Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock

Ashenafi Milkesa Fekensa

A Dissertation Submitted to the Department of Applied Biology

College of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology (Specialization in Health Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

December, 2025

Adama, Ethiopia

Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock

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DEDICATION

I dedicate this entire work to the beautiful, indelible memory of my second daughter, Elgrace, my “Barredduu”. Her time with us was heartbreakingly short, spanning just less than two years, and ended as I stood on the verge of defending this PhD dissertation paper. During her difficult, early life, she displayed extraordinary fortitude while confronting multiple, devastating congenital heart defects including Ebstein Anomaly and a Ventricular Septal Defect (VSD). These core conditions triggered severe secondary issues, such as pulmonary hypertension, cardiomegaly, and Severe Acute Malnutrition (SAM) are among the many. The sheer willpower she exhibited in her fight for survival, often without sleep, became the singular motivation that fueled my academic perseverance through my darkest hours.

She was an unparalleled source of inspiration and possessed a spirit wise beyond her years. I love you, my daughter. May this academic achievement honor your memory until that glorious day we meet again. It is a striking, somber parallel that this dissertation studies *Coxiella burnetii*, a bacterium known to cause heart disease in humans.

Rest in eternal peace, bareedduu Koo!

DECLARATION

I hereby declare that this Dissertation entitled "**Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock**" is my original work conducted at the Applied Biology Department, Adama Science and Technology University under the supervision of Professor Hunduma Dinka and Dr. Tesfaye Rufael. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this dissertation have been duly acknowledged through appropriate citations.

Ashenafi Milkesa	_____	_____
Name of student	Signature	Date

RECOMMENDATION

We, the supervisors of this dissertation, hereby certify that we have read and revised the dissertation entitled "**Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock**" prepared under our guidance by **Ashenafi Milkesa Fekensa** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in **Applied Biology (Health Biotechnology)**. Therefore, we recommend the submission of the dissertation to the department for further review and defense.

Professor Hunduma Dinka (PhD)		
Major Supervisor	Signature	Date
Tesfaye Rufael (PhD)		
Co-supervisor	Signature	Date

APPROVAL SHEET

We hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled "**Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock**" by **Ashenafi Milkesa Fekensa**.

Professor Hunduma Dinka (PhD)		
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Co-supervisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the dissertation open defense by **Ashenafi Milkesa Fekensa** have read and evaluated the dissertation entitled "**Serological and Molecular Investigation of *Coxiella burnetii* Infection and Associated Risk Factors in Selected Sites of Ethiopian Livestock**" and examined the candidate during open defense. This is, therefore, to certify that the dissertation is accepted for partial fulfillment of the requirement of the degree of Doctor of Philosophy in Biotechnology (specialization in **Health Biotechnology**).

Chairperson	Signature	Date
Internal Examiner1	Signature	Date
Internal Examiner 2	Signature	Date
External Examiner 1	Signature	Date
External Examiner 2	Signature	Date

Finally, approval and acceptance of the dissertation is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the candidate's Department Graduate Council (DGC) and College Graduate Committee (CGC).

Department Head	Signature	Date
College Dean	Signature	Date
Office of Postgraduate Studies, Dean	Signature	Date

BIOGRAPHICAL SKETCH

Ashenafi Milkesa Fekensa was born on January 1, 1997, to Mr. Milkesa Fekensa Bedasa and Mrs. Alem Haile Dubaro, in the modest locality of Bote/Alemtena, East Shoa Zone, Oromia, Ethiopia. This area, situated at approximately 100 kilometers southeast of Addis Ababa, was marked by significant resource scarcity, including limited educational infrastructure and challenges with safe drinking water (High Fluorine content) are among the many. Despite these fundamental constraints, the Candidate embarked on an academic journey, standing as a pioneer from his birthplace to attain the PhD level of education.

His foundational schooling began with preparatory education (locally known as "ቄስ ትምህርት ቤት") followed by attendance at Bote Primary School (2003–2010) and Bote High School (2011–2012). The necessity of pursuing advanced secondary education beyond his immediate area led him to relocate and attend Bishoftu Preparatory High School (2013–2014). This period exemplified his resilience and dedication, requiring him to manage all personal and domestic needs independently, including cooking and baking, while maintaining his focus on academic rigor despite a background of limited financial means.

The Candidate commenced his university career at Debre Tabor University in 2015. However, following the initial year, he successfully transferred to Jimma University in 2016. There, his exceptional dedication to the life sciences culminated in a Bachelor of Science (BSc) degree in Applied Biology in 2017, graduating with Great Distinction (CGPA: 3.97/4.00). His undergraduate research was further recognized for its excellence, achieving a score of 99/100 and earning him a prestigious Gold-coated pen award and many recognitions and prizes in the academic arena.

Building on this superlative performance, he pursued specialized graduate studies at Adama Science and Technology University (ASTU), where he was awarded a Master of Science (MSc) degree in Biotechnology in 2018/2019. His performance at the Master's level was even more outstanding, concluding with a Perfect CGPA of 4.00/4.00 and an excellent thesis score of 97/100, achievements recognized with a distinguished Gold-coated Watch prize and many more.

Recognizing his sustained academic brilliance, the Senate of Adama Science and Technology University conferred a unique honor: direct admission into the Doctoral Program coupled with a concurrent appointment as a Lecturer. Hence, this opportunity paves the way to be the first PhD candidate at African Level to start PhD at the age of 23 and was witnessed and reported by many national (22 medias) and international Medias (like BBC etc.). Throughout his PhD coursework, seminar presentations, qualification examination, and independent studies, the Candidate continued to achieve exemplary results, maintaining a course-work CGPA of 3.99/4.00. This doctoral dissertation stands as the culmination of years of focused, high-achieving research, demonstrating an unwavering commitment to advancing scholarly knowledge within the field of Biotechnology (Health Biotechnology).

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"There is a time for everything, and a season for every activity under the heavens"

- Ecclesiastes 3:1

My gratitude begins with the Lord Jesus Christ of Nazareth, the Son of Almighty God, whose grace; wisdom, protection, and sustained strength have been the foundation of my life and the guiding light throughout this demanding academic journey. The successful culmination of this dissertation is intrinsically linked to the collective spirit and invaluable support provided by various institutions and individuals, whose generous contributions I now gratefully recognize.

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Finally, my deepest appreciation is reserved for my beloved family. To my wife, Tsion Bultuma, and my daughters, Akeelahel Ashenafi and Elgrace Ashenafi, thank you. For 7 years, your unwavering emotional, spiritual, and material support has sustained me through every triumph and tribulation of this long academic life. This dissertation is as much a testament to your patience and enduring belief in me as it is to my own efforts.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAA	Addis Ababa Abattoir
AA	Adama Abattoir
AHI	Animal Health Institute
ARSERC	Animal Research Scientific and Ethics Review Committee
ASTU	Adama Science and Technology University
BZFEDO	Borena Zone Finance and Economic Development Office
BZPDO	Borena Zone Pastoral Development Office
C. burnetii	*Coxiella burnetii*
CDC	Centers for Disease Control and Prevention
CFT	Complement Fixation Test
CI	Confidence Interval
CSA	Central Statistical Agency
Ct	Cycle Threshold
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FAM	Fluorescein Amidite (a fluorescent dye)
GPS	Global Positioning System
IACUC	Institutional Animal Care and Use Committee
iELISA	Indirect Enzyme-linked Immunosorbent Assay
IF	Immunofluorescence
MA	Modjo Abattoir
MoLF	Ministry of Livestock and Fisheries
MST	Multispacer Sequence Typing

NCBI	National Center for Biotechnology Information
NZDs	Neglected Zoonotic Diseases
OD	Optical Density
OIE	Office International des Epizooties (World Organisation for Animal Health)
OR	Odds Ratio
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen (measure of acidity)
PO	Pastoral Oromia
PPE	Personal Protective Equipment
Q fever	Query Fever
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SCV	Small-cell Variant
SOPs	Standard Operating Protocols
UAE	United Arab Emirates
USA	United States of America
WHO	World Health Organization

ABSTRACT

Coxiella burnetii (C. burnetii) infection is a significant global public health, veterinary, and economic concern. Despite the global public health and veterinary importance, information regarding its distribution, prevalence and risk factors is scarce in Ethiopia. Hence, a comprehensive sero-epidemiological and molecular investigation was conducted to characterize C. burnetii infection, the causative agent of Q fever, and its associated risk factors in livestock from Ethiopia. We carried out this study to bridge some of the knowledge and information gaps by focusing on the geographical distribution of serological evidence of infection in livestock slaughtered at major abattoirs in Addis Ababa, Adama, and Modjo, as well as pastoral areas of Oromia, Ethiopia. A total of 4,140 whole blood samples were collected from livestock species at three abattoirs (Addis Ababa, Adama, and Modjo) and three pastoral districts (Mega, Dubuluk, and Yabello) from January 2021 to May 2022. In addition, 264 biological samples consisting of milk, urine, vaginal swabs, aborted fetal tissues and whole blood were collected from livestock species in three pastoral districts (Moyale, Boku Luboma and Elweye) where there is evidence of reproductive problems. Moreover, 292 ticks were collected from infested livestock during the biological sample collection. During sample collection geographical data was collected using GPS for mapping the origin of livestock species sampled. Georeferencing was performed to accurately delineate the sample locations. The sera samples were analyzed for the presence of anti-C. burnetii antibodies using an indirect Enzyme Linked Immunosorbent Assay (iELISA) kit. Additionally, buffy coat from iELISA serum positive livestock, milk, urine, vaginal swabs, aborted fetal tissues, and ticks were analyzed by real time quantitative polymerase chain reaction (RT-qPCR) targeting cytochrome c oxidase subunit 1 (COX1) gene. Logistic regression analysis was used to quantify the association between seroprevalence of C. burnetii infection and various potential risk factors. Out of the 4,140 serum samples tested, 777 (18.77%; 95% CI: 17.59, 19.99) were found positive for C. burnetii antibody. The sero-prevalence estimate was 27.17% at Addis Ababa abattoir, 19.41% at Adama abattoir, 19.13% at Modjo abattoir and 12.1% in livestock tested from Mega, Dubuluk, Yabello pastoral areas. Sera analysis at the animal species level showed that cattle exhibited the lowest sero-prevalence estimate (11.83%; 95% CI, 10.27–13.53%), while the highest was observed in camels (28.39%; 95% CI, 25.16–31.80%). The sero-prevalence estimate was 21.34% (95% CI, 18.86–23.99%) in goats and 20.17% (95% CI, 17.49–23.07%) in sheep. Female livestock were observed to have a higher seroprevalence (26.65%; 95% CI, 23.37 30.13%) than male livestock (17.21%; 95% CI, 15.97–18.51%). The prevalence was also higher in adult livestock (25.02%; 95% CI, 23.26–26.85%) than their younger counterparts (11.03%; 95% CI, 9.64–12.54%). Higher seroprevalence of C. burnetii infection was also observed in livestock infested with ticks (carry ticks on their body parts) (80.99%; 95% CI, 73.55–87.08%) than those livestock which were not infested by ticks at all (7.05%; 95% CI, 6.52–7.59%). The results of multivariable logistic regression analysis showed that species, age, and tick infestation were important risk factors for C.

burnetii infection. The odds of infection were 3.22 times higher in camels and almost twice as high in goats and sheep compared to cattle. Adult livestock were infected more likely (OR = 3.23) than young ones. Interestingly, a significant difference was observed in the sero-prevalence of infection between livestock that were infested with ticks (OR = 16.32) and those which were tick-free. Furthermore, out of 384 buffy coat samples randomly selected from the iELISA sera positive livestock and tested by real-time quantitative PCR (RT-qPCR), none of them showed positive result. Similarly, none of the 264 biological samples (aborted fetal tissues, urine, milk, buffy coat, and vaginal swabs) tested by RT-qPCR showed positive result. The prevalence of tick infestation was highest in camels, with 143 ticks (49.0% of the total collection) while cattle had the lowest prevalence of infestation (23 ticks, 7.9%). The tick fauna was dominated by Rhipicephalus pulchellus (30.5%) and Amblyomma variegatum (15.4%), but Rhipicephalus pravus (1.0%) and Hyalomma truncatum (2.7%) being the least frequently observed tick species. Similar to the biological samples collected from livestock, all ticks (292) analyzed using RT-qPCR showed negative results. This suggests a potential low-level infection, intermittent pathogen shedding, or challenges with detection using the chosen gene target. In conclusion, this study provides the first comprehensive sero-epidemiological data for C. burnetii infection in a multi-species livestock population in Ethiopia, while also highlighting the challenge of molecular detection. The serological study revealed the widespread exposure of livestock to the C. burnetii (seropositive in the absence of vaccination), identified important risk factors, and warrants the need for enhanced surveillance and the implementation of biosecurity measures to safeguard both animal and public health.

Keywords: Buffy Coat, Coxiella burnetii, COX1 gene, iELISA, Livestock, RT-qPCR

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Coxiella burnetii (*C. burnetii*) is a deadly bacterium that cause zoonotic disease called Q fever or coxiellosis **(**van Schaik *et al*., 2013; Shaw and Voth, 2019; Mezouar *et al*., 2025**)**. The pathogen is considered a significant global public health concern although it is of greater concern in developing countries due to conducive environment, a considerable risk to both human and animal populations and poor public awareness **(**Oyston and Davies, 2011; Robi *et al*., 2023**)**. The disease is listed among emerging diseases declared by international organizations including WHO, FAO, EFSA, and CDC (Muhammad *et al*., 2023). A systematic review and meta-analysis showed that the pooled global prevalence was higher (17.38%) in sheep (Ahaduzzaman and Reza, 2024) ranging from 15.04% in North America to 19.14% in Africa. Higher prevalence was recorded in adult (20.93%) and female livestock (24.29%) and in sheep raised under an extensive production system (20.52%). The occurrence of evidence of infection with *C. burnetii* in several animal species was linked to its zoonotic importance (Robi *et al*., 2023).

In humans, Q fever is featured by an acute, self-limiting illness varying from mild to potentially life-threatening illness (Eldin *et al*., 2017; Mezouar *et al.*, 2025). Clinically it is characterized by pneumonia, hepatic disorders, and if infection occurs during pregnancy, it results in reproductive problems (Mezouar *et al*., 2025). Q fever rarely progresses to chronic form which leads to endocarditis and vascular infection with associated high morbidity and mortality as a result of immune-mediated complications (Al Suqri *et al*., 2024). This form of the disease is mostly seen in individuals with predisposing conditions (Angelakis and Roult, 2010). It is occupational disease commonly reported in veterinarians, slaughterhouse workers, animal owners, and scientists involved in animal studies (Van der Hoek *et al*., 2011).

Humans acquire the infection mainly by aerosols arising from parturition or slaughter materials. Domestic livestock such as cattle, sheep, goats and camels are considered the main

reservoir of the bacterium for humans (Rahal *et al*., 2025). Although there is no definitive evidence that ticks transmit *C. burnetii* to humans, the possible role of ticks in the epidemiology and transmission of the disease cannot be ruled out (Körner *et al*., 2021). This is supported by evidence that genotyping of *C. burnetii* isolates from ticks has revealed the circulation of strains of *C. burnetii* known to cause severe infection (Kumsa *et al*., 2015).

In livestock, the disease primarily affects the reproductive system, often resulting in spontaneous abortions and infertility resulting in considerable losses particularly in ruminants and camels (Robi *et al*., 2023). It has been commonly reported in ruminants and camels in (Celina and Cerný, 2022). Additionally, it has been reported in pigs, horses, buffaloes, rodents and various wildlife species (Celina and Cerny, 2022; Fatima and Upadhyay, 2025). In livestock the main outcomes of the disease are reproductive disorders (Agerholm, 2013; Gisbert *et al*., 2024).

In livestock, the transmission of *C. burnetii* is mainly via respiratory route in which droplets of infection that arise from genital secretions of infected livestock are inhaled (Knobel *et al*., 2013; Celina and Cerný, 2022). The bacterium is shed via birth products (placenta and fetal fluids), vaginal secretions, milk, urine, feces and semen (Rahal *et al*., 2025). Additionally, transmission by ticks has also been demonstrated in livestock and more than 40 tick species mostly Mediterranean ticks have been shown to transmit *C. burnetii* in livestock (Duron *et al*., 2015).

The occurrence of the disease in domestic livestock species has been reported in several countries of the world (Celina and Cerný, 2022). Serological investigation of *C. burnetii* has been conducted in Europe, the Americas, and many African countries. The occurrence of *C. burnetii* in wildlife has also been documented (Cekani *et al*., 2008; Knobel *et al*., 2013; Burns *et al*., 2023). The reservoirs of *C. burnetii* also have been largely investigated in many countries, especially in Europe (Körner *et al*., 2021). In Africa, the seroprevalence of *C. burnetii* infection varies considerably with higher prevalence recorded in countries with pastoral land use, including Chad, Egypt, Tanzania, Niger, and Kenya (Dupont *et al*., 1995; Vanderburg *et al*., 2014; Maina *et al*., 2016).

The disease has significant implication for the agricultural production and public health crisis (van Asseldonk *et al*., 2015; Robi *et al*., 2023). For example, over 58,000 livestock were culled as a result of the outbreak that occurred in the Netherlands between 2007 and 2010 and over 4,000 human cases were recorded associated with goat farms (Whelan *et al*., 2011; Schneeberger *et al*., 2014). The disease is endemic in Europe (France, Germany, Spain and Italy); In Africa, prevalence ranging from 32% (Egypt) to greater than 70% (Algeria) has been documented in livestock species; and the circulation of *C. burnetii* has been documented in the Americas, Asia and Australia (Rahal *et al*., 2025). The burden of *C. burnetii* infection could be more than what is reported due to poor surveillance in several countries and limited molecular diagnostic facilities (Milkesa *et al*., 2024).

In Ethiopia, the first report of evidence of occurrence of *C. burnetii* infection dates back to the reports of Philip and colleagues (1966) who detected the pathogen in ticks and ruminants. Subsequently, serological evidence of infection in humans was published in 1990 with prevalence of 6.5% in abattoir workers in Addis Ababa (Abebe, 1990). Subsequent to those earlier reports, few studies had been conducted on evidence of *C. burnetii* infection in livestock species revealing seroprevalence of 31.6% in cattle, 90% in camels and 54.2% in goats (Gumi *et al*., 2013). Study conducted in Borana pastoral area revealed seroprevalence of 28.46% in sheep and goats (Tesfaye *et al*., 2020). Similarly, a seroprevalence of 30.13% has been reported in Somali regions of the country (Ibrahim *et al*., 2021). Additionally, evidence of circulation of *C. burnetii* has been documented in tick species from pastoral regions of Ethiopia revealing the occurrence of different genotypes including two novel genotypes in addition to those known to cause severe diseases in humans, as well as spontaneous abortion in livestock (Hornok *et al*., 2014; Sulyok *et al*., 2014).

Despite the reports of the occurrence of the disease in both human and animal population, there are several knowledge gaps regarding the epidemiology and the genotypes of *C. burnetii* circulating in livestock species and tick vectors in Ethiopia (Sulyok *et al*., 2014). Most of the reports on the occurrence Q fever/coxiellosis were based on serological evidence (Robi *et al*., 2023). Molecular evidence regarding *C. burnetii* infection is extremely rare. Moreover, association of *C. burnetii* infection with reproductive disorders have not been established despite evidence of different reproductive problems in herds/flocks where serological evidence

has been reported. Prevention and control of the disease, however, requires thorough understanding of its epidemiology including the prevalence of infection in various host species, vectors and important biological secretion and excretions through which the bacterium is transmitted (Plummer *et al*., 2018). Studies carried out elsewhere in the world revealed the occurrence of high genetic diversity with possible geographical patterns (Gil-Zamorano *et al*., 2025). In several countries molecular techniques have been employed to identify *C. burnetii* in biological samples and establish its genotype. However, molecular evidence of *C. burnetii* infection is scarce in Ethiopia. Moreover, the serological studies conducted previously were limited either in geographic areas or populations. Therefore, this study was designed to bridge the knowledge gaps in the epidemiology and molecular diagnosis of *C. burnetii* in different livestock species from diverse geographical areas.

1.2. Statement of the Problem

Q fever is one the globally recognized zoonotic diseases causing severe economic loss in livestock industry and illness in humans. The disease is common in domestic ruminants and camel populations. Ethiopia has huge livestock resources manage under diverse production systems (ranging from pastoralism to commercial). Furthermore, the pervasive interface between humans, domestic livestock, and wildlife driven by traditional husbandry practices and close-contact management creates a high-risk environment for zoonotic spillover. This interaction is often exacerbated by the consumption of unpasteurized animal products and proximity to birthing areas. This is coupled with very poor veterinary and public health surveillance. All these poses greater risk of acquiring *C. burnetii* infection among human and animal populations. Few studies have been carried out in Ethiopia in livestock although they are limited in space and host species studied. As a result, there is limited knowledge on the distribution of the disease and the associated risk factors. Reliable and comprehensive information is needed for devising effective control, and prevention strategies.

Reproductive failures such as intermittent abortions, stillbirths, and infertility are frequently reported in livestock species particularly domestic ruminants in developing countries including Ethiopia (Ebani, 2022). The differential diagnosis of such problems is often not established due to lack of diagnostic capacity (Milkesa *et al*., 2024). It is, however, shown from various

reports globally, that coxiellosis is one of the causes of such reproductive failure (Agerholm, 2013). The worst thing is, livestock mostly remain asymptomatic carriers, shedding the pathogen through various excretions and secretions complicating control efforts and poses a continuous risk to herds (Knobel *et al*., 2013; Eldin *et al*., 2017).

The asymptomatic nature of the disease and the variable clinical manifestations contribute to the widespread occurrence and underreporting of its occurrence (Roest *et al*., 2013). The disease also causes a serious zoonotic infection (Q fever) in humans (Mezouar *et al*., 2025). The true prevalence of Q fever also remains undetermined in Ethiopia since data on the incidence in livestock is sparse (Milkesa *et al*., 2024). The sub-clinical nature of the disease in livestock and the shading of the pathogen in various fluids without showing clinical signs pose a serious risk to public health (Mezouar *et al*., 2025). The situation can be exacerbated by the lack of awareness among healthcare providers, misdiagnosis or overlooking of Q fever compared to other endemic diseases, which contribute to its widespread occurrence (Roest *et al*., 2013; Vanderburg *et al*., 2014).

Coxiellosis is one of the important infectious diseases with a potential to impact livestock productivity through reproductive impairment (Robi *et al*., 2023). A better knowledge of the epidemiology of the disease, the type of livestock infected, the ticks carrying the pathogen under various agro-ecological conditions and the identity of the pathogen are crucial for rational control (Plummer *et al*., 2018). The public health sector also requires reliable epidemiological information to understand and devise effective control of Q fever. Hence, serological and molecular investigation of *C. burnetii* infection and its associated risk factors in Ethiopian livestock was carried out to generate scientific information that would contribute to the understanding of the disease.

1.3. Objectives of the Study

1.3.1. General Objective

The general objective of this research is to investigate the Sero-epidemiological and Molecular analysis of *Coxiella burnetii* Infection and its Associated Risk Factors in Livestock from Addis Ababa, Adama, Modjo Abattoirs and Yabelo, Dubuluk, Mega, Boku Luboma, Elweye, Moyale Pastoral areas of Oromia, Ethiopia

1.3.2. Specific Objectives

The specific objectives of this research are:

- To estimate seroprevalence of *C. burnetii* infection across major domestic livestock species (cattle, sheep, goats, and camels) sourced from from Addis Ababa, Adama, Modjo Abattoirs and Yabelo, Dubuluk, Mega, Boku Luboma, Elweye, Moyale Pastoral areas of Oromia, Ethiopia
- To identify and quantify the associated risk factors significantly contributing to the seroprevalence of *C. burnetii* infection in the investigated livestock populations
- To detect and confirm the presence of *C. burnetii* DNA in various biological specimens (including the buffy coat from seropositive livestock, milk, urine, vaginal swabs, and aborted fetal tissues), using RT-qPCR
- To screen for the presence of *C. burnetii* DNA in tick species collected from livestock in the study areas using RT-qPCR

1.4. Scope of the study

The scope of this study is delimited to provide a focused and in-depth investigation into the epidemiology of *Coxiella burnetii* infection in livestock pastoral and agro-pastoral regions of Ethiopia. The study encompasses estimation of prevalence of coxiellosis in domestic ruminants and camels slaughtered at three major abattoirs in the country namely: Addis Ababa, Adama, and Modjo abattoirs, as well as pastoral areas of Oromia, Ethiopia. The abattoirs chosen represent the majority of livestock rearing areas of Ethiopia as livestock slaughtered at these abattoirs originate from all corners of the country. In addition to prevalence, risk factors of infection were identified.

The methods used include serological assays using indirect ELISA and molecular analysis targeting COX1 gene of *Coxiella burnetii*. Real time quantitative polymerase reaction targeting a single copy COX1 gene was used for detection of *Coxiella burnetii* DNA. The molecular assay targets biological samples such as milk, urine, vaginal swab, aborted fetal materials, placenta collected from seropositive livestock and ticks. Therefore, the current study was delimited to molecular detection using real-time RT-PCR and determination of the seroprevalence of *Coxiella burnetii* in Ethiopia. However, the prevailing national peace and

security challenges precluded the inclusion of other regions, Tigray and Western Ethiopia. Moreover, the study didn't include the identification of circulating *Coxiella burnetii* strains in Ethiopia by sequencing and bioinformatics aspect, since the results obtained by RT-qPCR were negative.

1.5. Significance of the Study

This study holds significant value for both advancement of scientific information and practical application of the information in animal-public health interventions in Ethiopia, particularly within livestock rearing regions and households. It represents the first comprehensive investigation into the sero-epidemiology and molecular characteristics of *Coxiella burnetii* the causative agent of Q fever/coxiellosis across major abattoirs and pastoral areas. Since the livestock slaughtered at those abattoirs originate from various areas of the country, the information provided by this study highlight the occurrence of *Coxiella burnetii* infection throughout the country. By establishing the prevalence of infection and identifying key risk factors in different livestock species, the research findings offer critical insights into the geographic distribution of the disease, host species infected, and the zoonotic potential of the bacterium.

Importantly, the data generated will serve as a foundational resource for veterinarians, public health officials, policymakers, and researchers. These findings can help the relevant authorities in designing of targeted disease surveillance, evidence-based control programs, and improved biosecurity protocols at abattoirs**.** Strengthening the understanding and management of coxiellosis in livestock not only reduces the risk of outbreaks but also minimizes economic losses among pastoral and agro-pastoral populations that are particularly vulnerable to the socio-economic impacts of animal disease. This, in turn, will contribute to improved rural livelihoods and improved food security**.**

Moreover, as *Coxiella burnetii* is a zoonotic pathogen**,** control of the occurrence and spread in animal populations has direct and important implications for human health. Outbreaks of Q fever in human population often originate from livestock, especially in areas where occupational exposure is high, such as abattoirs and pastoral settings. Identification of the geographical distribution of the occurrence of *Coxiella burnetii* in livestock maps the risk of

the disease in humans. By elucidating the epidemiological link between livestock and humans, the study supports a One Health approach, fostering integrated strategies that promote the well-being of both animal and human populations. Ultimately, this research addresses a neglected tropical zoonosis, advancing local and global efforts to mitigate its public health burden.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Historical Aspect

The first documented evidence on the occurrence of Q fever dated back to the 1937 outbreak of a febrile illness among abattoir workers in Brisbane, Queensland, Australia, from which the name Q fever was derived. At that time the agent causing the disease was not elucidated despite efforts made to isolate it from blood cultures and application of agglutination tests prompting a thorough investigation (Derrick, 1983; Maurin and Raoult, 1999). Derrick (1983) documented the clinical disease in humans detailing the duration of febrile illness and was able to reproduce the illness in guinea pigs after inoculating them with blood or urine from infected human patients although isolation of the causative agent failed in axenic culture. The work of Derrick made specific diagnosis of Q fever possible and proving that Q fever is both a pathological and clinical entity. Several scientists had contributed to the identification of the etiologic agent of the disease though three of them made remarkable inventions and worth mentioning. Figure 1 depicts the images of scientists who made crucial millstone in the discovery and understanding of the Q fever.

As a result of the failure of the agent to grow in axenic culture, the etiologic agent was proposed to be a virus. However, tissue samples from experimentally infected guinea pigs submitted to the Walter and Eliza Hall Institute of Medical Research, Melbourne where rickettsia-like bodies were identified within the splenic samples, suggested that the causative agent was a bacterium rather than a virus (Burnet and Freeman, 1983). This discovery was a significant milestone in the understanding of Q fever and paved the way for further research on the disease (Mezouar *et al*., 2025).

Around the same time, Davis (1938) and Cox (1939) encountered ticks transmitting febrile disease that is different from the usual disease they have been studying “Rocky Mountain Fever" caused by rickettsia in Montana, USA. The findings of these workers contributed substantially to the establishment of the etiological agent of a febrile disease, which they named "Nine Mile Fever" (Davis *et al*., 1938; Cox, 1939). In the year 1938, which had specially a

significant impact on the development of infectious agents, a member of the Montana research team contracted an illness that resembled Nine Mile Fever, which was later reproduced in guinea pigs by injecting the patient's blood. Rickettsia-like organisms were found in spleen samples from these infected guinea pigs, which allowed these researchers to draw a connection between their observations and the work of Derrick and Burnet in Australia.

A B C

Dr. Herald Rea Cox

(1907–1986**)**

Sir Frank Macfarlane Burnet

(1899 –1985)

Edward Holbrook Derrick (1898–1976)

Figure 1. Key scientists involved in the discovery and understanding of Q fever and *Coxiella burnetii*.

Source: NobelPrize.org Photo Gallery; Science Photo Library.

This figure highlights the three principal researchers who are instrumental in the initial identification and characterization of the organism that causes Q fever: A) Dr. Herald Rea Cox (American bacteriologist) who isolated the causative agent from ticks. B) Sir Frank Macfarlane Burnet (Australian virologist and later Nobel laureate) along with C) Dr. Edward Holbrook Derrick (Australian pathologist who first documented the clinical illness in abattoir workers) and conducted crucial investigations that classified the organism as a rickettsia-like bacterium rather than a virus, paving the way for its formal naming as *Coxiella burnetii*.

Cox noted that although the organism causing Q fever shared many similarities with rickettsia, there were distinguishing attributes such as the ability to pass through filters that other

rickettsia could not. As a result, the causative agent of Q fever was classified as a separate genus, *Coxiella* which was placed in the Rickettsiaceae family of the order Rickettsiales. However, this classification became redundant after 16S rRNA gene sequencing analysis, which led to the reclassification of *C. burnetii* into the order Legionellales, family Coxiellaceae within the Gamma subdivision of Proteobacteria in later years (Stein *et al*., 1993; Roest *et al.,* 2013).

2.2. Etiology

Coxiella burnetii is a Gram-negative, non-motile, pleomorphic, coccobacillary bacterium that belongs to the family Coxiellaceae. Its size ranges from 0.2-0.4 µm in length and 0.4-1.0 µm in diameter (Shaw and Voth, 2019; Fatima and Upadhyay, 2025) (Figure 2). It is an obligate intracellular bacterium, which reproduces quickly within parasitophorous vacuoles of eukaryotic host cells, with a doubling time of 20-45 hours (Howe and Heinzen, 2008; Samanta *et al*., 2019). During its course of development two morphologically distinct forms are produced (Fatima and Upadhyay, 2025). 1). Small cell variant, which is infective to livestock and usually released from infected to extracellular environment. 2). Large cell variant that develops within the cells after infection by small cell variant, metabolically active and multiplies by division (Fatima and Upadhyay, 2025). *Coxiella burnetii* (small cell variant) is well-known for its ability to persist in the environment resisting adverse conditions such as heat, drying and a number of disinfectants (Shepherd *et al.,* 2023). *C. burnetii* was previously believed to be the only member of the *Coxiella* genus. However, new species have been identified in reptiles (*C. cheraxi*), birds *(C. avium*), and humans (*C. massiliensis*) (Hackert *et al*., 2012).

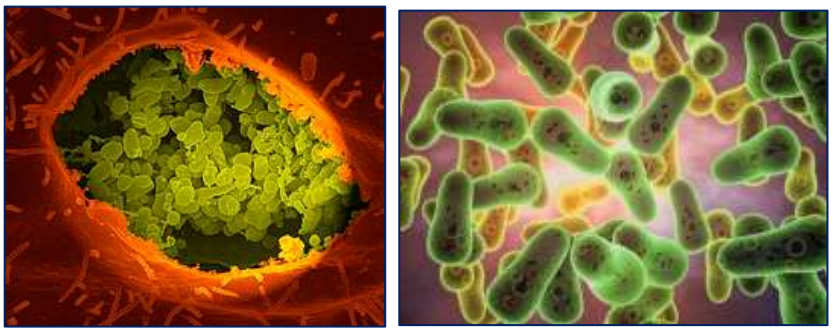


Figure 2. 3D Visualization of the Intracellular Habitat of *Coxiella burnetii*. This illustration (derived from a dry fracture technique on a Vero cell) depicts *C. burnetii* bacteria actively replicating within the parasitophorous vacuole (PV). The image highlights the unique, acidic intracellular niche essential for the pathogen's survival and growth.

Source: Science Photo Library; CDC Public Health Image Library (PHIL).

2.2.1. Biological Properties and Genotypic Diversity of *Coxiella burnetii*

The etiological agent of Q fever, *Coxiella burnetii*, is a highly specialized obligate intracellular pathogen whose unique biological attributes underpin its transmissibility and disease manifestation (Adhvaryu *et al*., 2025). In addition to the morphological forms described above, *C. burnetii* exists in two phase variants based on the structure of its surface lipopolysaccharide. These are Phase I organisms also known as smooth form, which have the full-length lipopolysaccharide and are infective to livestock causing disease. The phase I forms are often isolated from infected livestock and the environment. Phase II organisms have shorter lipopolysaccharide arising from deletion of genes involved in the formation of the lipopolysaccharide during passages in culture or cell lines rendering them non-virulent (Hackert *et al*., 2012). That is, the lipopolysaccharide is the only virulent factor for *C. burnetii* (Abnave *et al*., 2017).

The replication of *C. burnetii* occurs inside the parasitophorous vacuole (PV), a defining acidic, phagolysosome-like compartment maintained by the bacteria using a sophisticated Type IV Secretion System (Figure 3) (Ghigo *et al*., 2012; Shaw and Voth, 2019). *C. burnetii* has a circular chromosome of approximately 2 Mbp in size made up of six genomic groups, designated as I to VI. Moreover, all isolates of *C. burnetii* were shown to have either an autonomously replicating plasmid or plasmid sequences integrated into their chromosome with four distinct plasmid types known so far (Minnick and Raghavan, 2011). The persistence of the organism in the environment is reinforced by its genetic and physicochemical resilience. The bacterial genome is relatively compact, typically consisting of a chromosome and a virulence-associated plasmid such as QpH1, which enhances its pathogenic potential (Table 1) (Seshadri *et al*., 2003). Crucially, the SCV morphology confers exceptional resistance to environmental stresses, including desiccation, heat, and chemical agents. This allows the

bacteria to survive for extended periods often months in dried birth products, dust, soil, and manure, establishing inhalation of contaminated aerosols as the primary route of human infection (Mezouar *et al*., 2025).

Table 1. Genomic Features of *Coxiella burnetii* (Nine Mile Strain) Chromosome and Virulence Plasmid (QpH1)

Genomic Feature	**Chromosome**	**Plasmid (QpH1)**
Size (Base Pairs, bp)	1,995,275	37,393
G + C Content (%)	42.6	43.9
Coding (%)	89.1	78.8
Average ORF Size (bp)	849	736
Protein-Coding Genes (Total)	2,094	40
Genes similar to known proteins	1,022	11
Genes similar to proteins of unknown function	179	5
Genes of conserved hypotheticals	200	1
Genes of hypotheticals	693	23
rRNA (Number)	3	0
tRNA (Number)	42	0

In terms of genotypic and antigenic diversity, *C. burnetii* exhibits two primary features. Antigenically, it displays a phase variation corresponding to Lipopolysaccharide (LPS) structure: the virulent, smooth Phase I LPS form is found in natural infections, while the non-virulent, rough Phase II LPS form emerges after multiple *in vitro* passages (Hackert *et al*., 2012). Furthermore, molecular characterization has identified approximately 20 distinct *Coxiella* genotypes grouped into six clusters (Group I to VI) (Russell-Lodrigue *et al*., 2009). Historically, genotypes from Groups I, II, and III were linked predominantly to acute infections, while Groups IV and V were associated with chronic disease (Glazunova *et al*., 2005). However, recent investigations by the World Organisation for Animal Health suggest that the link between a single genotype and a specific acute or chronic outcome, or a particular host, may not be exclusive (WOAH, 2024). Consequently, the combination of the PV survival mechanism, the compact genome, and SCV-mediated environmental stability establishes *C. burnetii* as a highly infectious agent of significant public and veterinary health concern (Tiwade *et al*., 2025).

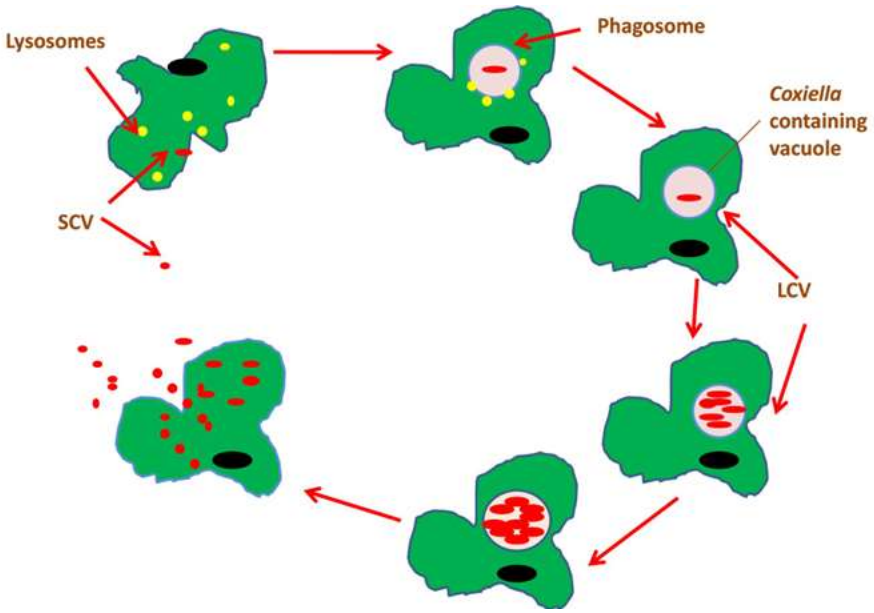


Figure 3. The biphasic development cycle of *C. burnetii*.

Source: Fatima and Upadhyay, 2025.

2.3. Taxonomic classification

Coxiella burnetii belongs to the Rickettsiales order, Coxiellaceae family, and Rickettsiae tribe, along with the Rickettsia and Rochalimaea genera. However, it has been reclassified from Rickettsiales to Legionellales and is now considered part of the Proteobacteria gamma group. Within this group, its phylogenetic neighbors include *Legionellae* spp., *Francisella tularensis*, and *Rickettsiella* spp. (Figure 4) (Seshadri *et al*., 2003). Despite being previously thought of as a member of the *Rickettsia* genus, gene-sequencing research has placed the *Coxiella* genus (as shown below) within the Legionellale order, Coxiellaceae family, alongside Rickettsiella (Shaw and Voth, 2019).

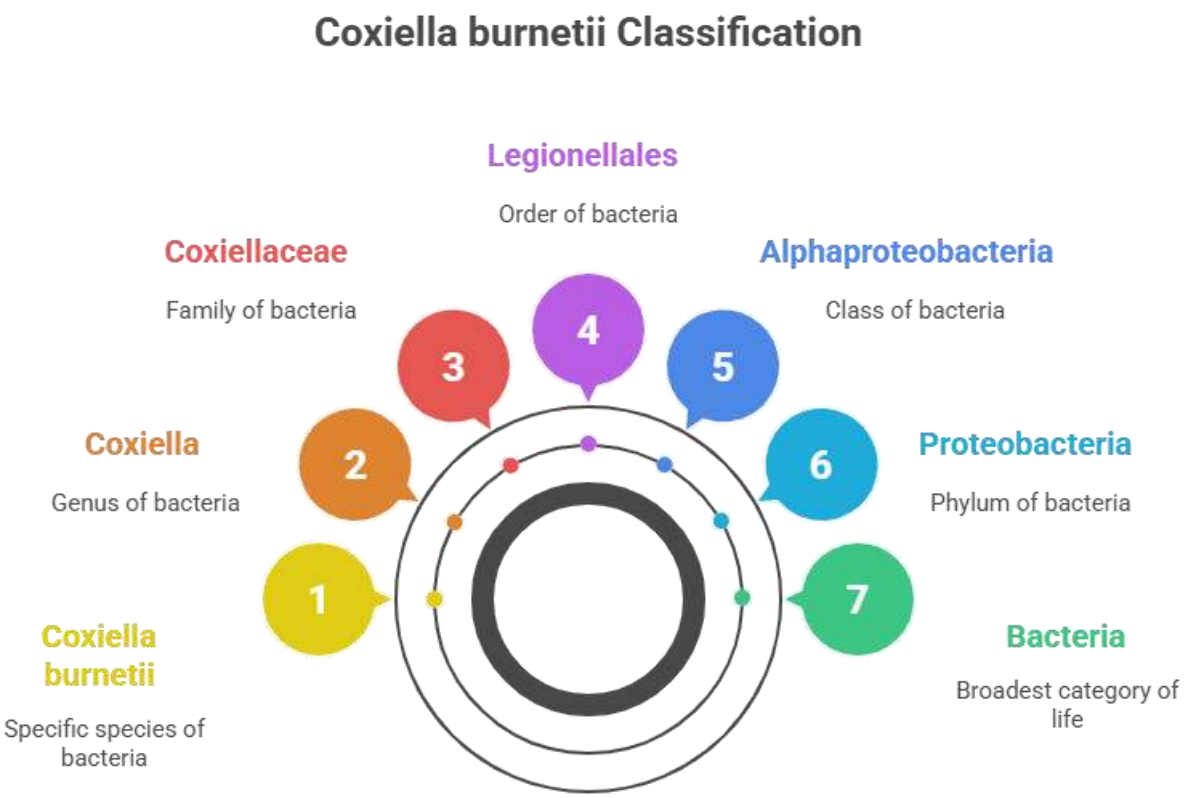


Figure 4. Taxonomic classification of *Coxiella burnetii*.

Source: NCBI.

2.4. The disease: Q fever / Coxiellosis

Q fever is an infection disease caused by *Coxiella burnetii*, which is transmitted from livestock to humans (Eldin *et al*., 2017). The disease is commonly considered an occupational hazard for those who work with livestock or animal products. Q fever mostly presents as a self-limiting flu-like illness that is characterized by atypical pneumonia and an unexplained fever (Honarmand *et al*., 2012). In humans it can occur as an acute debilitating illness lasting 2–4 weeks in which some of the cases develop chronic form. The main route of infection in humans is inhalation of dust particles containing *C. burnetii*. Although most infections remain clinically inapparent, very few cases of acute infections with hospitalization due to pneumonia, hepatitis or prolonged fever and rare cases of localized pericarditis, myocarditis, meningitis, meningoencephalitis, bone marrow necrosis, pancreatitis or orchitis have been reported

(Tolpinrud *et al.,* 2025). In those individuals with weak immunity the infection persists causing chronic illness featured by infective endocarditis or vascular lesions or Q fever fatigue syndrome (Hawker *et al*., 1998).

Naturally infected livestock rarely manifest clinical signs. The notable signs observed in domestic livestock include reproductive failure and late-stage abortions that result from placentitis and metritis particularly in small ruminants. In infected livestock the clinical signs usually persist and observed in subsequent reproductive cycles resulting in persistent economic losses (Angelakis and Raoult, 2008). The outcomes of infection include abortion, stillbirth, premature delivery, and delivery of weak offspring (sheep and goats); infertility, metritis and mastitis (cattle and camel) (Pal *et al*., 2025).

The Centers for Disease Control and Prevention has classified Q fever as a "Category B" critical biological agent, indicating its potential use as a bioterrorism weapon (Das and Kataria, 2010). Except for the Arctic and New Zealand, Q fever has been reported in over 59 countries worldwide as depicted in Figure 5 (Stevenson *et al*., 2015). Recently, there is increasing evidence that *C. burnetii* is a cause of non-malaria febrile illness and community-acquired pneumonia in many developing countries, and it has been identified as a re-emerging pathogen that poses a growing public health threat (Eldin *et al*., 2017). It is a widespread disease having a global impact and can affect people in diverse geographical regions (Mohammadi *et al*., 2025).

Figure 5. Evidence for *Coxiella burnetii* exposure has been confirmed across the globe, with the notable exception of the Arctic regions and New Zealand.

2.4.1. Epidemiology

2.4.1.1. Reservoirs of infection

The bacterium *Coxiella burnetii* has a wide range of reservoir hosts. The primary reservoirs of the pathogen are domestic ruminants including sheep, goats, camel, and cattle, which serve as the major source of human outbreaks (Celina and Cerny, 2022, Pal *et al*., 2025). People acquire the infection through inhalation of aerosols from infected animal feces, urine, milk, and birth products (Figure 6). Exposure to infected domestic ruminants and their products poses a significant risk for acquiring the pathogen in humans especially farmers and veterinarians. Apart from Cattle, sheep, and goats, a diverse range of other domestic livestock such as dog, cat, rabbit, pig, horse, buffalo, rodent, and birds have been reported to harbor *C. burnetii* and spread the pathogen to humans (Pal *et al*., 2025). Moreover, more than 100 wild mammal species have been known to serve as reservoirs of *C. burnetii* (Celina and Cerny, 2022).

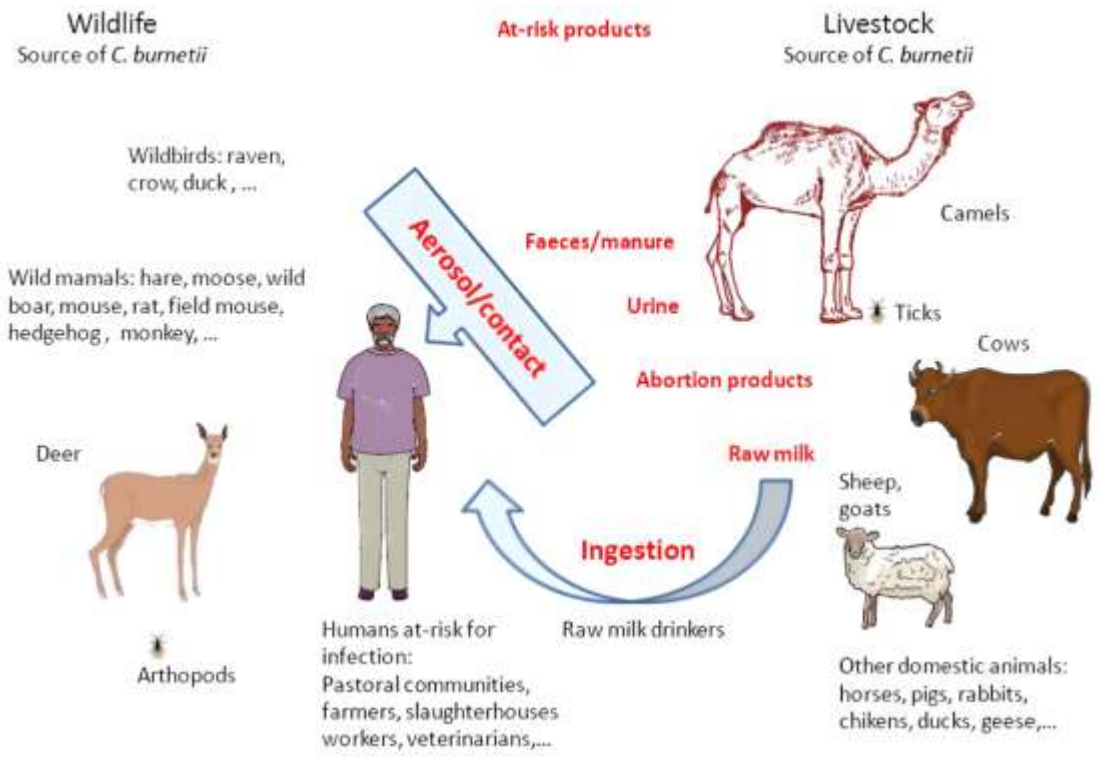


Figure 6. Pictorial Representation of the Major Natural Reservoirs and Sources of *Coxiella burnetii.*

Ticks are both carriers and reservoirs of the disease, with over 70 tick species known to carry *C. burnetii*. Studies have shown that a gram of tick faeces contains more than a billion Coxiella (Pal *et al*., 2025). Although more than 40 ixodid ticks were shown to harbor *C. burnetii*, special affinity has been reported for Mediterranean ticks such as Ixodes, *Rhipicephalus*, *Hyalomma*, and *Dermacentor* (Pal *et al*., 2025). Once infected, individual ticks may carry the bacteria for the rest of their lives. Ticks are known to play two roles: maintenance of the organisms in nature and transferring the bacterium from wild hosts to domestic livestock. Although ticks may have a role in environmental maintenance, it is unknown if they play a role in human infection (Olivieri *et al*., 2021).

2.4.2. Risk Factors for *Coxiella burnetii* Infection

The occurrence and distribution of Q fever are dictated by a sophisticated interplay of pathogen characteristics, host susceptibility, and environmental dynamics. Risk factors are broadly segmented into Agent/Environmental, Animal Host, and Human Exposure domains (Mezouar *et al*., 2025).

2.4.2.1 Agent and Environmental Determinants

A major driver of Q fever epidemiology is the exceptional environmental resilience of the Small Cell Variant (SCV) of *C. burnetii*. The bacterium is resistant nor the physical and chemical stresses and easily dispersed. It is resistant to acids up to pH 4.5, temperature of 62°C and UV light and pressure up to 300,000 kPa (Pal *et al*., 2025). This spore-like stability allows the pathogen to survive for extended periods in dried feces, dust, and soil, facilitating highly efficient long-distance aerosol transmission (Carrieri *et al*., 2002). This mode of spread is significantly enhanced by high winds, and local environmental features such as paddy fields can further contribute to risk in specific locales (Choi *et al*., 2024).

The seasonal dynamics of Q fever are closely linked to contamination events. While high human incidence is often reported in spring and early summer, correlating with peak lambing and shearing seasons (Tan *et al*., 2024), cases have also been documented during the dry season in other endemic areas, demonstrating variability based on regional climate (Tissot-Dupont *et al*., 2007; Prabhu *et al*., 2011). Rainfall and infected animal density particularly livestock are important environmental determinants of Q fever in humans. The disease occurs mostly during kidding season. The involvement of diverse reservoir hosts, including livestock, pets, and wild/domestic birds (which can transmit the disease via droppings or ectoparasites), means that human cases can occur even in urban environments lacking direct livestock contact (Figure 7).

2.4.2.2 Animal Host and Management Factors

In livestock, management factors such as housing types practice of isolation of newly introduced livestock and other husbandry practices are important drivers of infection. In animal populations, which serve as the pathogen's primary amplifying reservoirs, risk is dictated by husbandry practices and physiological status. The most critical biological risk factor is the

female ruminant's late-stage pregnancy and parturition, as the bacterium concentrates massively in the placenta, resulting in extreme environmental shedding during abortion or birthing (Celina and Cerny, 2022). Consequently, a herd history of abortion is strongly associated with active *C. burnetii* infection. Poor hygiene practices, such as the unsanitary disposal of aborted materials and a failure to isolate livestock during parturition, amplify local contamination (Keshavamurthy *et al*., 2020).

Herd management and demographics are crucial contributors to risk. The risk of infection consistently increases with age, as older livestock particularly adult females exhibit higher seroprevalence due to cumulative exposure (Bwatota *et al*., 2022). Management factors promoting density, such as large flock sizes or a high number of goats per care, enhance aerosol concentration and horizontal transmission (Rizzo *et al*., 2016; Hou *et al*., 2022). The greatest risk of introduction and propagation comes from unregulated animal movement, including the purchase of new livestock with unknown infection status, and uncontrolled cross-border livestock trade (Celina and Cerny, 2022; Wolf *et al*., 2020). Additionally, management practices like year-round lambing maintain continuous contamination, while co-infection with other pathogens may compromise herd health, indirectly influencing *C. burnetii* risk (Wolf *et al*., 2020; Bwatota *et al*., 2022).

2.4.2.3. Human and Occupational Exposure Factors

Human risk is heavily stratified by occupational exposure and residential proximity to animal sources. Individuals working as farmers, veterinarians, slaughterhouse workers, and animal researchers are recognized as high-risk groups due to direct contact with livestock and contaminated materials (Fatima and Upadhyay, 2025).

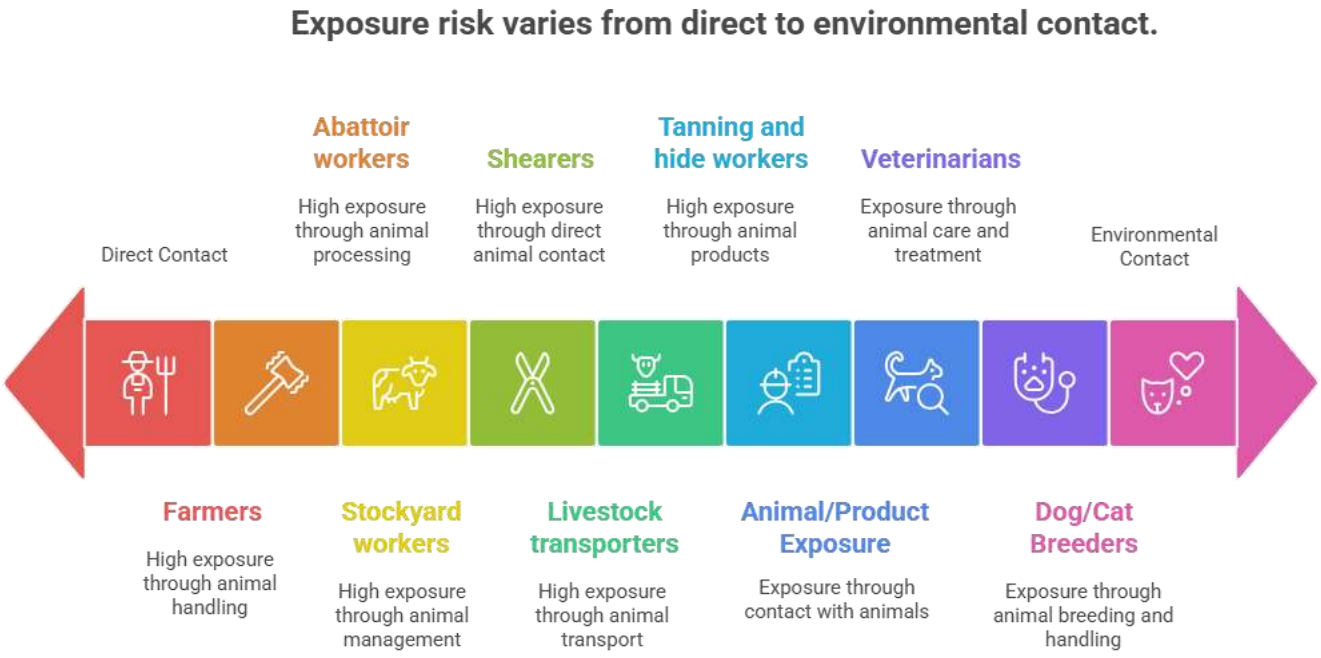


Figure 7. Occupational risk groups for Q fever infection in human population.

Source: Nahed and Khaled (2012).

This risk extends to those in related fields, such as the wool industry, and even to laboratory and hospital settings through contaminated samples (Anderson *et al*., 2011). Residential proximity to ruminants is a key non-occupational risk factor; both living in rural areas and dwelling near areas of high animal seropositivity increases the likelihood of infection (Eldin *et al*., 2017; Pouquet *et al*., 2020).

Individual host factors also modulate susceptibility and disease progression. While individuals of all ages can be infected, the risk of developing clinical disease and disease severity is highest in the active working age range (45–65) and among those over 40 (Anderson *et al*., 2011; CDC, 2013). Males are generally more susceptible to infection than females. Crucially, individuals with underlying health issues, including pregnancy, heart disease, or immunodeficiency, are at a heightened risk of developing severe or chronic Q fever complications. Environmental exposure events, such as military personnel being exposed to *C. burnetii* aerosols generated by helicopters in endemic areas, further illustrate the diverse exposure pathways (Faix *et al*., 2008).

2.4.3. Mode and Route of Transmission

C. burnetii can easily spread through the air, and aerosol transmission has been documented over a mile away being carried by wind. In wild livestock, the most common route of infection is through tick bites, while in domesticated livestock, the bacteria can be inhaled in aerosolized form (Eldin *et al*., 2017). While many livestock show no symptoms, the bacterium can be excreted via reproductive fluids and aborted materials. In addition, the pathogen multiplies in the epithelial cells of the intestine and midgut before excreted in feces contaminating the livestock' hair coat (Mohabati Mobarez *et al*., 2021). Aerosol can be formed from those secretions and execrations to be transmitted mainly through respiratory routes (Figure 8). However, vectorial transmission by tick bite has been demonstrated (Duron *et al*., 2015).

Humans primarily contract *C. burnetii* through inhaling bacteria present in air contaminated by excreta from infected livestock. Consuming raw milk or milk products from diseased livestock or unpasteurized milk from infected livestock can also lead to infection through the oral route, as the pathogenic material present in these products is sufficient to cause an infection (Lejeune and Rajala-Schultz, 2009). The bacterium can spread through transovarial and transplacental routes, and large numbers of organisms can be present in amniotic fluids and the placenta during birth, sometimes exceeding 10^9 bacteria per gram of placenta although less than 10 bacteria can cause infection (Sprong *et al*., 2012). This remarkably low infectious dose of *C. burnetii* greatly increases the likelihood of transmission.

In humans, *C. burnetii* infection is highly infectious, and as few as one to ten organisms can cause an infection. Transmission to humans can occur through tick bites, contact with droplets or fomites, and other mechanisms. Maurin and Raoult (1999) observed both sexual transmission and indirect transmission through contact with contaminated wool, straw, clothing, linens, and other items. However, coughing and sneezing are not known to transmit Q fever, and there is no evidence of transmission through casual contact such as hugging, shaking hands, kissing, or sharing food (Roest *et al*., 2013). Person-to-person transmission is extremely rare (Fatima and Upadhyay, 2025).

In nature *C. burnetii* is ultimately maintained in wild livestock (rodents and birds) via tick transmission. The tick population can also maintain the bacterium through transovarian

transmission. Spread to domestic livestock can occur through contact with tick excreta or tick bite. Human can acquire the infection from domestic livestock, the environment and ticks as depicted in Figure 8.

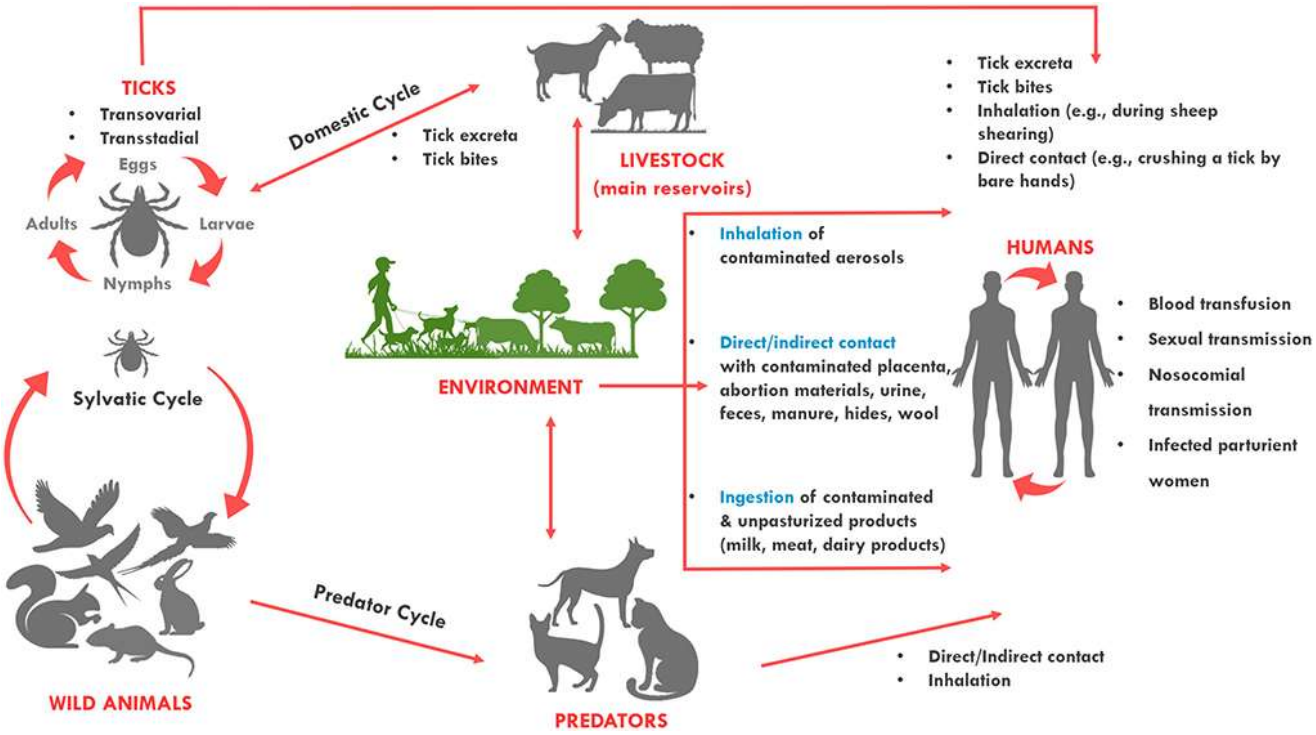


Figure 8. Schematic presentation of the transmission routes of *C. burnetii* from the reservoirs to human and animal hosts.

Source: Celina and Cerný, 2022; Fatima and Upadhyay, 2025.

2.4.4. Global Distribution of Q fever Outbreaks

Outbreaks of the disease have been reported in several countries mostly industrialized nations, including in Australia ($n = 9$), UK ($n = 8$), France and the Netherlands (each $n = 7$) and the USA ($n = 6$) (Figure 9) (Tan *et al*., 2024). The median number of cases per outbreak ranged from 2 to 4107. The duration of the outbreaks ranged from 4 to 1722 days (Tabita *et al*., 2024). The majorities of the outbreaks (43/81) were recorded in communities outside of traditional at-risk occupational settings and were frequently associated with living in proximity to livestock holdings. The largest outbreak of Q fever was recorded in the Netherlands during 2007–2010 with 4000 confirmed acute human cases arising from endemically infected dairy

goat herds by *C. burnetii* (Tolpinrud A *et al.,* 2025). Previous studies showed that variable persistence of infection, source of infection and methods of propagation can influence the size of the outbreak (Chiesa *et al*., 2023). Exposure to domestic ruminants (sheep, goats, and cattle) and their products was the most common source of infection (Tabita *et al*., 2024).

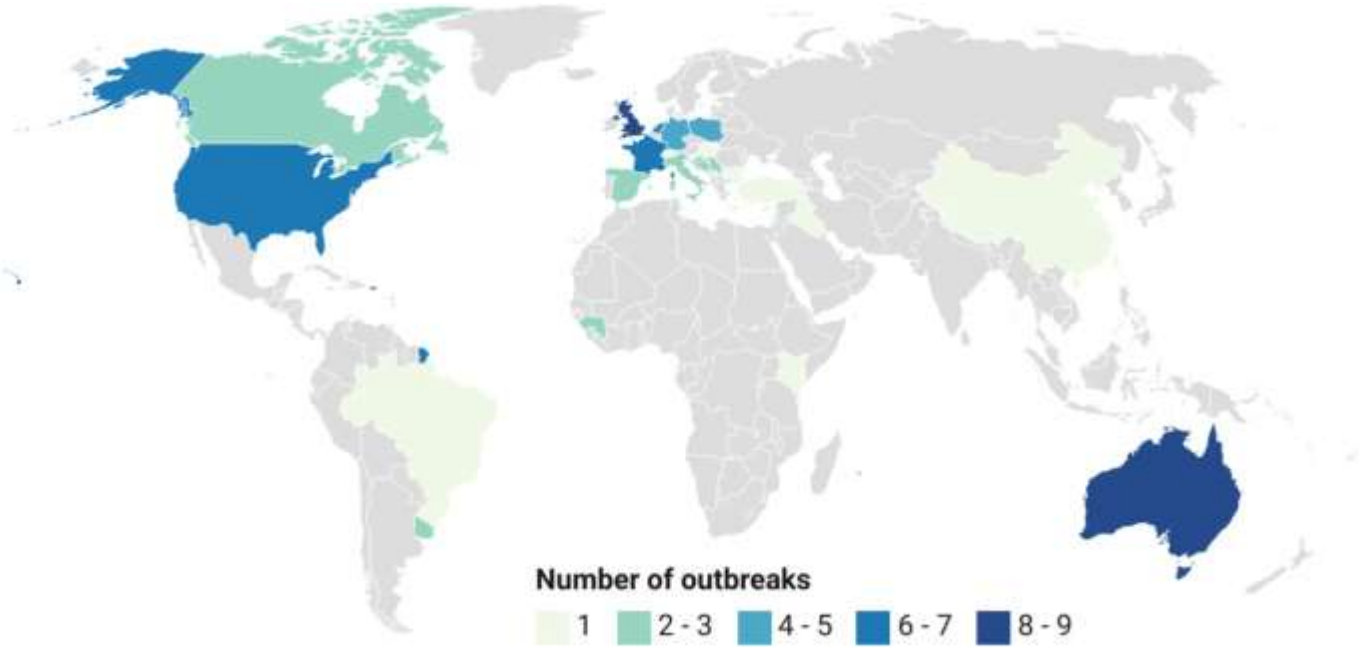


Figure 9. Global Distribution of *Coxiella burnetii* Outbreak Events Affecting Humans.

Source: Tan *et al*., 2024.

2.4.5. Global Seroprevalence of Q fever

Q fever is an endemic disease occurring worldwide with the exception of New Zealand and Antarctica (Tolpinrud *et al.,* 2025). According to Stevenson *et al*. (2015), serological evidence of Q fever is available in every region of the world except in those countries where it never occurred. That is, the disease is widespread although it is particularly prevalent in certain regions, such as the Mediterranean, where it is considered mostly endemic. This global distribution of Q fever is important to consider for public health purposes, as it can help guide prevention and control efforts. The seroprevalence of Q fever mostly ranges from 5% to 50% in livestock and 8% to 30% in humans globally (Hena *et al*., 2022). However, the seroprevalence of the disease ranges from 13% to 33% in integrated human and animal studies

in Egypt (Pal *et al*., 2025). The global sero-prevalence of the disease in livestock and humans is given in Table 2 (Konputtar *et al*., 2024) and Figure 10.

Table 2. The global sero-prevalence ranges of *Coxiella burnetii* infection in livestock and humans

Country	**Species/Population**	**Study Type**	**Study Year(s)**	**Sample Type**	**Total Sample (n)**	**Number Positive**	**Prevalence (%)**
USA	Bulk Tank Milk (Bovine/Caprine)	Serology (Prevalence)	Varies	BTM*	293	182	62.1
Poland	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	720	80	11.1
Algeria	Bulk Tank Milk (Bovine/Caprine)	Serology (Prevalence)	Varies	BTM	200	74	37.0
India	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	359	33	4.3
Turkey	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	48	17	35.4
Australia	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	49	6	12.2
Italy	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	92	63	68.5
China	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	1140	381	33.4
Sweden	Bulk Tank Milk (Bovine/Caprine)	Serology (Prevalence)	Varies	BTM	153	126	82.4
El Salvador	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	33	27	81.8
Germany	Individual Serum (Human/Animal)	Serology (Prevalence)	Varies	Serum	603	435	72.1

*Bulk Tank Milk

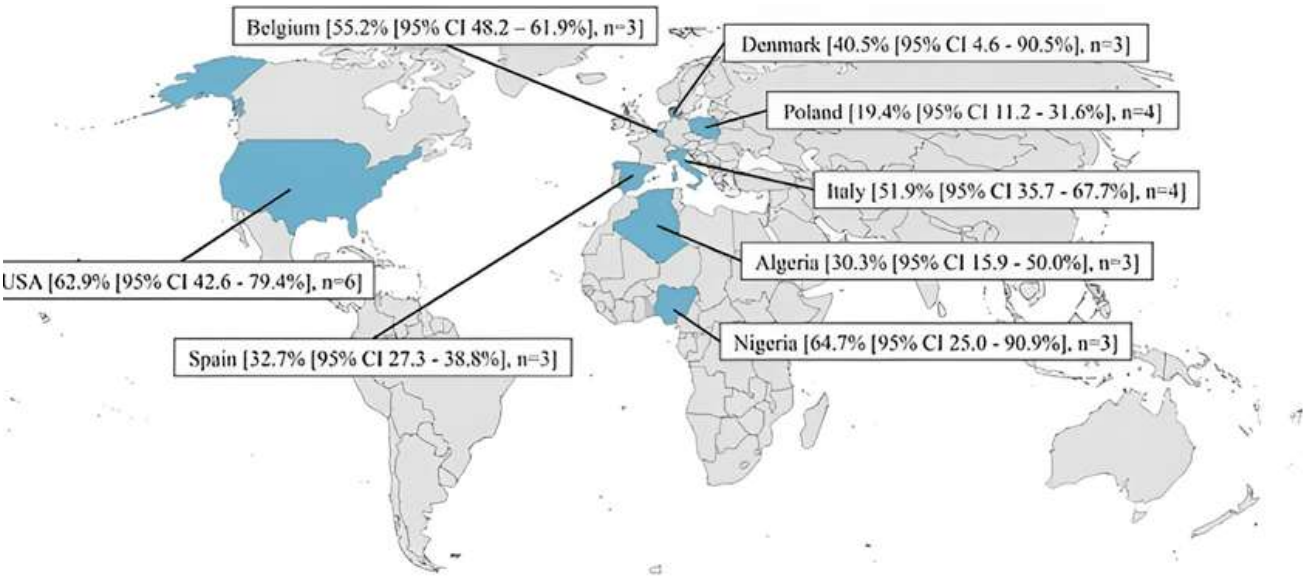


Figure 10. Schematic Overview of the Global Geographical Distribution of Q fever Serological Evidence.

Source: Konputtar *et al*., 2024

2.4.6. Seroprevalence in Africa

Vanderburg and colleague reviewed serological evidence on the occurrence of Q fever throughout the African continent across the north, south, east, west, and central regions (Figure 11) although the cases are underreported (Vanderburg *et al*., 2014). The reasons for underreporting are limited awareness about the disease, inadequate diagnostic facilities, and low levels of surveillance and reporting and limited access to healthcare and veterinary services in some areas. The serological evidence of the occurrence of infection with *C. burnetii* in Africa is summarized in Table 3,4,5 and 6 (Vanderburg *et al*., 2014). Higher seroprevalence up to 80% was reported in Chad while lower prevalence (lower than 4%) seroprevalence of was documented in Cameroon. In South Africa a seroprevalence of 8% was published although the prevalence of 100% was recorded in aborted materials. In eastern African countries published evidence showed varying prevalence based on the host species studied, test method employed and geographical location (Table 7).

Table 3. Seroprevalence of *Coxiella burnetii* Infection in Livestock and other animals in Northern Africa

Sub-Region	Country	Species/Population	Study Type	Study Year(s)	Sample Type	Total Sample (n)	Number Positive	Prevalence (%)
Northern	Egypt	Buffalo	Prevalence	2012	Serum	45	0	0%
Northern	Tunisia	Sheep (Controls)	Disease Association	1997	Serum	517	35	7%
Northern	Egypt	Cattle	Prevalence	2012	Serum	54	7	13%
Northern	Morocco	Sheep (Controls)	Disease Association	1986–1987	Serum	156	23	15%
Northern	Tunisia	Goats & Sheep (Abortion)	Disease Association	1997	Serum	118	22	19%
Northern	Tunisia	Goats & Sheep (Abortion)	Disease Association	2009	Birth Products/Vaginal Fluids	72	14	19%
Northern	Egypt	Cattle	Prevalence	2009	Milk	100	22	22%
Northern	Egypt	Dogs	Prevalence	1998–1999	Serum	150	34	23%
Northern	Egypt	Goats	Prevalence	2012	Serum	30	7	23%
Northern	Sudan	Goats	Prevalence	2010–2011	Serum	460	109	24%
Northern	Morocco	Sheep (Prior Abortion)	Disease Association	1986–1987	Serum	115	38	33%
Northern	Egypt	Sheep	Prevalence	2012	Serum	55	18	33%

Table 4. Seroprevalence of *Coxiella burnetii* infection in Livestock and other animals in Western Africa

Sub-Region	Country	Species/Population	Study Type	Study Year(s)	Sample Type	Total Sample (n)	Number Positive	Prevalence (%)
Western	Senegal	Cattle	Prevalence	2007–2008	Serum	196	7	4%
Western	Ghana	Cattle	Prevalence	2012	Serum	166	30	18%
Western	Nigeria (Study 2)	Cattle	Prevalence	1983–1984	Milk	169	41	24%
Western	Niger	Goats (Controls)	Disease Association	1971–1972	Serum	47	12	29%
Western	Niger	Goats (Prior Abortion)	Disease Association	1971–1972	Serum	75	24	32%
Western	Nigeria (Study 1)	Cattle	Prevalence	1983–1984	Milk	84	44	52%
Western	Nigeria (Study 1)	Cattle	Prevalence	1983–1984	Serum	306	169	55%
Western	Nigeria (Study 2)	Cattle	Prevalence	1983–1984	Serum	88	48	55%

Table 5. Seroprevalence of *Coxiella burnetii* infection in Livestock and other animals in central African countries

Sub-Region	Country	Species/Population	Study Type	Study Year(s)	Sample Type	Total Sample (n)	Number Positive	Prevalence (%)
central	Cameroon	Cattle (Prior Abortion)	Disease Association	1968	Serum	116	0	0%
central	Cameroon	Cattle (Prior Abortion)	Disease Association	1985	Serum	490	14	3%
central	Chad (Study 2)	Cattle	Prevalence	1999–2000	Serum	195	8	4%
central	Chad	Cattle	Prevalence	1985	Serum	193	13	7%
central	Cameroon	Cattle (Controls)	Disease Association	1985	Serum	193	13	7%
central	Chad (Study 2)	Sheep	Prevalence	1999–2000	Serum	142	16	11%
central	Chad (Study 2)	Goats	Prevalence	1999–2000	Serum	134	18	13%
central	Cameroon	Cattle	Prevalence	2000	Serum	1,377	431	32%
central	Chad (Study 2)	Camels	Prevalence	1999–2000	Serum	142	114	80%

Table 6. Seroprevalence of *Coxiella burnetii* infection in livestock Southern Africa

Sub-Region	Country	Species/Population	Study Type	Study Year(s)	Sample Type	Total Sample (n)	Number Positive	Prevalence (%)
Southern	South Africa	Cattle	Prevalence	1985–1986	Serum	8,900	692	8%
Southern	South Africa	Cattle and Sheep Farms (Abortion Cases)	Disease Association	1972–1976	Fetal Tissue	12 Farms	12 Farms	100% (of farms tested)

Table 7. Epidemiological overview of *Coxiella burnetii* prevalence in Livestock and other animals in Eastern Africa.

Country	Species/Population	Diagnostic Technique	Sample Type	Total Sample (n)	Prevalence (%)
Kenya	Livestock (Goats, Sheep, Cattle, Camels)	Serology (ELISA)	Serum	2,157	47.9%
Tanzania	Dairy Cattle	Serology (ELISA)	Serum	2,049	3.9%
Uganda	Ticks (from Dogs/Cats, Livestock, Vegetation)	Real-time PCR	Pooled Ticks (859 pools)	9,602 (Individual Ticks)	5.5% (Pool Positivity)

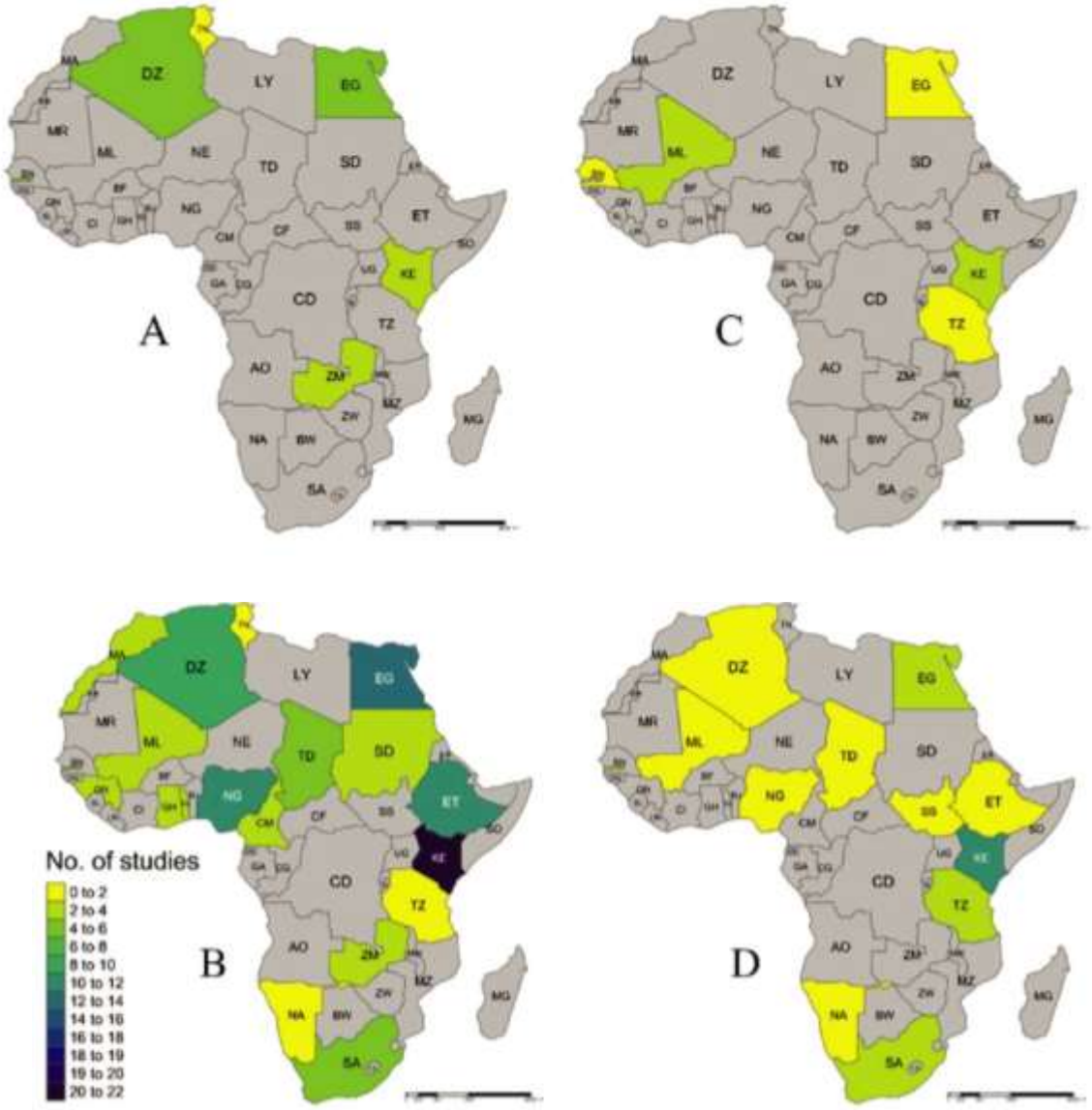


Figure 11. Maps of Africa demonstrating the number of studies in respective countries where seroprevalence and molecular studies were performed. Molecular and seroprevalence studies in livestock (cattle, sheep, and goats) are respectively shown in A & B. Molecular and seroprevalence studies in humans are respectively shown in C & D.

Source: Bwatota *et al*. (2022).

2.4.7. Occurrence of Q fever in Ethiopia

Table 8 presents the results of studies carried out thus far on the occurrence of infection with *C. burnetii* in Ethiopia. Only limited serological studies has been carried out in Ethiopia compared to other African countries such as Kenya and Egypt resulting in the lack of information on the prevalence and distribution of the disease. Limited availability of resources, lack of infrastructure, and poor awareness of both professionals and the community about the disease are possible reasons for limited studies. The few studies conducted however, reports the occurrence of infection mostly in domestic livestock. Seroprevalence ranging from 6.5% in abattoir workers (Abebe, 1990) to 92.22% in livestock was documented (Alemayehu *et al*., 2021). The seroprevalence was highest in sheep and goats (Alemayehu *et al*., 2021) and camels (Gumi *et al*., 2013). In contrast, lower prevalence was recorded in cattle (Deressa *et al*., 2020; Ibrahim *et al*., 2021).

The previous studies are geographically limited; focus mostly on few study samples and livestock that originated from one or two agroecological zones or production systems. They did not provide information on the distribution of the infection across the country. Moreover, the majority of the studies were conducted on sheep and goats, while only three studies include cattle; two studies included camels and two studies included humans. Comprehensive epidemiological information is lacking. However, such information is important for public health and animal health authorities to develop targeted interventions to efficiently prevent and control the disease. In general, there are knowledge and awareness gaps on the occurrence *C. burnetii* in livestock and humans in Ethiopia.

Table 8. Comprehensive overview of documented seroprevalence studies for *Coxiella burnetii* infection in Ethiopia

Author(s) & Year	Study Population / Location	Design	Total Sample Size (n)	Lab Method(s)	Positive Prevalence (%)
Abebe (1990)	Humans at Addis Ababa Abattoir	Not Indicated	465 Humans	Complement Fixation Test (CFT)	Humans: 6.5%
Deressa *et al*. (2020)	Cattle in Jimma Town (Dairy/Slaughterhouse)	Cross-Sectional	Not Indicated	i-ELISA (ID.vet)	Cattle: 8.77%
Ibrahim *et al*. (2021)	Multi-Host Study in Somali Region (Humans, Cattle, Camels, Goats, Sheep)	Multi-Stage Cross-Sectional Cluster	1,062 Total	Indirect ELISA (ID.vet)	Humans: 27.0% Cattle: 9.6% Sheep: 28.9% Goats: 48.8% Camels: 55.7%

Proboste *et al*. (2021)	Cattle in South-Western Ethiopia Dairy Farms	Not Indicated	227 Cattle	i-ELISA (ID.vet)	Cattle: 20.0%
Tesfaye *et al*. (2020)	Sheep and Goats in Borana Pastoral Area	Cross-Sectional	506 Total	ELISA	Overall: 28.46%
Balako Gumi *et al*. (2013)	Pastoral Livestock in Southeast Ethiopia (Cattle, Camels, Goats)	Cross-Sectional	368 Total	ELISA Kit (IDEXX)	Cattle: 31.6% Goats: 54.2% Camels: 90.0%
Alemnew *et al*. (2021)	Goats in Ataye Boer Nucleus Site, North Shewa Zone	Not Indicated	513 Goats	i-ELISA	Goats: 65.7%
Gebretensay *et al*. (2019)	Sheep in the Highlands (Reproductive Disorders/Abortion)	Cross-Sectional	445 Sheep	ELISA Kit (IDEXX)	Sheep: 68.33%
Alemayehu *et al*. (2021)	Sheep and Goats in Three Agroecology Zones (Abortion Risk Factors)	Cross-Sectional	223 Total	ELISA Kit (IDEXX)	Sheep: 65.41% Goats: 92.22%
Getachew *et al*. (2024)	Cattle, Goats, Sheep in South Omo Zone (Pastoral Area)	Cross-Sectional	1,350 Total (450 Cattle, 450 Goats, 450 Sheep)	ELISA	Cattle: 37.6% Small Ruminants: 28.7%

2.4.8. Molecular Epidemiology

2.4.8.1. Molecular Epidemiology, Genotypes, and Pathogenicity

Molecular characterization, primarily through advanced genotyping methods and comparative genomic assays, has unveiled significant genetic diversity within *C. burnetii* populations globally, revealing the existence of numerous distinct strains (Table 9). This molecular epidemiology is crucial for understanding the transmission, evolution, and strain-specific pathogenicity of Q fever, directly informing the development of targeted diagnostics and intervention strategies (Mohammadi *et al*., 2025). Genomics and sequencing techniques have profoundly enhanced the study of *C. burnetii*, providing deeper insights into its genetic makeup and its relationship to disease outcome (Figure 13).

Table 9. Comprehensive overview of molecular techniques available for genotyping *Coxiella burnetii* isolates

Year Developed	Full Name	Purpose of the Technique
1990	Restriction Fragment Length Polymorphism typing (RFLP)	To identify differences in fragment lengths of DNA after digestion with specific restriction enzymes.
1997	Com1 encoding genes sequencing	To differentiate strains using sequence analysis of the *Com1* (or *MCM*) encoding gene.
1999	Com1 and MucZ encoding genes sequencing	To differentiate strains with increased resolution by combining sequence analysis of both *Com1* and *MucZ* encoding genes.
2005	Multispacer Sequence Typing (MST)	To differentiate strains based on DNA sequence variation in short, non-coding intergenic regions (spacers) across the genome.
2006	Multiple Locus Variable Number Tandem Repeats Analysis (MLVA)	To identify variation in the number of tandemly repeated DNA elements (VNTRs) at multiple, distinct genomic loci.
2007	IS1111 repetitive element PCR-based differentiation typing	To differentiate strains by identifying the location and presence of different IS1111 insertion elements throughout the genome using PCR.
2009	Randomly Amplified Polymorphic DNA (RAPD)	To differentiate strains through the analysis of randomly amplified DNA fragments of the entire genome using short, arbitrary primers.
2011	Single Nucleotide Polymorphism typing (SNP)	To differentiate strains by detecting a single nucleotide difference (SNP) at specific genomic loci.

2.4.8.2. Genotype Classification and Disease Outcome

The genetic diversity within *C. burnetii* is intrinsically linked to the variation in its clinical presentation across both human and animal hosts. Genotyping techniques, such as Multiple Locus Variable-number Tandem-repeat Analysis (MLVA) and Single Nucleotide Polymorphism (SNP) analysis, have successfully delineated distinct genotypes. These genetic variants exhibit differences in virulence, host preference, and geographical association (Truong *et al*., 2022; Khademi *et al*., 2024). For instance, while many genotypes can cause the standard, self-limiting Acute Q Fever in humans (presenting as a flu-like illness or pneumonia), certain specific strains are disproportionately linked to severe disease. Notably, genotypes associated with particular virulence plasmids are frequently overrepresented in Chronic Q Fever cases,

such as endocarditis, indicating that the strain's genetic composition determines its capacity for long-term persistence and severe illness in humans (Afrasiabian *et al*., 2024).

This genetic-clinical correlation is equally critical in veterinary medicine. Specific clonal complexes of *C. burnetii* are responsible for the large-scale Q fever outbreaks characterized by devastating abortion storms in livestock (Gisbert *et al*., 2024), exemplified by the strains involved in the massive Netherlands outbreak. The capacity of these strains to induce high morbidity and reproductive failure underscores the importance of molecular characterization. Consequently, identifying the precise circulating genotype is a crucial step: it offers vital information for prognosis in human patients and provides essential data for risk assessment and targeted control strategies within livestock populations.

2.4.8.3. Geographical Distribution and Importance

C. burnetii genotypes exhibit non-uniform geographical distribution, which often reflects regional animal populations and trade routes. Some genotypes may be highly endemic to specific livestock populations (e.g., particular sheep or goat complexes), making them primary concerns for veterinary authorities in those areas (Celina & Cerný, 2022). The global movement of livestock and animal products has facilitated the spread of certain highly pathogenic clones across continents, complicating control efforts.

In nutshell molecular characterization is essential not only for tracing the source of outbreaks but also for recognizing that *C. burnetii* is not a single entity. The various genotypes function as distinct pathotypes, each carrying a unique risk profile for causing severe disease in humans and significant economic losses due to reproductive failure in livestock. Continued evolution in genomics will further refine our understanding of these genotype-phenotype relationships, leading to more targeted diagnostics and preventative measures.

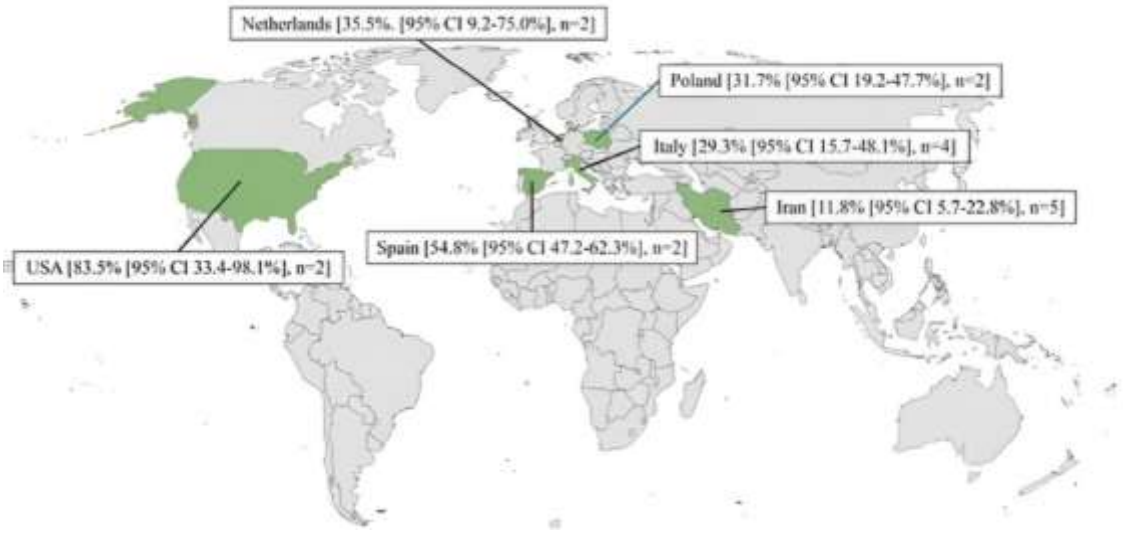


Figure 12. Global Distribution of *Coxiella burnetii* Confirmed by Molecular Evidence.

Source: Konputtar *et al*., 2024.

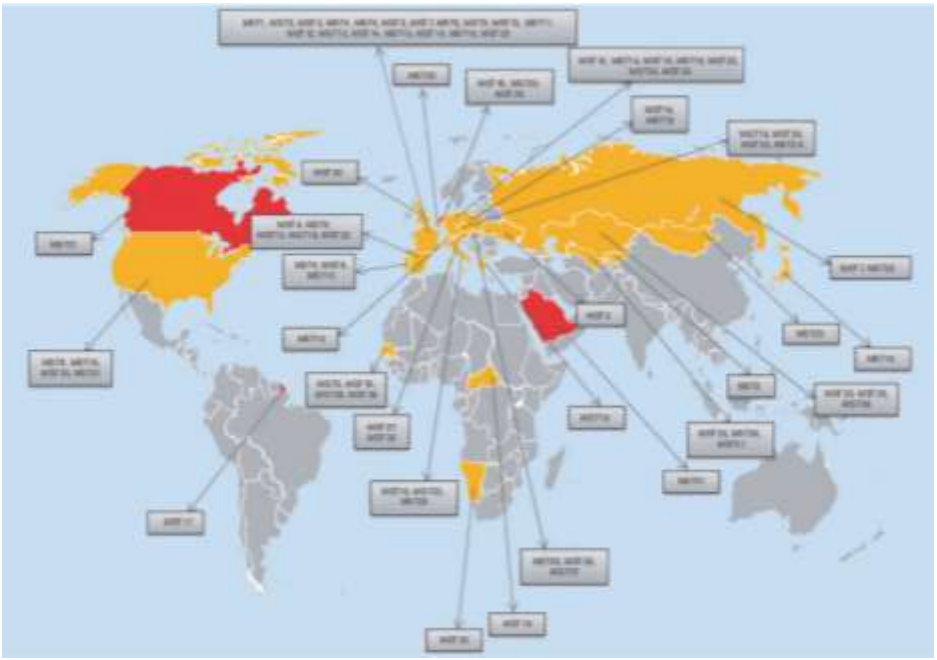


Figure 13. Geographical distribution of *Coxiella burnetii* detected genotypes. In red, countries characterized by a unique circulating clone. In yellow, countries where other MST genotypes have been described.

In Africa, the available literature suggests that the understanding of *C. burnetii* infection has mainly relied on observational studies on the prevalence of the disease, with little emphasis on genetic/molecular epidemiology. A review of genotyping studies conducted using SNP, MST, VNTR, and MLVA techniques via PubMed and Web of Science revealed that relatively few of these studies have been conducted in Africa, as shown in Figure 14. The studies have identified up to ten different MST genotypes from various hosts and countries, including ticks, cattle, goats, dogs, rodents, and lice. MST 2, 6, 16, and 19 genotypes have been found in humans, while some genotypes (2, 30, and 52) are unique to Africa, and others (such as MST16 and MST20) are found in non-African countries as well (Salifu *et al*., 2019).

Figure 14. Distribution of Various *C. burnetii* Genotypes in Africa by Host Species. Genotypes are distinguished by color, while host origin is specified by the ribbon shape: circle (tick), star (human), square (louse), and triangle (dogs/rodents).

Source: Salifu *et al*. (2019).

Establishing the genetic profiles of *Coxiella burnetii* strains circulating within African vectors remains a priority, yet data from Ethiopia are notably sparse, anchored by only two foundational molecular investigations characterizing the organism's genotypes in ticks recovered from domestic livestock. These critical studies, published by Sulyok *et al*. (2014)

and Kumsa *et al*. (2015), not only confirmed the involvement of ticks in local epidemiology but also elucidated the presence of both familiar and entirely novel genotypes. The initial effort by Sulyok and colleagues, which screened 296 tick samples, focused on the IS1111 region via real-time PCR. Eight highly positive cases, encompassing *Amblyomma cohaerens* and *A. variegatum*, were subjected to Multispacer Sequence Typing (MST) and Multiple-Locus Variable-number Tandem Repeat Analysis (MLVA). This meticulous work successfully identified a novel sequence type, designated ST52 by MST, which MLVA-6 further resolved into two newly defined MLVA genotypes: type 24 (AH) and type 26 (AI).

The subsequent, larger-scale investigation conducted by Kumsa *et al*. (2015) built upon these findings, analyzing DNA from 842 ticks using qPCR targeting two distinct genes. Their results established an overall *C. burnetii* prevalence of 6.4% (54/842), with the pathogen detected across seven different tick species. Specifically, *Amblyomma gemma* and *Rhipicephalus pulchellus* demonstrated significantly higher infection frequencies than other species. Most importantly, the MST analysis conducted by Kumsa *et al*. revealed a distinct geographic association of genotypes, demonstrating that the known MST genotype 18 was circulating predominantly in ticks from southeastern districts (Arero, Moyale, and Yabelo), whereas MST genotype 20 was confined to ticks collected in central districts. Collectively, the work by Sulyok *et al*. and Kumsa *et al*. is paramount, clearly indicating that ticks serve as significant reservoirs for genetically diverse *C. burnetii* strains, and establishing a baseline for tracking the pathogen's molecular epidemiology in Ethiopia.

2.4.9. *C. burnetii* as a Biological Weapon

Since *C. burnetii* is a highly infectious pathogen with as little as one organism able to cause clinical disease, it attracts attention or use as a biological weapon. The route of infection of the pathogen favors its use as a weapon for warfare (Madariaga *et al*., 2003). *C. burnetii* can be aerosolized over distance, being carried by wind currents, and has potential to infect people (Meles *et al*., 2024). This suggests the huge potential of the agent to be used for terrorist threat. It may be surprising why Q fever is considered a preferred bio weapon. The answer lies in the fact that Q fever is highly infectious and can cause clinical infection. Additionally, chronic Q fever can lead to endocarditis, hepatitis, or chronic fatigue syndrome, making it a highly hazardous agent (Miller *et al*., 2021).

Evidence showed that *C. burnetii* has been previously weaponized for use in biological warfare (CDC, USA). According to estimates of WHO, 50 kg of *C. burnetii* can cause acute illness in 125,000 people with 9,000 deaths if aerosolized over urban area having 500,000 inhabitants (WHO, 1970). There have been concerns that Q fever could potentially be utilized as a biological warfare agent due to its high level of infectiousness and ability to spread over a wide area since the 1970s (SIPRI, 1971). In 1964, pathogens causing Q fever were studied for their potential as biological weapons, along with other agents such as Anthrax, Glanders, Brucellosis, and various animal and plant pathogens. Q fever was also considered an excellent "incapacitating" agent by bio-weapon developers in the U.S. during the 1960s, and the U.S. Army historically used it to.

If used as an aerosolised biological weapon, *C. burnetii* could provoke acute disabling disease, which can be complicated by fatal (e.g., endocarditis) or debilitating (e.g., chronic fatigue syndrome) disorders (Madariaga *et al*., 2003). Additionally, there have been developments indicating that Q fever can cause acute disabling disease without a high mortality rate (Maradiaga *et al*., 2003). Diagnosis is often made through serology due to its hazardous nature (Kagawa *et al*., 2003).

2.5. Diagnosis

Definitive diagnosis of infection with *C. burnetii* can only be established through laboratory testing. Laboratory confirmation of the disease can be achieved either directly by culture and molecular techniques or indirectly through serological methods. To identify the organism, methods such as isolation, staining procedures, and specific detection techniques like immunohistochemistry, fluorescence in-situ hybridization, and polymerase chain reaction (PCR) assays are utilized. In addition to isolation and PCR, genotyping technologies are now valuable as an adjunct, enabling comprehensive strain identification (Miller *et al*., 2021; Zhang *et al*., 2022).

2.5.1. Isolation of the agent in culture

Due to the non-specific symptomatology or clinically inapparent infection of Q fever in humans and livestock, laboratory tests are the only way to reach a definitive diagnosis. Isolation and identification of the organism can be achieved by inoculating laboratory livestock, particularly guinea pigs, with properly prepared specimens. Isolation of *C. burnetii* in culture can be performed either in tissue culture using human embryonic lung fibroblast cell lines, embyonated eggs or in a cell-free bacteriological medium (Miller *et al*., 2020).

However, isolation of the agent in culture is rarely performed except in research laboratories licensed to work with these highly contagious organisms. However, relying solely on isolating *C. burnetii* may miss many current and past infections. Culture is typically avoided due to the difficulty, risk, and time-consuming nature of isolating *C. burnetii*, which requires a biosafety Level 3 facility (Field *et al*., 2000; Masala *et al*., 2004). The shell vial cell culture, originally intended for viral culture, has been adapted to isolate strict or facultative intracellular bacteria such as *C. burnetii*. A similar approach has been outlined for *C. burnetii* by Raoult *et al*. (1990).

2.5.2. Microscopy /Staining

There are several staining techniques available for identifying *Coxiella burnetii*, including Stamp, Gimenez, Macchiavello, Giemsa, and modified Koster (Quinn *et al*., 1999). Among these techniques, the Stamp method is commonly used in veterinary diagnostic laboratories, and the agent can be observed using microscopy (Table 10). Microscopic examination of stained smears is usually used to establish diagnosis of Q fever in livestock (Pal *et al*., 2025). *C. burnetii* stains poorly using conventional stains. In several countries diagnosis of Q fever in livestock relies on modified Ziehl-Neelsen (MZN) stain. For instance, stamp-stained smears can be made shortly after an abortion using an aborted fetus, placenta, or vaginal discharges. On the other hand, the Gimenez method is widely used in research laboratories for monitoring infected culture cells, as it does not require an acidic solution for differentiation (Shepherd *et al*., 2023).

Table 10. Summary of direct diagnosis of *Coxiella burnetii*

Diagnostic Category	Method	Description
Culture	Isolation (Cell Culture/Embryos/Livestock)	This is a relatively slow method that requires strict Biosafety Level 3 (BSL-3) facilities. *C. burnetii* is isolated using cell cultures, 6–7-day-old chick embryos, or laboratory livestock
Microscopic Methods	Staining (Direct Visualization)	Utilizes standard histological staining techniques, including Gimenez, Giemsa-Romanowski, and other specialized methods for visualization in tissue samples
Immunodetection	Immunofluorescence and Immunoperoxidase	These techniques involve using labeled antibodies to directly detect the pathogen in clinical samples. They are most applicable for early detection in the acute stage of the disease

2.5.3. Serological tests

Since antibodies persist longer after infection serological techniques are better suited for detection of exposure to *C. burnetii* in unvaccinated individuals or animal populations (Tolpinrud *et al*., 2025). Serological techniques remain the main tool for diagnosis of *C. burnetii* infection. Seroconversion can be detected in infected individuals within 3-6 weeks after the onset of symptoms. Serological tests can differentiate between acute (Phase II) and chronic infections (Phase I) through differential detection of antibodies against phase I and phase II antigens of *C. burnetii* (Pal *et al*., 2025). Serological methods such as Indirect immunofluorescence antibody test (immunofluorescence assay, IFA), complement fixation (CFT), enzyme – linked- immunosorbent assay (ELISA) and microagglutination are most used

to diagnose Q fever in both livestock and humans (as presented in Table 11). This approach has been described in studies by Slaba *et al*. (2005) and Rousset *et al*. (2009).

2.5.3.1. Indirect fluorescent antibody test (IFA)

The most commonly used serological test for diagnosing acute and chronic Q fever, as well as monitoring individuals who may develop chronic Q fever, is highly favored (Wegdam-Blans *et al*., 2012; Herremans *et al*., 2013). It remains the reference serological test since it is simple and the result is accurate. This test is straightforward to conduct, requires minimal reagents, and has rapidly become the preferred method for screening large numbers of sera. To confirm acute Q fever diagnosis, a fourfold increase in Phase II IgG antibody titer is necessary in paired serum samples (CDC, 2011; Wegdam-Blans *et al*., 2012).

However, cross-reactions with *Legionella* and *Bartonella* species have been reported (Musso and Raoult, 1997). The main disadvantage of IFA is the difficulty associated with cutoff values as various laboratories have developed their own IFA test. Although it has been considered that acute Q fever is defined by the presence of Phase II IgM ≥ 50, it became very difficult to make distinction between acute Q fever and past infection. However, Cléa and colleague (2019) demonstrated that avidity test can help in differentiating acute and past infection using IFA (França *et al*., 2023).

2.5.3.2. Complement fixation Test (CFT)

Despite still being in use, the OIE reference test is not very sensitive and may not detect antibodies in some livestock due to the antigen used in the test. Complement-fixing antibodies, on the other hand, can remain present for years after infection, making the CFT test highly specific for *C. burnetii* and useful in determining previous exposure to the organism (Fournier *et al*., 1998; Wegdam-Blans *et al*., 2012).

2.5.3.3. Enzyme-linked Immunosorbent assays (ELISA)

The selection of clinical diagnosis methods has been discussed in a study by Niemczuk *et al*. (2014). Currently, the most effective method for diagnosing Q fever in ruminants is a

combination of PCR and ELISA, which may have contributed to the rise in positive diagnoses of Q fever (Berri *et al*., 2000; Gutiérrez-Bautista *et al*., 2024).

Table 11. Comparative performance of serological methods of investigation and diagnosis of *Coxiella burnetii*

Diagnostic Method	Description and Utility	Sensitivity, Specificity, & Practicality
Indirect Immunofluorescence	Reference method for the serodiagnosis of Q fever. It determines antibodies against both Phase I and Phase II antigens.	Highly specific and sensitive; requires only a very small amount of antigen.
Microimmunofluorescence	A variation of IF that is also considered the reference serological test for Q fever.	High specificity and sensitivity.
Enzyme Immunoassay	Widely used for seroepidemiological studies and large-scale testing.	Generally, more specific and sensitive than CFT; highly practical for high-throughput screening.
Immunoblot Test	A confirmatory test that separates individual *C. burnetii* antigens.	Specific and sensitive, but generally time-consuming due to the technique involved.
Dot Immunoblot Assay	A simplified, rapid version of the immunoblot technique.	Equally specific and sensitive as ELISA and MIF, but more specific than CFT.
Complement Fixation Test	An older, traditional method for detecting Phase I and Phase II antibodies.	Less specific and less sensitive than IF/ELISA. Its use has been largely superseded.
Microagglutination	A simple test often used to detect early antibody response to *C. burnetii*.	Simple and sensitive for early detection; requires a relatively large amount of antigen.
Indirect Haemolytic Test	A method proposed primarily for the diagnosis of chronic Q fever.	Primarily used for diagnosing long-term infection.
Radioimmunological Test	A specialized assay based on the use of radioactively labelled components.	Historically important; generally complex and specialized.

2.5.4. Molecular methods

2.5.4.1. Polymerase Chain Reaction

Polymerase chain reaction (PCR) has been used to detect *C. burnetii* DNA or RNA in various samples including blood, serum and fresh tissues. The technique involves detecting the presence of the specific sequences in the genome of the organism in question, which in this case is *C. burnetii*. The two most frequently used specific repeated sequences for detection of *C. burnetii* are IS1111 and IS30a (Duron *et al*., 2015). Advances have also been made in such a way that lyophilization improves the sensibility of detection of *C. burnetii* by PCR since it concentrates bacterial DNA. Several established PCR diagnostic tests are used to detect *C. burnetii* DNA in clinical samples, as listed in Table 12 (Garrity *et al*., 2005). Tissue-based PCR is a highly accurate and sensitive method for detecting *C. burnetii* infection (Jones *et al*., 2006). A positive PCR assay on bodily secretions, such as birth products, vaginal secretions, milk, or feces, indicates that the animal is currently infected.

2.5.4.2. Real-time PCR

Diagnostic and research techniques have advanced and now have the capability to identify and measure the amount of *C. burnetii* DNA (Klee *et al*., 2006). When studying bacterial shedding, researchers can collect samples from individual sources such as the vagina, milk, or colostrum, as well as from the milk tank. However, identifying the livestock that are shedding the bacteria is challenging due to the uncertain patterns of shedding, as noted in studies by Rousset *et al*. (2009) and Guatteo *et al*. (2012).

2.5.4.3. Light Cycler Nested PCR

Fenollar and Raoult have adapted a nested PCR method that targets a 20-copy htpAB-related sequence which is present in multiple copies. This modified technique can diagnose both acute and chronic Q fever using serum as the sample and the Light-Cycler as the thermal cycler (Fenollar and Raoult, 2007; Stemmler and Meyer, 2002).

2.5.4.4. MLVA (multi-locus variable number of tandem repeats analysis) and multispacer sequence typing (MST)

These methods enable the characterization of *C. burnetii* without requiring the separation of the organism and are recognized as the most precise techniques for *C. burnetii*, permitting the identification of as many as 36 unique genotypes.

One of the most commonly used typing methods is multispacer sequence typing (MST), which targets multiple intergenic regions of the *C. burnetii* genome (Truong *et al*., 2022). This method has been shown to be highly discriminatory and can identify up to 36 distinct genotypes of *C. burnetii*. Other molecular typing methods for *C. burnetii* include single nucleotide polymorphism (SNP) analysis, multilocus sequence typing (MLST), and whole-genome sequencing (WGS). Molecular typing techniques have greatly contributed to our understanding of the diversity and evolution of *C. burnetii*, and have been useful in tracking the spread of outbreaks and tracing the source of infections.

Table 12. Polymerase chain reaction methods of investigation and diagnosis of *Coxiella burnetii*

Diagnostic Method	**Description and Primary Application**	**Key Strength/Utility**
DNA Amplification	Used successfully for the qualitative detection of *C. burnetii* DNA in both cell cultures and clinical specimens.	Highly sensitive for confirming the presence of the pathogen.
Real-Time PCR	Used to simultaneously detect and quantify the amount of *C. burnetii* DNA. Essential for investigating bacterial shedding in livestock.	Provides quantitative data (bacterial load) in addition to detection.
Light Cycler Nested PCR	A highly sensitive, two-step PCR method for amplifying minute amounts of target DNA.	Very efficient method for early diagnosis when bacterial load may be low.
Genotyping (MLVA and MST)	Methods such as Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) and Multispacer Sequence Typing (MST).	Extremely useful for epidemiological investigation to clarify links and trace the source of infection.

2.6. Treatment Strategies for *Coxiella burnetii* Infection

The therapeutic management of Q fever necessitates distinct approaches depending on the host (animal or human) and the stage of the disease (acute or chronic). Treatment protocols focus on reducing bacterial load, preventing severe complications, and minimizing environmental shedding (Eldin *et al*., 2016).

2.6.1. Treatment in Animal Hosts

In domestic ruminant herds where Q fever is confirmed, treatment primarily aims to reduce pathogen shedding rather than eliminate infection entirely, thereby lowering the risk of human exposure. The common practice involves administering tetracyclines (e.g., 8 mg/kg orally) via water for several weeks prior to the anticipated parturition date. While the effectiveness of this preventive approach in entirely stopping shedding is not fully established, it is intended to mitigate the massive bacterial load released in birthing products. For individual livestock, particularly during late gestation, parenteral administration of long-acting oxytetracycline (e.g., two injections of 20 mg/kg spaced 20 days apart) has been suggested to potentially prevent adverse pregnancy outcomes, though supporting clinical data remains scarce. Outside of direct animal treatment, a critical public health control measure is the pasteurisation of raw milk (63°C for 30 minutes or 72°C for 15 seconds), which is effective at eliminating *C. burnetii* and other milk-borne pathogens (Bisgin *et al*., 2011).

2.6.2. Treatment in Humans

Pharmacological management of human Q fever utilizes antibiotics capable of penetrating and acting within the acidic phagolysosome (parasitophorous vacuole) where *C. burnetii* replicates. Antibiotic therapy yields good results against the acute presentation of this infection. However, the development of the chronic form necessitates more complex treatment protocols and is frequently marked by relapse, contributing significantly to elevated mortality rates (Kovácová and Kazár, 2000).

2.6.2.1. Acute Disease

Doxycycline is the established and preferred first-line antibiotic for treating acute Q fever in adults and children over the age of eight, as well as sick children of any age (Anderson *et al*., 2013). The standard regimen for adults involves 100 mg oral doxycycline every 12 hours for 14 days. Pediatric dosing is weight-based: children under 45 kg receive 2.2 mg/kg twice daily, while those over 45 kg receive the adult dosage. While doxycycline is highly effective, other agents like the macrolides clarithromycin and roxithromycin, and certain fluoroquinolones (FQs) have shown efficacy in *in vitro* and limited clinical settings. Conversely, macrolides such as erythromycin have proven unreliable. Early detection and complete treatment are crucial to prevent progression to chronic disease (Hartzell *et al*., 2008). Special consideration is given to pregnant women, who are typically treated with trimethoprim/sulfamethoxazole 160 mg 800 mg twice daily) throughout gestation to avoid fetal risks associated with tetracyclines.

2.6.2.2. Chronic Disease

Chronic Q fever, most commonly manifesting as endocarditis or vascular infection, necessitates a significantly more prolonged and aggressive therapeutic approach due to the persistence of the bacteria. The recommended long-term treatment is a combination therapy of doxycycline (100 mg twice daily) and hydroxychloroquine (200 mg every eight hours) (Fenollar *et al*., 2001). Hydroxychloroquine is used not primarily for its direct antimicrobial effect, but because it raises the pH of the parasitophorous vacuole, thereby enhancing the bactericidal activity of doxycycline.

When doxycycline is medically contraindicated specifically in cases of drug allergy, gestation, or pediatric patients under the age of 8 the treatment of Q fever may be managed using efficacious alternatives like rifampin or cotrimoxazole. It is standard practice to administer cotrimoxazole, along with folic acid supplementation, to pregnant women for a minimum of five weeks (Tissot-DuPont *et al*., 20004; Angelakis and Raoult, 2010; Ford *et al*., 2014). This regimen requires long-term patient monitoring and periodic diagnostic testing. In cases where chronic endocarditis has resulted in severe valve damage or heart failure, surgical intervention remains a critical component of the overall treatment plan (Hartzell *et al*., 2008).

2.7. Prevention and Control Strategies

Effective control of Q fever requires a multi-faceted approach targeting the pathogen in animal reservoirs, the environment, and human populations. This strategy centers on accurate diagnosis, rigorous biosecurity, robust surveillance, and the integration of the One Health concept (Sheek-Hussein *et al*., 2025).

2.7.1. Core Prevention Measures and Biosecurity

Controlling the disease in animal populations is paramount due to the high concentration of *C. burnetii* shed by infected ruminants (Robi *et al*., 2023). Biosecurity protocols must be strictly enforced, particularly around the birthing process (Blacksell *et al*., 2025). In infected or seropositive flocks, parturition should be restricted to designated, easily sterilized areas, and all associated delivery utensils and equipment must be thoroughly disinfected immediately after use. The most critical step is the immediate and complete removal and destruction of placentas, foetuses, and all products of conception to prevent environmental contamination and consumption by domestic or wild carnivores (Zarza *et al*., 2021). Furthermore, strict hygiene is necessary for all personnel; hands must be thoroughly washed with soap and water after any contact with livestock or their bodily excretions.

While tick control is important, the primary transmission route remains aerosol, emphasizing the need to limit environmental pathogen load (Celina and Cerný, 2022). Regarding herd management, official animal quarantine and mass extermination of infected herds are not recommended, nor is the prophylactic use of tetracycline in feed during gestation due to its limited oral absorption (Reinbold *et al*., 2010).

2.7.2. Diagnostic Accuracy, Surveillance, and Vaccination

The foundation of Q fever control relies on accurate diagnosis and effective surveillance (Miller *et al*., 2021). Prompt and reliable testing is necessary to identify infected individuals and flocks, which then informs movement restrictions; for instance, livestock from seropositive flocks should only be introduced into other seropositive or vaccinated flocks after confirming the seller's flock status. Surveillance activities should monitor prevalence trends and guide vaccination programs.

While the US Food and Drug Administration has not approved a human Q fever vaccine, highly effective options exist internationally. Australia, for example, approved the whole-cell *C. burnetii* vaccine Q-Vax in 1989. This vaccine, which requires a mandatory pre-screening skin test and serology due to the risk of severe reactions in previously exposed individuals, has demonstrated high effectiveness (over 95% efficacy documented over five years) in high-risk groups such as veterinarians, abattoir workers, and laboratory professionals (Hartzell *et al*., 2008). In livestock, effective vaccines can be used to protect ruminants from developing clinical symptoms, ideally administered before their first offspring to prevent shedding during high-risk reproductive events.

2.7.3. The One Health Approach to Control of Q fever

Given that *C. burnetii* is a ubiquitous zoonotic pathogen transmitted from livestock to humans and facilitated by environmental reservoirs, the One Health approach is indispensable for effective prevention and control (Farinhas *et al*., 2024). This strategy mandates multi-sectoral cooperation between veterinary, public health, and environmental agencies to holistically manage the disease. For example, effective management of Q-fever outbreaks through One Health approach has been documented in the Netherlands. The implementation of One Health principles allows for shared surveillance data, unified risk assessment, and coordinated intervention strategies such as targeting vaccination campaigns in areas where high animal prevalence coincides with high-risk human occupations (Figure 15) (Williams-Macdonald *et al*., 2023). Early detection and treatment of acute Q fever in humans is crucial for preventing the development of severe chronic Q fever complications, particularly in vulnerable populations like pregnant women, those with compromised immune systems, or individuals with pre-existing valvular heart issues (Reeves *et al*., 2017). Ultimately, a coordinated One Health effort is essential to mitigate the risk across the entire host-environment interface (Rahaman *et al*., 2019).

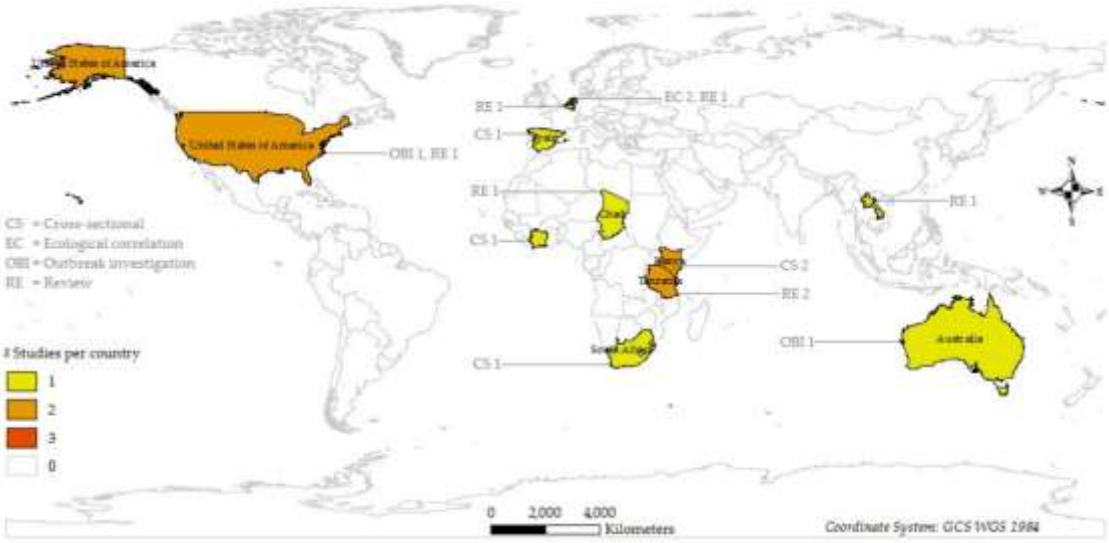


Figure 15. Distribution of studies that used a One Health approach to Q fever by location and study design.

Source: Rahaman *et al*., 2019.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

This study was carried out on livestock destined for slaughter at Addis Ababa, Adama, and Modjo abattoirs, as well as those livestock reared in the Borana pastoral zone of Oromia Regional State, as depicted in Figure 16.

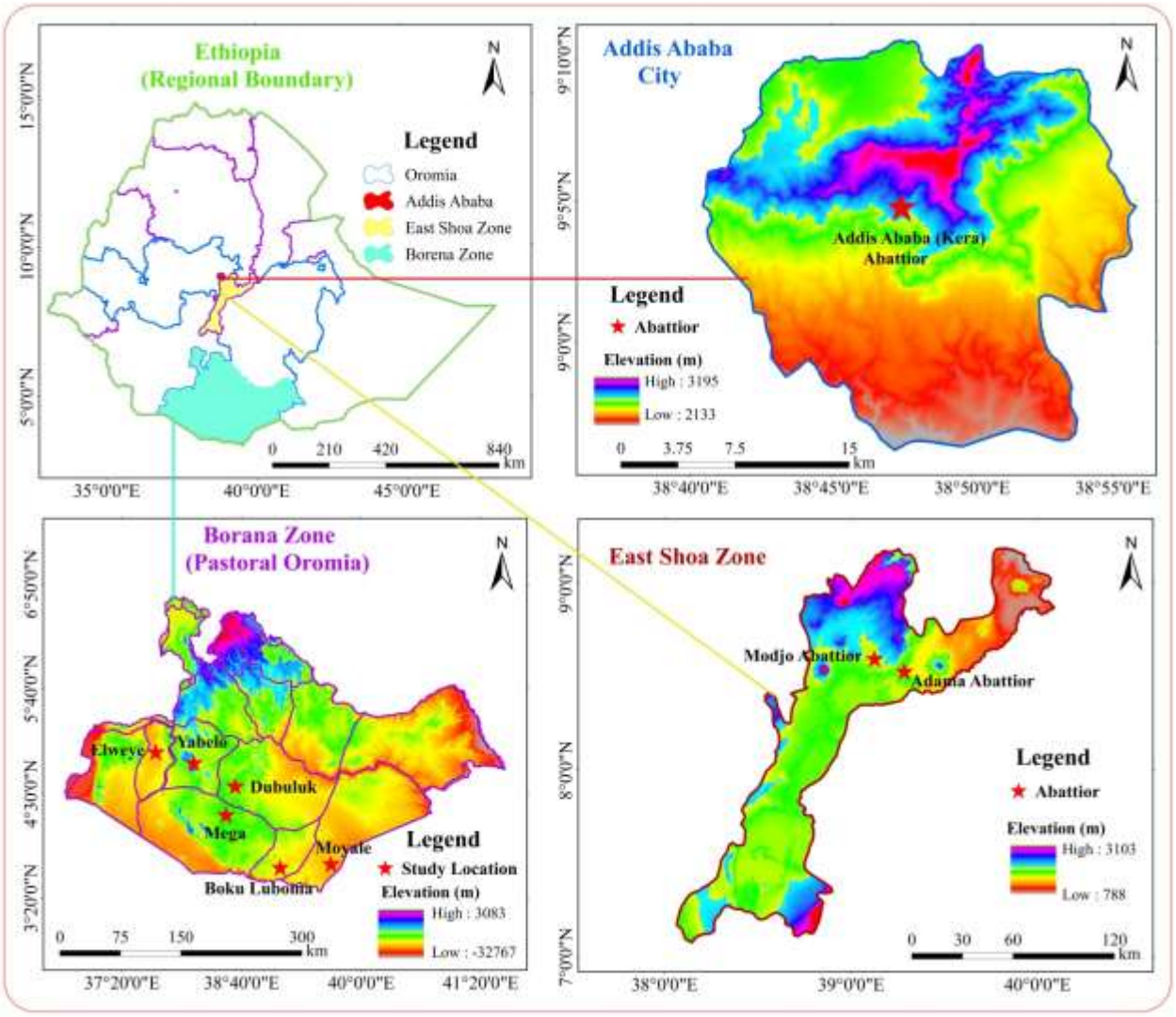


Figure 16. Map of Ethiopia showing the geographic locations of the study districts and abattoirs. The map was generated using ArcGIS 10.4.1 (Redlands, California, USA).

The Borana pastoral zone is located in the southern part of Ethiopia's Oromia Regional State. The capital of Borana Zone, Yabello, is situated 575 kilometers south of Addis Ababa. The Borana Zone has thirteen districts and share borders with Kenya in the south via Moyale, Miyo, Dirre, and Teltelle districts. For this study, six districts (Yabelo, Dubuluk, Mega, Boku Luboma, Elweye and Moyale) were chosen based on the reports of occurrence of reproductive problems such as abortion in livestock (Edao *et al*., 2020).

The Borana Zone has a semi-arid to arid climate and is geographically located between 4˚ to 6˚ N latitude and 36˚ to 42˚ E longitude, featured with isolated mountains and valleys. The altitude of the Borana Zone ranges from 1,000 to 1,700 meters above sea level, and the mean annual rainfall ranges from 250 to 700 mm. The average annual temperature in the area ranges from 19℃ to over 25℃. Pastoralism is the main means livelihood for the Borana pastoral community. The main livestock species reared in Borana zone comprises cattle, goats, sheep, and camels. The Borana Zone Pastoral Development Office reports that the zone has livestock population of 1,416,180 cattle, 1,262,782 goats, 776,870 sheep, 237, 205 camels, 306,057 poultry, 102,767 donkeys, 1,841 horses, and 4,433 mules (BZPDO, 2018). As of 2015, the human population was reported to be 1,283,925 (BZFEDO, 2015).

Adama is located in the East Shoa Zone of Ethiopia's Oromia Regional State, situated at 8.54˚N and 39.27˚E, with an elevation of 1712 meters and approximately 99 kilometers southeast of Addis Ababa. It is strategically located along the Ethio – Djibouti transaction. It is one of the largest metropolitan cities in Ethiopia, with an estimated population of 450,000 humans and many resorts that make it suitable for conferences and tourism. Adama Municipal abattoir has the capacity to slaughter approximately 150 cattle and 500 sheep and goats per day (CSA, 2016). The majority of the feed lots of the country are concentrated in and around Adama. Livestock slaughtered at Adama abattoir mostly originate from the eastern, central and south eastern parts of Ethiopia (Getaw *et al*., 2010).

The Addis Ababa abattoir enterprise is situated in Addis Ababa, the capital of the country at an altitude of 2500 meters above sea level. The average annual temperature and rainfall of Addis Ababa city is 21℃ and 1800 mm, respectively. The relative humidity of the area ranges from 70 to 80% during rainy season and 40 to 50% during dry season. According to CSA, an

average of 183,000 cattle, 42,200 sheep, 4,700 goats, and 830 pigs are slaughtered at Addis Ababa abattoir each year (CSA, 2016). Generally, the cattle, sheep, and goats that are slaughtered at Addis Ababa abattoir originate from different regions and agro-ecological zones across the country (Oljira *et al*., 2022).

Modjo is the administrative center of Lume district, which is located in the East Shoa Zone of Oromia Regional State. It is located 66 kilometers southeast of Addis Ababa, at a latitude of 8˚35'N and a longitude of 39˚7'E, with an elevation of 1790 meters above sea level. The area receives rainfall twice a year, during the long and short rainy seasons. The rainy season lasts from June to September. The average annual rainfall, temperature, and mean relative humidity of the area are 776 mm, 19.4℃, and 59.9%, respectively (CSA, 2016). Since the abattoir is export abattoir, livestock slaughtered at this abattoir originate from all corners of the country mainly Arsi, Borana, Jimma, Somali, Southern Ethiopia and South Wollo. Livestock slaughtered at Modjo abattoir were reared under diverse agro-ecologies and different husbandry systems (Teklu and Negussie, 2016). They were acquired from various local markets and transported to the abattoirs, where they were given food, water, and rest for a period ranging from 24 to 72 hours before being slaughtered (Deresa *et al*., 2013).

3.2. Study Population and Design

A cross-sectional study was conducted in two distinct phases. The initial data collection took place from January 2021 to May 2022. During this time, blood samples were collected from cattle, sheep, goats and camels at abattoirs and from pastoral area. The second phase of the study was conducted during January and February, 2025 in which biological samples such as milk, urine, vaginal swabs, aborted materials and placenta were collected from clinically affected and sero-positive livestock. In addition, ticks were collected from infested livestock for molecular detection of *C. burnetii*.

The study population comprises cattle, sheep, goat and camel population raised in the catchment areas of the abattoirs and Borana pastoral area. Cattle, sheep, goats and camels that were older than 6 months and indigenous Ethiopian breeds constitute study livestock. During the phase one of data collections, all sampled livestock were clinically healthy. Livestock

sampled during the second phase of the study were those having clinically reproductive disorders.

During the first phase a total of 4,140 livestock were sampled for serological investigation using iELISA. During the second phase 648 samples were collected for the molecular analysis. For the first phase livestock of both sexes were included while only females having reproductive problems were included during the second phase. The age of the study livestock was estimated as previously explained by Schelling *et al*. (2003). Study livestock were chosen for sampling from each pastoral area by selecting various households and their herds. In the Borana pastoral area, the majority of livestock included in the study were females as they were used for milk production purpose. In contrast, the majority of livestock sampled at abattoirs were male livestock. All the study livestock were not vaccinated against *C. burnetii*.

3.2.1. Sample size determination

For estimation of prevalence and identification of risk factors, the minimum sample size was computed using the formula developed by Thrusfield (2013) with expected prevalence of 50%, 95% confidence interval and absolute precision of 0.05 for each animal species as follows:

$$n = \frac{(1.96)^2 \times \mathrm{P}_{exp}(1 - \mathrm{P}_{exp})}{d^2} \quad (1)$$

where, n is the sample size, P_{exp} is expected prevalence (50%), d is absolute precision (0.05) at 95% CI.

$$n = \frac{(1.96)^2 \times 0.5(1 - 0.5)}{(0.5)^2} = 384$$

The study required a minimum of 1536 livestock (384×4). However, a total of 4,140 livestock sampled (1,564 cattle, 736 camels, 1,012 goats, and 828 sheep), which is more than double to increase the accuracy of the study. Of these, 1,012 samples were obtained from Addis Ababa abattoir, 1,511 from Modjo abattoir, and 237 from Adama abattoir, while 1,380 livestock were sampled from Borana pastoral areas.

For the molecular analysis component, 384 samples were randomly selected from 777 seropositive livestock. In addition, 264 biological samples including milk, urine, vaginal swab, aborted materials were collected from clinically infected livestock. Moreover, 292 tick samples were collected for molecular detection of *C. burnetii*. During each sample type collection, GPS coordinates, location names, and geographical distribution data were carefully documented.

3.3. Sample Collection and Sampling Technique

3.3.1. Sample Collection

3.3.1.1. Collection of Blood Samples

A total of 4,208 whole blood samples were collected, consisting of 4,140 samples for serological analysis and 520 blood samples for molecular analysis. The blood samples were collected from livestock early in the morning before they were taken out for grazing in case of pastoral herds and before slaughtering at abattoirs. The livestock were properly restrained (Appendix I). Then approximately 6 – 8 mL of blood samples were collected from jugular vein of each animal using sterile plain vacutainer tubes and needles. The samples were placed in a slant position overnight at room temperature to allow clotting. The next morning, the sera were separated from the blood and stored in 1.8 mL disposable screw-capped cryovials (Cryo.STM; Greiner Bio-one, GmbH Frickenhausen, Germany).

Samples were transported to the Animal Health Institute (AHI), Sebeta, Ethiopia, for serological analysis. During transportation of the samples the temperature was maintained at 4°C using a portable refrigerator that was plugged into a car. Upon arrival at the AHI laboratory the samples were stored at -20°C until they were analyzed. The serum samples were used for antibody detection through the indirect ELISA technique. For molecular analysis, collected blood samples were centrifuged to separate the serum from the cellular components where the buffy coat layer was used for molecular detection of *C. burnetii* DNA. Each sample was labeled and identified with individual identification code given to identify the host species. Information was also collected on area, sex, age, species, and tick infestation status of livestock during sample collection. The tubes containing the samples were protected from direct sunlight (Kaygisiz *et al*., 2011).

3.3.1.2. Collection of Milk Samples

A total of 71 milk samples were collected from lactating livestock, including 11 cows, 15 goats, 37 camels, and 8 sheep. During sampling, the udderof each animal was washed and dried and their teats were disinfected with 70% alcohol (Appendix 1). The first strip of milk was discarded and the sample was collected directly into sterile collection tube. Each tube was labelled with sample identification code. While collecting the milk, contact between the milk and hands were avoided. A total of 20 mL of milk was collected and kept at 4°C until testing. In the laboratory, the milk samples were centrifuged at 3,000 g for 10 min. The supernatant was discarded and 1.5 mL of the sediment/pellet was transferred to a labelled 2 mL cryotube and stored at -20ºC. The frozen specimens were transferred to AHI and processed for DNA extraction and PCR for the detection of *C. burnetii* specific DNA fragments (Kasa *et al*., 2020)**.**

3.3.1.3. Collection of Aborted Materials

Aseptically, a total of 4 aborted fetal tissues and placenta were collected from 2 goats and 2 cattle (Appendix 1) in labelled sterile universal bottles and preserved on ice and were stored at 4°C prior to processing (Jones *et al*., 2010).

3.3.1.4. Collection of Vaginal Swabs

A total of 62 vaginal swabs were collected aseptically from 18 goats, 13 cattle, 23 camels, and 8 sheep (Appendix 1). Each swab sample was placed in a labelled sterile cryogenic vial containing preservative and stored at -20°C (Sánchez-Rodríguez *et al*., 2024).

3.3.1.5. Collection of Urine Samples

A total of 59 urine samples were collected from female camels in clean and sterile labelled bottles (Appendix 1). Approximately 20 to 300 mL of urine was collected and centrifuged at 3,000 x g for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 300 µL of phosphate-buffered saline (pH 7.2).

3.3.1.6. Ticks sample collection

Ticks were collected from study livestock following proper restraint techniques to ensure animal and personnel safety (Appendix 1). Each animal was thoroughly examined for ticks on all body parts including the ears, dewlap, udders, perineum using gloves and fine-tipped tweezers for precision. Ticks were grasped near their mouthparts and removed with consistent, upward pressure to minimize the risk of breaking the specimen or releasing any infectious fluids. Care was taken to avoid twisting, jerking, or crushing the ticks during removal. In cases where mouthparts remained embedded, they were either gently extracted or left in place if removal posed a risk of injury. A total of 292 ticks were collected from all livestock included in the study and pooled per host species resulting in 30 pools. Each pool contained a maximum of 10 ticks.

Post-collection, hands and the affected skin areas were sanitized using alcohol to prevent contamination. The collected ticks were subsequently placed in sealed vials containing a 70% ethanol and glycerol solution, ensuring complete submersion to prevent desiccation. Each vial was carefully sealed and labelled with pertinent information, including host species, date of collection, location, collector's name, and the anatomical site of attachment. The samples were then transported to the AHI, Parasitology Laboratory for identification and molecular analysis.

3.3.2. Sample transport, storage and disposal

All samples were shipped to AHI by laboratory personnel trained in Biorisk management. The samples were properly packaged following the standard operating protocols (SOPs) developed by AHI to ensure sample integrity and the safety of the transporting personnel. Cryoboxes (coolers) were used to keep samples chilled. Upon arrival, the samples were accessioned and registered at the AHI sample reception unit following the sample registration SOP. The samples were then uniquely labelled and dispatched to testing laboratories. The serum samples were retained during the research period at -20°C ± 5°C while the buffy coat samples were kept at -80°C ± 5°C until tested by PCR. After completion of the assay, the samples were safely dispatched to the washing and sterilization unit of AHI, registered on the sample disposal log book, decontaminated by steam sterilization (autoclave) and properly packed and transported

to incineration facility for incineration. The transportation and disposal of the samples was carried out by experienced personnel who were trained in biological safety and Biorisk management.

3.4. Laboratory Procedures

3.4.1. Serological analysis

The commercial indirect enzyme-linked immunosorbent assay (iELISA) (IDEXX® Switzerland AG, CH3097, Liebefeld-Bern, Switzerland) was used to detect anti- *C. burnetii* antibodies in the serum samples. The antigen coated wells of the ELISA plates were incubated with the diluted serum samples according to the manufacturer's instructions (Appendix 2). Breifly, the negative and positive controls were diluted at 1:400 using wash solutions and dispensed into duplicate wells, while the serum samples were added to the remaining wells. The plate was covered and gently shaken and incubated for 60 min at 37 °C in a humid chamber.

After incubation, the wells were emptied and washed three times with 300 µL of wash solution, and then 100 µL of secondary antibody conjugate was added and incubated as described above. The plate contents were discarded, and the plate was washed three times using 300 µL of wash solution. Subsequently, 100 µL of substrate was added, and the plate was incubated at room temperature for 15 min. Finally, 100 µL of stop solution was added, and the absorbance was read at 450 nm. The Agilent BioTek ELx800 absorbance microplate Reader was employed to measure the absorbance values as Optical density (OD).

The S/P-ratio, which represents the percentage of the ratio between the sample OD and the positive control OD, was calculated as follows: Samples with an S/P percent ≥40% were considered seropositive for *C. burnetii*, while those with values between 30–40% were considered dubious, and those ≤30% were considered negative. Based on the sensitivity (99%) and specificity (98%) of the commercial ELISA kit we used, the true prevalence of *C. burnetii* infection was also calculated using the formula previously described by Habibzadeh *et al*. (Habibzadeh *et al*., 2022). The Dell desktop PC was used to take measurements with a photometer at 450 nm. The data analysis program BioTek Gen5TM 2.0 was used. The Agilent

BioTek ELx800 absorbance microplate Reader machine was utilized. Finally, it was exported to an excel sheet and the optical density value was determined (OD).

3.4.2. Molecular study method and procedure (Laboratory analyses)

3.4.2.1. Extraction of DNA

DNA was extracted from all samples (buff coat, urine, milk, aborted fetal tissues, vaginal swabs and ticks) using the Qiagen DNeasy extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions at AHI Molecular biology and Genomics laboratories (Appendix 3). The eluted bacterial DNAs were preserved at −80°C for subsequent analysis (Figure 17).

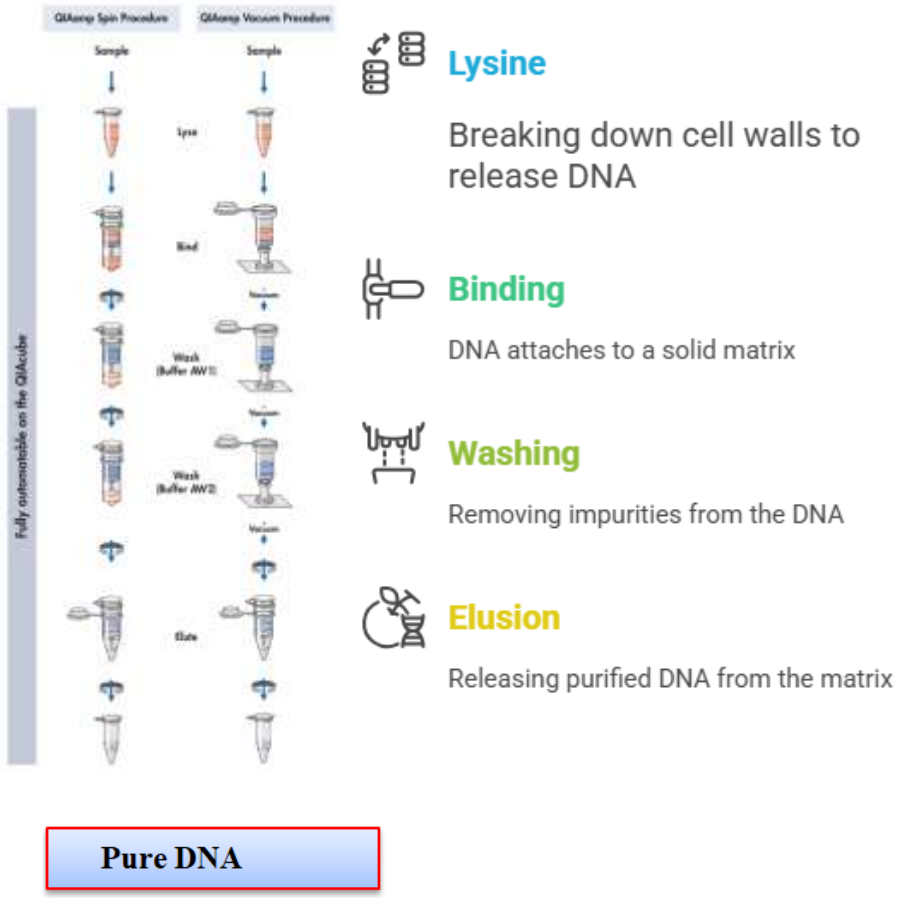


Figure 17. Basic Steps for *Coxiella burnetii* DNA Extraction from Samples Utilized in the Study (QIAamp® DNA Mini Kit Protocol).

3.4.2.2. Quantitative Real –Time Polymerase chain reaction (RT-qPCR)

The extracted DNA was analyzed by quantitative real-time polymerase chain reaction (RT-qPCR), targeting the COX1 gene of *C. burnetii*, utilizing an Applied Biosystems 7500 Fast real-time quantitative PCR (RT-qPCR) system (Applied Biosystems, USA) (Appendix 4). The primers and probes used are displayed in Table 13.

Table 13: Components and Specifications of the Oligonucleotides (Primers and Probe) Employed in the RT-qPCR Assay for *Coxiella burnetii* COX1 Gene Detection

Target Gene	**Name**	**Oligonucleotide Sequence 5'-3'**	**References**
COX1	Cox-F	GTC TTA AGG TGG GCT GCG TG	
	Cox-R	CCC CGA ATC TCA TTG ATC AGC	ADAFSA, 2024
	Cox-Probe	FAM-AGC GAA CCA TTG GTA TCG GAC GTT TAT GG-TAMRA	

The reaction was carried out in a total volume of 15 µL, comprising 0.5 µL of water, 7.5 µL of master mix, 0.8 µL of forward primer, 0.8 µL of reverse primer, and 0.4 µL of probe. All reagents were mixed thoroughly within a single reaction tube to ensure uniform distribution. Subsequently, 10 µL aliquots of this mixture were transferred to an Applied Biosystems plate, tailored to the sample count. To complete the 15 µL reaction volume, 5 µL of extracted DNA was added to each well (ADAFSA, 2024).

Known *C. burnetii* isolates, obtained from UAE, served as positive controls, while DNase-free water was used as a negative control. The plates were sealed to prevent evaporation and placed in the thermal cycler, which was connected to a computer running the Applied Biosystems sequence detection software (version 1.4.0). The PCR reaction conditions consist of 40 cycles, with a 3-minute denaturation step at 95ºC, followed by a 3-second annealing phase at 95ºC, and a 30-second extension at 60ºC. During each extension phase, the signals from the reporter dye (FAM) and quencher dye (Tamra) were recorded, allowing for the calculation of the threshold cycle (Ct) for each sample. Samples exhibiting Ct values less than 35 were classified

as positive, while those with Ct values exceeding 35 were deemed negative for *C. burnetii* (Amin *et al*., 2024).

3.5. Sample Preparation for Identification of Ticks

Ticks were cleaned using either ultrasonic cleaning or immersion in a moderate detergent solution to effectively remove surface contaminants without compromising the integrity of the specimens. Following an initial detergent wash, the ticks were transferred to a 5% sodium hydroxide (NaOH) or potassium hydroxide (KOH) solution. An ultrasonic cleaner was employed for five to ten minutes to eliminate any embedded debris. In the absence of an ultrasonic cleaner, a small, stiff artist's brush with trimmed bristles was used to manually clean the ticks while submerged in the NaOH or KOH solution (Dixon *et al*., 2000).

After cleaning, the ticks were placed in petri dishes for identification using a camera-equipped stereomicroscope. Proper personal protective equipment (PPE), including gloves and eye protection, was worn at all times when handling the ticks and chemicals. Waste disposal was conducted in accordance with the biosafety guidelines established by the Animal Health Institute to ensure safety and compliance.

3.5.1. Morphological Tick identification procedure

Tick samples were identified utilizing established taxonomic keys, specifically those outlined by Walker and Bouattour (2003) for African ticks, along with other relevant regional references. Identification was conducted using camera-equipped stereomicroscopes (Olympus SZ model) allowing for detailed examination of the specimens. Key morphological traits were assessed, including the size, shape, and coloration of the scutal ornamentation, as well as the structural characteristics of the capitulum, encompassing the basis and palps. Additionally, the segmentation and presence of spurs on the legs were noted, along with the position of the anal groove whether it was situated anteriorly, posteriorly, or was absent. The presence or absence of festoons along the posterior edge of the scutum also played a critical role in the definitive identification of each tick species (Chitimia-Dobler *et al*., 2022).

3.6. Geo-referencing (Mapping)

The livestock that were sampled at abattoirs were distributed across the areas/districts from where they originated. During the study period, they were sampled and analyzed using thematic density maps created using ArcGIS 10.0 (*Environmental Systems Research Institute,* ESRI, 2011). These maps visually represented both the distribution of the samples collected and the locations of those that tested negative for *C. burnetii*. Three thematic maps were created to show the distribution of the samples: one indicating the total number of samples, and second indicating the number of serologically-positive samples identified by using iELISA and third indicating area with geographical coordinate's serologically-positive samples identified by using iELISA and RT-qPCR negative samples.

3.7. Data Management and Analyses

The laboratory test data was filtered, edited, and entered into Microsoft® Excel, Windows 2019. The STATA software version 16.0 (Stata Corp, 4905 Lake way Drive, College Station, Texas 77845 USA) was used to analyze the data and estimate the effects of risk factors on the prevalence of *C. burnetii* infection. Multivariable logistic regression was employed to compute the associations between the risk factors and prevalence. The variables were manually fitted to the model to check for confounding. To evaluate the fitness of the logistic regression model, the Hosmer-Lemeshow and Pearson methods were used. The measure of association between prevalence and risk factors was reported as the odds ratio. The effect of clustering was evaluated using multilevel mixed-effects generalized linear models. A P value of < 0.05 and a 95% confidence interval were considered to be significant in this study. Descriptive statistics was used to summarize the results of molecular analysis.

3.8. Ethics statement (Approval)

The sampling of livestock was conducted in accordance with the protocols and guidelines set forth by Animal Health Institute (AHI) and Adama Science and Technology University (ASTU), which complies with the international laws and regulations regarding ethical considerations in research was also dully considered. This study was carried out after obtaining ethical clearance with Reference number: RECSoANS/BIO/11/2021 (Appendix 5).

CHAPTER FOUR

4. RESULTS

4.1. Seroprevalence of *C. burnetii* infection

The present study analyzed a total of 4140 sera from domestic livestock species to estimate the prevalence of anti *C. burnetii* antibodies. The overall seroprevalence of *C. burnetii* infection was 18.77% (95% CI: 17.55–19.99) (Table 14). Considering the 99% sensitivity and 98% specificity of the commercial ELISA kit we used, the estimated true prevalence of *C. burnetii* infection was 17.29%. The lowest seroprevalence was recorded in livestock from pastoral areas (12.1%; 95% CI: 10.43–13.94) while the highest prevalence observed in livestock from Addis Ababa abattoir (27.17%; 95% CI: 24.45–30.03). Analysis of the infection at the animal species level showed that cattle exhibited the lowest seroprevalence (11.83%; 95% CI: 10.27–13.53), while the highest was observed in camels (28.39%; 95% CI: 25.16–31.80). The seroprevalence was 21.34% (95% CI: 18.86–23.99) in goats and 20.17% (95% CI: 17.49–23.07) in sheep.

Table 14. The seroprevalence of *Coxiella burnetii* infection estimated in livestock species from diverse geographic localities.

Variable	Category	No. tested	No. positive	Prevalence (%)	X^2	95% CI	
						Lower	Upper
Area	Addis Ababa Abattoir	1012	275	27.17	87.32	24.45	30.03
	Modjo Abattoir	1511	289	19.13		17.17	21.2
	Adama Abattoir	237	46	19.41		14.57	25.03
	Borana pastoral areas	1380	167	12.1		10.43	13.94
Species	Cattle	1564	185	11.83	99.63	10.27	13.53
	Camel	736	209	28.39		25.16	31.8
	Goat	1012	216	21.34		18.86	23.99
	Sheep	828	167	20.17		17.49	23.07
Sex	Female	683	182	26.65	33.31	23.37	30.13
	Male	3457	595	17.21		15.97	18.51
Age	Young	1850	204	11.03	131.46	9.64	12.54
	Adult	2290	573	25.02		23.26	26.85
Tick infestation	Yes	142	115	80.99	378.48	73.55	87.08
	No	3998	621	7.05		6.52	7.59
Total		**4140**	**777**	**18.77**		**17.59**	**19.99**

Higher seroprevalence was observed in female livestock (26.65%; 95% CI: 23.37 30.13) than in male livestock (17.21%; 95% CI: 15.97–18.51). The prevalence was also higher in adult livestock (25.02%; 95% CI: 23.26–26.85) than their younger counterparts (11.03%; 95% CI: 9.64–12.54). Higher seroprevalence of *C. burnetii* infection was also observed in livestock infested with ticks (carry ticks on several body parts) (80.99%; 95% CI: 73.55–87.08) than those livestock which were not infested by ticks at all (7.05%; 95% CI: 6.52–7.59).

4.2. Geo-referencing the seroprevalence of *C. burnetii* in Abattoirs

Abattoirs serve as a valuable source of information on the prevalence of *C. burnetii* infection in different regions of the country, as the livestock that are slaughtered there come from various parts of the country. In order to provide insight into the distribution of seroprevalence results across the country, the data obtained from the livestock slaughtered at different abattoirs were

geo-referenced by thematic density maps created by using QGIS 10.0 and depicted in Figure 18. This spatial information is critical in identifying areas with a higher risk of *C. burnetii* infection, which may serve to inform targeted interventions to mitigate the spread of the bacterium and reduce the risk of transmission to humans. Consequently, these data may play a pivotal role in enhancing public health outcomes and reducing the burden of zoonotic diseases.

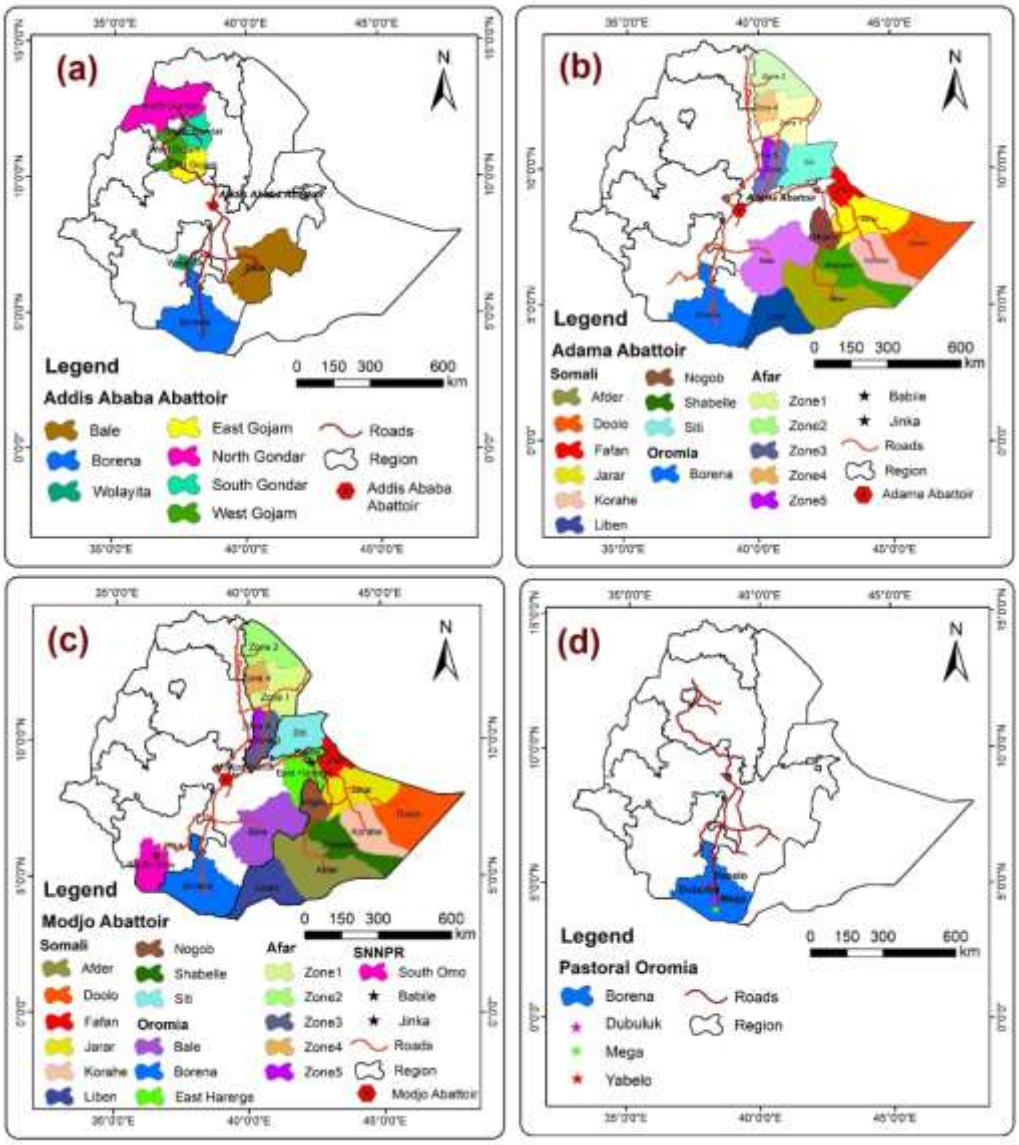


Figure 18. The serum samples collected from livestock were systematically categorized according to their respective sampling areas and sources. The four sampling areas were as follows: (a) Addis Ababa Abattoir (AAA), which is located in the capital city of Ethiopia, (b) Adama Abattoir (AA), which is situated in the city of Adama, (c) Modjo Abattoir (MA), located in the town of Modjo, and (d) Pastoral Oromia (PO), which is a pastoral region in Ethiopia.

In the context of Addis Ababa Abattoir (AAA), livestock (sample size, 1012) were predominantly sourced from three distinct regions, which are Amhara region / north western part of Ethiopia (East and West Gojam and North and South Gondar zones), Oromia region (south eastern part mainly Bale and Borana zones), and Southern Ethiopia region (Wolayita zone). These regions were identified and are graphically represented in Figure 18A. For Adama abattoir (AA) and Modjo abattoir (MA) livestock originate from four regions including Somali, Oromia, Afar, Southern Ethiopia, as illustrated in Figure 18B and Figure 18C, respectively. These regions were identified and mapped to provide a comprehensive understanding of the geographical origins of the livestock. In the Borana Pastoral zone of Oromia, livestock were sampled from Dubuluk, Mega, and Yabelo districts, as depicted in Figure 18D.

This geographical information clearly showed that infection with *C. burnrtii* is widely distributed through Ethiopia except the western and north western parts, from where livestock were not sampled. The widespread infections in livestock pose a risk to human health. By utilizing this data, targeted interventions may be designed to mitigate the risk of zoonotic disease transmission, thereby improving public health outcomes. The prevalence was found to be higher in livestock at the Addis Ababa Abattoirs than those at the other two Abattoirs and pastoral region. The tested sample size and prevalence in case of Addis Ababa Abattoir were 1012 (27.12%), Adama Abattoir 237 (19.41%), Modjo Abattoir 1511 (19.13%) and Borana Pastoral area 1380 (12.1%), respectively (Figure 19).

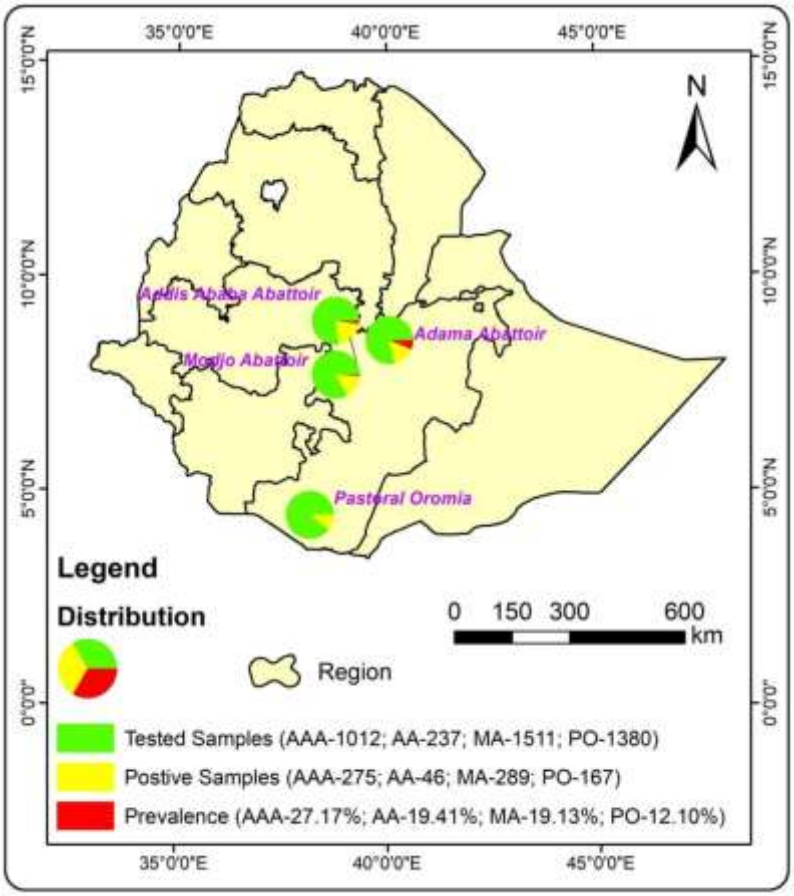


Figure 19. The seropositivity of livestock tested for *Coxiella burnetii* evaluated using the iELISA in four distinct sampling regions: Addis Ababa Abattoir (AAA), Adama Abattoir (AA), Modjo Abattoir (MA), and Pastoral Oromia (PO). The study presented the distribution of samples tested, positive, and prevalence percentage, which were represented using different colors.

4.3. Analysis of risk factors of *C. burnetii* infection

Statistically significant association ($p < 0.001$) was observed between livestock species and the seroprevalence of *C. burnetii* (Table 15). The odds of infection in camels were 3.22 (95% CI: 2.57–4.05) times that of cattle. Sheep and goats were twice more likely to be infected with *C. burnetii* than cattle (for sheep, OR = 2.15; 95% CI: 1.63–2.82; for goats, OR = 1.99; 95% CI: 1.53–2.6). Adult livestock had higher odds of infection than younger livestock (OR =

3.23; 95% CI: 2.63–3.96; p < 0.001). Those livestock infested with ticks were more likely to be seropositive to *C. burnetii* than livestock which were free of ticks. The odds of infection in livestock carrying one or more ticks were 16.32 times (95% CI: 10.17–26.18) the odds of infection in livestock that were not infested by ticks. Additionally, a higher prevalence was observed in the female livestock compared to male livestock, however, the difference in prevalence between the two sexes was not statistically significant (OR = 0.78; 95% CI: 0.59–1.02). The results of the multivariable logistic regression analysis are presented in Table 15.

Table 15. Results of a multivariable logistic regression analysis examining the association between various variables and seroprevalence of *Coxiella burnetii* infection in livestock in Ethiopia

Variables	**Category**	**OR**	**SE**	**Z**	**p-value**	**95% CI**	
						Lower	**Upper**
Species	Cattle	Ref					
	Camel	3.22	0.374	10.09	0.000	2.56835	4.04817
	Goat	1.99	0.27	5.11	0.000	1.5315	2.60322
	Sheep	2.15	0.299	5.5	0.000	1.63525	2.82048
Sex	Female	Ref					
	Male	0.78	0.108	-1.81	0.071	0.59126	1.02174
Age	Young	Ref					
	Adult	3.23	0.337	11.24	0.000	2.63228	3.96245
Tick infestation	Yes	16.32	3.935	11.58	0.000	10.1726	26.1781
	No	Ref					
	Constant	0.07	0.012	-15	0.000	0.04674	0.09504

OR, Odds Ratio; CI, Confidence Interval; Ref., Reference; SE, Standard Error; Z, z-score.

4.4. Detection of *C. burnetii* using RT-qPCR in serum samples from seropositive samples

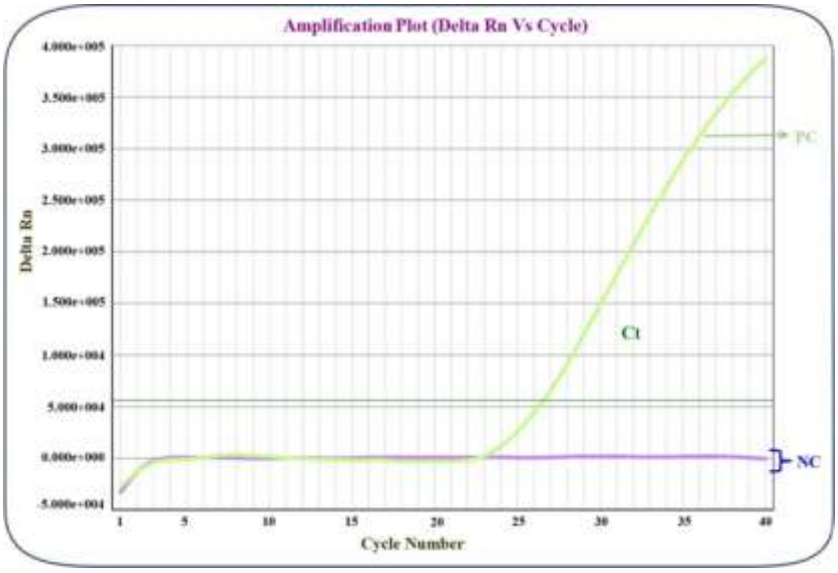
Out of a total of 4,140 serum samples tested, 777 (18.77%; 95% CI: 17.59 – 19.99) were identified as positive using the iELISA method. Of the 777 positive samples, 384 of them randomly selected and analyzed with Real-Time qPCR. None of them showed positive result

for *C. burnetii* DNA. The results of serological and molecular analysis are given in Table 16. In addition, the result of molecular analysis is depicted in Figure 20.

Table 16. Results of serological test and molecular analysis using Real-Time qPCR on samples collected from livestock species at abattoirs and from pastoral areas

Variable	Category	No. tested by iELISA	No. Positive by iELISA	No. Tested by RT-qPCR	No. Positive by RT-qPCR	Ct reading for COX1
Controls	Positive Control	0 (N/A)	0 (N/A)	1	1 (100.0%)	23.3
	Negative Control	0 (N/A)	0 (N/A)	1	0 (0.0%)	Not amplified
Area	Addis Ababa Abattoir	1012 (24.4%)	275 (27.2%)	90 (23.4%)	0 (0.0%)	Not amplified
	Modjo Abattoir	1511 (36.5%)	289 (19.1%)	90 (23.4%)	0 (0.0%)	Not amplified
	Adama Abattoir	237 (5.7%)	46 (19.4%)	46 (12.0%)	0 (0.0%)	Not amplified
	Borana Pastures	1380 (33.3%)	167 (12.1%)	158 (41.2%)	0 (0.0%)	Not amplified
Species	Cattle	1564 (37.8%)	185 (11.8%)	90 (23.4%)	0 (0.0%)	Not amplified
	Camel	736 (17.7%)	209 (28.4%)	102 (26.6%)	0 (0.0%)	Not amplified
	Goat	1012 (24.4%)	216 (21.4%)	102 (26.6%)	0 (0.0%)	Not amplified
	Sheep	828 (20.0%)	167 (20.1%)	90 (23.4%)	0 (0.0%)	Not amplified
Sex	Female	683 (16.5%)	182 (26.7%)	180 (46.9%)	0 (0.0%)	Not amplified
	Male	3457 (83.5%)	595 (17.2%)	204 (53.1%)	0 (0.0%)	Not amplified
Age	Young	1850 (44.7%)	204 (11.0%)	194 (50.5%)	0 (0.0%)	Not amplified
	Adult	2290 (55.3%)	573 (25.0%)	190 (49.5%)	0 (0.0%)	Not amplified
Tick Infestation	Yes	142 (3.4%)	115 (80.9%)	115 (30.0%)	0 (0.0%)	Not amplified
	No	3998 (96.6%)	621 (15.5%)			
Total		**4140 (100%)**	**777 (18.8%)**	**384 (100%)**	**0 (0.0%)**	Not amplified

A)



B)

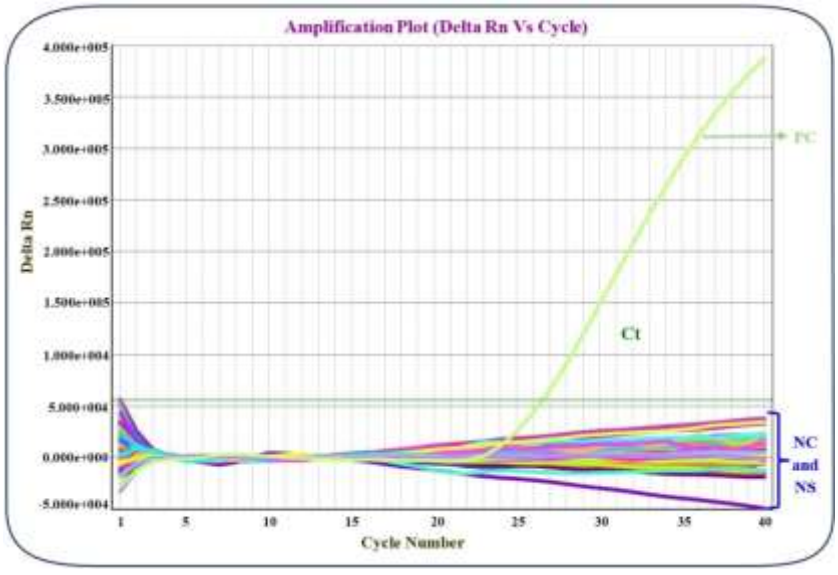


Figure 20. The graphs generated from the Real-Time quantitative PCR analysis. A) RT-qPCR amplification curve displays the positive and negative controls. B) RT-qPCR amplification curve, both the positive and negative controls are shown alongside the samples. This comprehensive depiction underscores the validity of the Real-Time qPCR procedures for *Coxiella burnetii*. Where NC and PC represent the

negative and positive controls, respectively. NS indicates a negative sample, and Ct stands for cycle threshold.

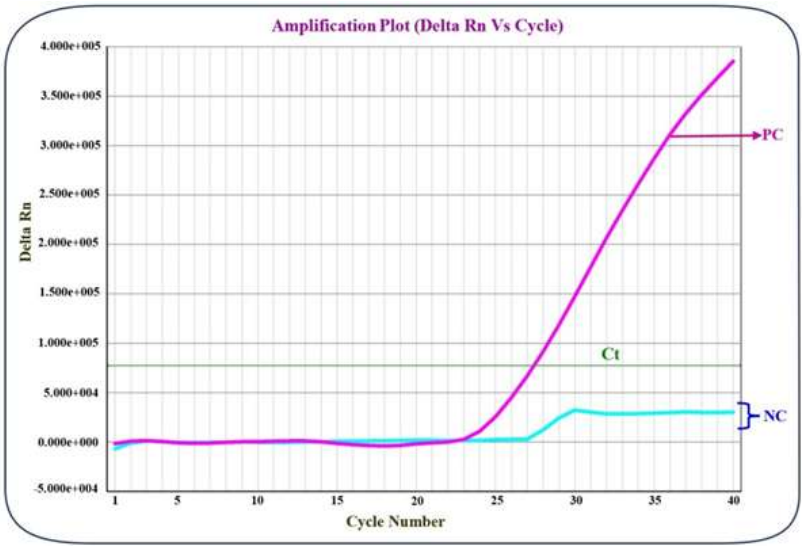
4.5. Detection of *C. burnetii* using RT-qPCR in biological samples collected from pastoral areas

Table 17 and Figure 21 present the results of molecular analysis using RT-qPCR on various biological samples collected from livestock species having different reproductive problems from pastoral areas. Out of 264 samples from cattle, camels, goats, and sheep analyzed, none of them showed positive results to *C. burnetii* COX1 gene.

Table 17. Results of analysis of biological samples collected from domestic livestock with various reproductive disorders from pastoral area using RT-qPCR

Variable	Category	No. tested by RT-qPCR	No. Positive by RT-qPCR	Ct reading for COX1
Controls	Positive Control	1	1 (100.0%)	23.5
	Negative Control	1	0 (0.0%)	Not amplified
Area	Elweye	94 (35.6%)	0 (0.0%)	Not amplified
	Boku Badiya	91 (34.5%)	0 (0.0%)	Not amplified
	Moyale	79 (29.9%)	0 (0.0%)	Not amplified
Species	Cattle	33 (12.5%)	0 (0.0%)	Not amplified
	Camel	99 (37.5%)	0 (0.0%)	Not amplified
	Goat	87 (32.9%)	0 (0.0%)	Not amplified
	Sheep	45 (17.0%)	0 (0.0%)	Not amplified
Sample Type	Milk	71 (26.9%)	0 (0.0%)	Not amplified
	Urine	59 (22.4%)	0 (0.0%)	Not amplified
	Vaginal Swabs	62 (23.5%)	0 (0.0%)	Not amplified
	Aborted Fetal Tissues	4 (1.5%)	0 (0.0%)	Not amplified
	Whole Blood	68 (25.8%)	0 (0.0%)	Not amplified
Sex	Female	241 (91.1%)	0 (0.0%)	Not amplified
	Male	23 (8.9%)	0 (0.0%)	Not amplified
Age	Young	186 (70.5%)	0 (0.0%)	Not amplified
	Adult	78 (29.5%)	0 (0.0%)	Not amplified
Tick Infestation	Yes	177 (67.0%)	0 (0.0%)	Not amplified
	No	87 (33.0%)		Not amplified
Total		**264 (100%)**	**0 (0.0%)**	Not amplified

A)



B)

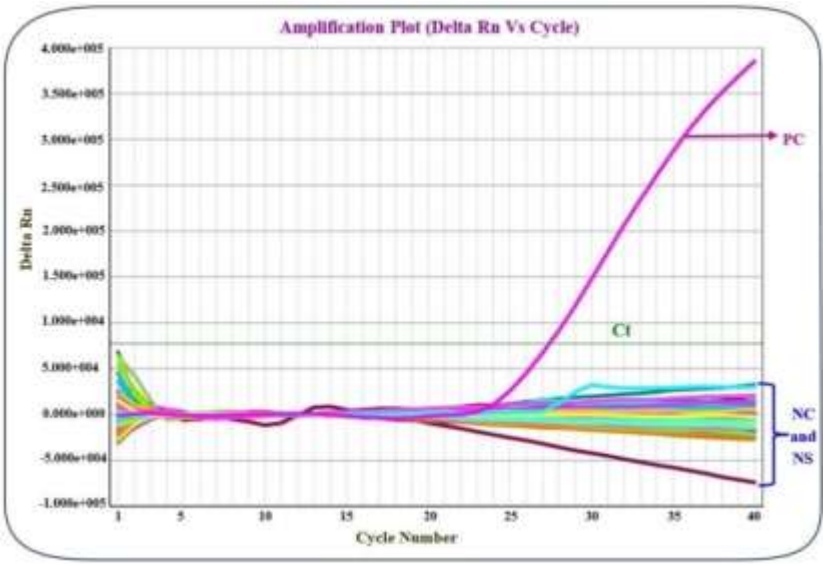


Figure 21. The graphs generated from RT-qPCR analysis on biological samples collected from cattle, camels, sheep and goats from Borana pastoral areas. A) RT-qPCR amplification curve displays the positive and negative controls used in the assay. B) RT-qPCR amplification curve presents both positive and negative controls alongside the samples analyzed in this study. Where

NC and PC represent the negative and positive controls, respectively. NS indicates a negative sample, and Ct stands for cycle threshold.

4.6. Prevalence of Tick Infestation in Study Livestock and Species Composition

Out of 292 ticks collected from the study livestock, three tick genera: *Amblyomma*, *Hyalomma*, and *Rhipicephalus* were identified (Figure 22). Tick infestation rates varied significantly among the different host species (Table 18). Camels had the highest infestation rate, with 143 ticks (49.0% of the total collection) followed by goats (79 ticks, 27.1%), sheep (47 ticks, 16.1%), and cattle, which had the lowest infestation rate (23 ticks, 7.9%). Overall, the most prevalent tick species was *Rhipicephalus pulchellus*, accounting for 89 ticks or 30.5% of the total collection. The next most common species were *Amblyomma variegatum* (45 ticks, 15.4%) and *Amblyomma lepidum* (44 ticks, 15.1%). Conversely, *Rhipicephalus pravus* was the least common species, with only 3 ticks identified.

Amblyomma lipidium

Amblyomma variegatum

Amblyomma cohaerens

Amblyomma gemma

Rhipicephalus pulchellus

Rhipicephalus pravus

Rhipicephalus evertsi

Hyalomma truncatum

Figure 22. Ticks species identified by stereomicroscopy. Images of ixodid (hard) ticks that infest cattle, sheep, goats, and camels in the Borana pastoral region of Ethiopia.

Table 18. Distribution and prevalence of tick species on different livestock hosts in the study area.

Tick Species	**Camel (n=143)**	**Goat (n=79)**	**Sheep (n=47)**	**Cattle (n=23)**	**Total (n=292)**
Rhipicephalus pulchellus	72 (50.3%)	8 (10.1%)	7 (14.9%)	2 (8.7%)	89
Amblyomma variegatum	7 (4.9%)	20 (25.3%)	10 (21.3%)	8 (34.8%)	45
Amblyomma lipidium	4 (2.8%)	20 (25.3%)	15 (31.9%)	5 (21.7%)	44
Rhipicephalus evertsi	7 (4.9%)	16 (20.3%)	9 (19.1%)	4 (17.4%)	36
Hyalomma dromedarii	30 (21.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	30
Amblyomma gemma	15 (10.5%)	4 (5.1%)	2 (4.3%)	1 (4.3%)	22
Amblyomma cohaerens	4 (2.8%)	8 (10.1%)	2 (4.3%)	1 (4.3%)	15
Hyalomma truncatum	4 (2.8%)	0 (0.0%)	2 (4.3%)	2 (8.7%)	8
Rhipicephalus pravus	0 (0.0%)	3 (3.8%)	0 (0.0%)	0 (0.0%)	3
Total Ticks	**143 (100%)**	**79 (100%)**	**47 (100%)**	**23 (100%)**	**292**

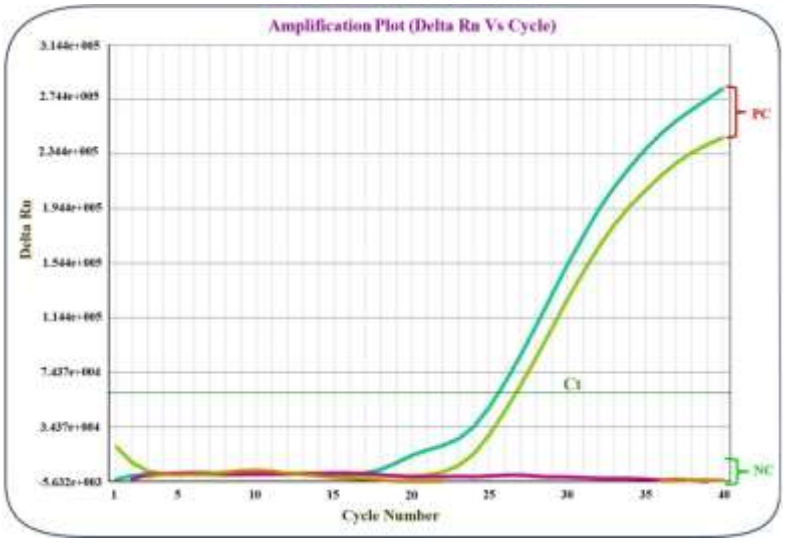
4.7. Molecular Detection of *C. burnetii* in Ticks Using RT-qPCR

Table 19 and Figure 23 present the RT-qPCR results for detection of *C. burnetii* in ticks collected from livestock species. None of the 292 ticks analyzed showed positive result for COX1 gene of *C. burnetii*.

Table 19. Results of detection of *C. burnetii* COX1 gene in ticks collected from Livestock species in the study areas

Variable	Category	No. tested by RT-qPCR	No. Positive by RT-qPCR	Ct reading for COX1
Controls	Positive Control	2	1 (100.0%)	19 and 22
	Negative Control	2	0 (0.0%)	Not amplified
Area	Addis Ababa Abattoir	40 (13.7%)	0 (0.0%)	Not amplified
	Modjo Abattoir	40 (13.7%)	0 (0.0%)	Not amplified
	Adama Abattoir	40 (13.7%)	0 (0.0%)	Not amplified
	Borana pastures	172 (58.9%)	0 (0.0%)	Not amplified
Species	Cattle	23 (7.9%)	0 (0.0%)	Not amplified
	Camel	143 (49%)	0 (0.0%)	Not amplified
	Goat	79 (27%)	0 (0.0%)	Not amplified
	Sheep	47 (16.1%)	0 (0.0%)	Not amplified
Sex	Female	222 (76.1%)	0 (0.0%)	Not amplified
	Male	70 (23.9%)	0 (0.0%)	Not amplified
Age	Young	183 (62.7%)	0 (0.0%)	Not amplified
	Adult	109 (37.3%)	0 (0.0%)	Not amplified
Total		**292**	**0 (0.0%)**	Not amplified

A)



B)

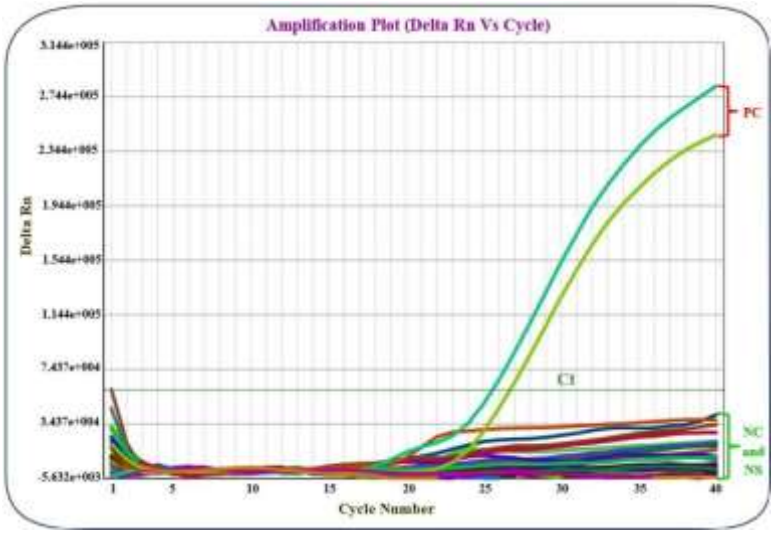


Figure 23. The graphs generated from RT-qPCR analysis of tick samples. A) RT-qPCR amplification curve of this figure shows the positive and negative controls used in the analysis. B) RT-qPCR amplification curve presents the results for the tick samples analyzed along with the positive and negative controls.

4.8. Geospatial Mapping of Molecular Detection for *C. burnetii*

Figure 24 illustrates the geo-referencing results for all study sites. This includes the initial sampling areas of Adama, Modjo, and Addis Ababa (Kera) Abattoirs, as well as the Borana pastoral area including Mega, Dubuluk, Yabello, Elweye, Boku Luboma, and Moyale.

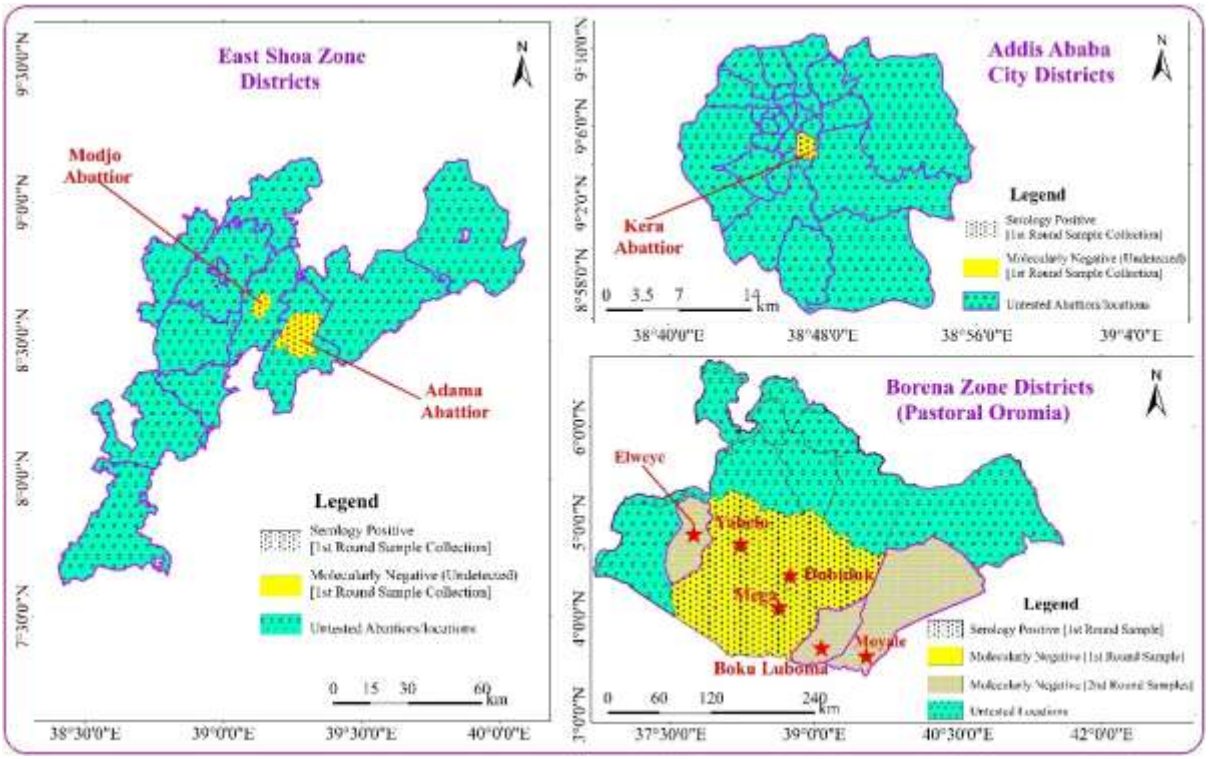


Figure 24. Geo-Referencing of samples collected including their geographical coordinates. It visually maps the locations of the sampling sites, which encompass various abattoirs and pastoral regions in Ethiopia. The figure provides a clear representation of the distribution of the collected samples, facilitating an understanding of the geographic context of the molecular detection of *C. burnetii*.

CHAPTER FIVE

5. DISCUSSION

The close interaction between humans and livestock and consumption of raw or undercooked animal products are public health challenges in Ethiopia, which heightens the risk of zoonotic diseases such as Q fever (Tsegaye *et al*., 2022). The prevalence of *C. burnetii* infection is largely unknown in livestock species and there are no control interventions in place in the country. On the other hand, there are widespread occurrence of abortion and infertility in domestic ruminant population (Alemayehu *et al*., 2021), where cases of endocarditis have also been reported in humans (Moges *et al*., 2015). As the differential diagnosis of reproductive problems has not been established particularly in pastoral and smallholder mixed-production systems, the occurrence of coxiellosis cannot be ruled out (Robi *et al*., 2023). This study presents data from cross-sectional observation that explored seroprevalence of *C. burnetii* infection and attempted the detection of *C. burnetii* DNA in various biological samples from four livestock species originated from diverse geographical locations.

The present study estimated the seroprevalence of coxiellosis and identified important risk factors of infection in four livestock species at major abattoirs (originating from various parts of the country) and Borana pastoral area of Ethiopia. Additionally, diverse biological materials including milk, urine, vaginal swabs, aborted fetal tissues, and blood (buffy coat) were tested for detection of *C. burnetii* DNA. Molecular detection of *C. burnetii* was also carried out in ticks infesting domestic livestock species. Understanding of the infection in livestock is crucial for elucidation of the epidemiology of the disease (Mwololo *et al*., 2022).

The results of serological investigation provide valuable information on the epidemiology of *C. burnetii* in livestock species of significance to Ethiopia. The study encompasses Borana pastoral area where diverse livestock species and wildlife share grazing areas and water resources, which is ideal for transmission of infectious agents among multiple hosts. The abattoir survey was conducted 56 years after the first evidence of the disease was reported in goats and sheep slaughtered at the Addis Ababa abattoir (Philip *et al*., 1966). The abattoir

study also focused on livestock that originate from diverse geographical areas of the country. The findings of present study revealed that serological evidence of coxiellosis is prevalent in domestic ruminant species and camels tested across a wider geographical area. Although human samples were not included in this study, the widespread occurrence of infection in domestic livestock suggest a public health risk given the close interaction between humans and livestock and habit of consumption of raw/under cooked animal products. It has also been shown that high herd level seroprevalence is associated with higher public health risks (Konputtar *et al*., 2024).

Overall, a seroprevalence of 18.77% (95% CI: 17.59–19.9%) with varying levels among the tested livestock species was revealed in the present study from various locations in Ethiopia. This is quite considerable as nearly 1 in 5 livestock are infected by the bacterium. Investigators from different countries reported variable seroprevalence in different animal species. Thus, our finding is in consent with the 20% prevalence reported from south-western Ethiopia using similar serological methods (Proboste *et al*., 2021). However, our observation is lower when compared to the results of some of the previous studies such as the seroprevalence reported by Gumi *et al*. (31.6%) and Tesfaye *et al*. (28.46%) among pastoral livestock species in southern part of Ethiopia (Gumi *et al*., 2013; Tesfaye *et al*., 2020). Lower seroprevalence of coxiellosis has also been documented elsewhere in the world including Albania (Cekani *et al*., 2008), Kenya (Mwololo *et al*., 2022), and Republic of South Africa (Gummow *et al*., 1987) with 9.1%, 12.80% and 7.78% %, respectively.

The variation observed in the seroprevalence between our study and the previous reports could be attributed to differences in sample size, sampling methodology, and the sensitivity and specificity of the diagnostic tests utilized. Although some of the previous studies used similar serological tests, the studies were conducted on small sample sizes (Mwololo *et al*., 2022; Muema *et al*., 2022). Besides, differences in the agro-ecological conditions, geographical locations and livestock management practices could have contributed to the observed difference in seroprevalence (Selim *et al*., 2023). Certain areas are highly vulnerable to airborne spread of *C. burnetii* (Mutisya *et al*., 2025). Similarly, previous studies revealed that seroprevalence varies depending on the species of livestock studied, geographical location and

the diagnostic tests used (Abbass *et al*., 2020). Alkenani and his colleagues (2024) demonstrated the effects of geographical locations on the seroprevalence