
	Do you keep dogsat the household?	Yes; No	Dogs	
	Which option bestdescribes the feeding management	Only zero grazed; generally zero grazed but occasionally graze at pasture; generally grazed at pasture	Management	

Section	Question	Choices	Label	hint
	Which option bestdescribes water provision for theherd	Well/bore hole; tap water; river or stream	Water	
	Do you vaccinatethe herd routinely against any diseases	Yes; No	Vaccinations	
If yes above	FMDV	Yes; No; Don’tknow	FMDV	
	Brucellosis	Yes; No; Don’tknow	Brucella	
	Leptospirosis	Yes; No; Don’tknow	Lepto	
	Q fever	Yes; No; Don’tknow	Q fever	
	Pasteurella	Yes; No; Don’tknow	Pasteurella	
	Black leg	Yes; No; Don’tknow	Blackleg	
	Anthrax	Yes; No; Don’tknow	Anthrax	
	Other	Free text	Other_vacc	
	Which option bestdescribes who milks the cows?	Respondent; Owner (if not respondent) Family member; Outside milker/contr actmilker	Milker	
If outside milker	Does the milkergo to multiple farms?	Yes; No	Milker_farms	
	Which best describes preparation ofmilk from thisherd before drinking?	Warm up on fire or stove; bring to the boilon fire or stove; consume without any heating	Milk_prep	

Section	Question	Choices	Label	hint
	Who normally assists with calving for theherd?	Respondent; Owner (if not Respondent);Family member; Outside help?	Calving_assist	
	How do you normally disposeof the after birth/placenta after a calving	Leave for cow to eat; Burn; Bury; Throw on rubbish heap; Feed to other animals (dogs/pigs)	Placenta	
	Has any cow aborted in the last12 months as far as you are aware?	Yes; No; Don’tknow	Abortion	
	In your view do you have trouble getting cows incalf?	Yes; No; Don’tknow	Calving_trouble	
Is yes above	Do you know whyyou are having this problem?	Free text?	Calving_trouble_reason	
	Do you observe rodents in or around the cattlehouse?	Yes; No	Rodents	
If yes	Do you use anyrodent control?	Yes; No	Rodent_control	
Genotype danimal	Picture ear tagnumber	Photo	Ear_tag	Clear photoof ear tag to cross reference with animal_ID

Section	Question	Choices	Label	hint
	Last 4 digits onanimal ID		Short_ID	Needed to help sample labelling
	Animal Age	Integer	Age (years)	Age in years if known. Leave blank if not known
	Animal breed	Shorthorn; Zebu; Shorthorn-Zebucross; Shorthorn-Zebucross Friesian; Shorthorn-Zebucross Ayrshire; Shorthorn-Zebucross Jersey; Local-Grade cross; Grade; Ankole; Unknown; Other	Breed	Select one stated by owner
	Dentition score	0;1;2;3;4;5	Dentition	See sheetfor dentition scoring
	Body condition score	1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5	BCS	See chartfor BCS scoring
	Animal Sex	Male; Female;not evaluated	Sex	Select one
If female	Which option bestdescribes this cow?	Heifer; Cow with 1 or more calves;	Cow_age	
If female	When was she lastserved	Never; Month/y ear	Service	
If had 1 or more calves				

Section	Question	Choices	Label	hint
	How many calves has this cow given birth to alive	Integer	Calf_number	
	When did she lastcalve?	Month/year	Calf_date	
	Which option bestdescribes the last calf?	Normal healthy; borne weak but survived; born weak and died within first month; don't know	Calf_status	
	Which option bestdescribes getting the cow back in calf after the last calving?	Not yet put to the bull; put to the bull but not pregnant; put tothe bull and pregnant	Calving_status	
	Which option bestdescribes her current pregnancy status?	Don't know; Inseminated butnot sure if pregnant; Pregnancy tested positive; Pregnancy tested negative	Pregnancy_status	
	Has this cow ever aborted/prematu redead calf?	Yes; No; Don'tknow?	Abortion_status	
If yes above	When did she abort/have premature calf	Month/year	Abortion_date	
	Genital discharge	No genital dischar ge;Sero us;Muc oid;Pur ulent;	Genital_discharge	

Section	Question	Choices	Label	hint
		Bloody; Other;Not Evaluated		
	Udder condition	Normal; Mastitic; Flabby; Other	Udder_status	
	Milk consistency	Normal; Bloody; Mucoid; Purulent; Other	Milk_status	
	Does the animalappear to be drooling	Yes; No; Not evaluated	Salivation	
	Does the animal appear lame or unwilling to move	Yes; No; Not evaluated	Lameness	
	FMD-like lesions	Mouth; Feet; Mouth and feet;None; Not evaluated	FMD_lesions	Useful to know for risk of spread to next far and to takerisk mitigating action
Sample collection	Serum Sample 1	Yes; No	Serum1	
	Serum Sample 1Barcode		Serum_code1	Scan bar code and hand write 4 digit animal ID and date ontube
	Reason for not collecting bloodsample		Serum1_reason	
	Serum Sample 2	Yes; No	Serum2	

Section	Question	Choices	Label	hint
	Serum Sample 2Barcode		Serum_code2	Scan bar code and hand write 4 digit animal ID and date ontube
	Reason for not collecting bloodsample		Serum2_reason	
	EDTA Sample 1	Yes; No	EDTA	
	EDTA Sample 1Barcode		EDTA_code	Scan bar code and hand write 4 digit animal ID and date ontube
	Reason for not collecting bloodsample		EDTA_reason	
	Vagina swab Sample 1	Yes; No	Swab	
	Vaginal Swab Sample 1 Barcode		Swab_code	Scan bar code and hand write 4 digit animal ID and date ontube
	Reason for not collecting vaginalswab		Swab_reason	
Please estimate distance tonext dairy farm	Please estimate distance to next dairy farm	Less than 100m;100-500m, more than 500m	Distance	
GPS northing				
GPS easting				

RESEARCH OUTPUTS

(i) Research Paper

Bwatota, S. F., Shirima, G. M., Hernandez-Castro, L. E., Bronsvoort, B. M. D. C., Wheelhouse, N., Mengele, I. J., Motto, S. K., Komwihangilo, D. M., Lyatuu, E., & Cook, E. A. J. (2022). Seroprevalence and risk factors for Q fever (Coxiella burnetii) exposure in smallholder dairy cattle in Tanzania. *Veterinary Sciences*, *9*(12), 662.

(ii) Systematic Review Paper

Bwatota, S. F., Cook, E. A. J., de Clare Bronsvoort, B. M., Wheelhouse, N., Hernandez-Castor, L. E., & Shirima, G. M. (2022). Epidemiology of Q fever in domestic ruminants and humans in Africa. A systematic review. *CABI One Health*.

(iii) Poster Presentation

Appendix 2: Poster Presentation

EPIDEMIOLOGIC STUDY OF Q FEVER IN SMALLHOLDER DAIRY CATTLE IN SELECTED REGIONS OF TANZANIA

Shedrack Bwatota[1], Gabriel Shirima[1] , Elizabeth Cook[2], Isaac Mengele[2], Shabani Motto[2], Bibiana Zirra Shallangwa[3], Luis Hernandez-Castro[3], Mark Bronsvoort[3]
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Introduction: In Tanzania, the dairy sector is mainly composed of smallholder dairy farmers and hugely contributes towards livelihoods by employing women and youths, and therefore helps to reduce poverty in the region. However, infectious, and zoonotic diseases like Q fever caused by *Coxiella burnetii* that cause infertility and abortion in cattle negatively impact the Tanzanian dairy sector.

Problem statement: The information about *C. burnetii* in Tanzanian livestock data is lacking especially those kept under the smallholder dairy system. Also, the disease has been detected in humans in Tanzania which raised a concern to animals and animal products thus calls for concrete studies to establish and device control interventions at animal level. Therefore, this study will generate data to the scientific community, policy makers and implementers on the best intervention measures to control the disease in the smallholder dairy sector, to reduce transmission, and thus the incidence of the disease in dairy cattle and ultimately reduce or prevent human infections.

Materials and Methods: A cross-sectional study was conducted to determine prevalence and the risk factors associated with *Coxiella burnetii* seropositivity in smallholder dairy cattle in six regions whereby blood samples and vaginal swabs were collected from dairy cattle.Interviews involving the cattle owner aging 18 years were conducted to understand the risk factors related to animal health, husbandry, and farmer socioeconomics. Serum was tested for antibodies against *C. burnetii* (ELISA) and *C. burnetii* DNA was identified from vaginal swabs using (qPCR) analyses.

Results: An overall animal seroprevalence of 3.86% (95% CI 3.06 - 4.78%) was estimated across the study regions. Molecular identification of *C. burnetii* from vaginal swabs from female cattle revealed an overall prevalence of 0.94% (18,1920) (95% CI 0.5-1.4%) (**Table 2**). The final multivariable logistic regression model showed that old animal age, extensive feeding system, and low precipitation were strongly associated with Q-fever seropositivity (**Fig 1**).

Table 1: Q fever seroprevalence in smallholder dairy cattle.

Region(s)	-	+	Total	seroprevalence%	95% CI	Pops	Weights
Arusha	314	4	318	1.26	0.34 - 3.12	78637	247
Tanga	481	43	524	8.21	6.0 - 10.89	41639	79
Kilimanjaro	505	16	521	3.07	1.77 - 4.94	161984	311
Mbeya	218	0	218	0	0.0 - 1.68	72724	334
Njombe	184	3	187	1.6	0.33 - 4.62	7177	38
Iringa	268	13	281	4.63	2.49 - 7.78	7081	25
Total	**1970**	**79**	**2049**	**3.86**	**3.06 - 4.78**	**369242**	

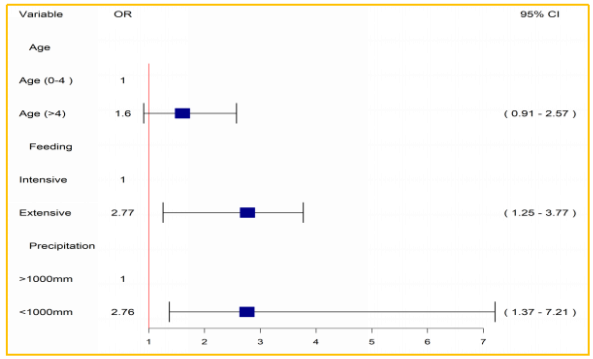

Fig 1: Forest plot for the final multivariable logistic model

Table 2: qPCR Prevalence of Q fever in smallholder dairy cattle (females).

Region	Samples collected	Positive samples	Prevalence %	95%CI
Arusha	300	7	2.3	0.9-4.7
Iringa	281	2	0.71	0.08-2.5
Kilimanjaro	517	2	0.39	0.04-1.4
Mbeya	218	0	0	0.0-1.6
Njombe	187	1	0.53	0.01-2.9
Tanga	417	6	1.44	0.5-3.1
Total	**1920**	**18**	**0.94**	**0.6-1.5**

Conclusion: Q fever exposure is high among smallholder dairy cattle in Tanga and Iringa regions. There is an active shedding of the *C. burnetii* via the vaginal swabs of the healthy-looking female animals with variable magnitude across the study areas. Old animals reared under extensive system and within dry areas are at higher risk of exposure of Q fever.

Acknowledgements: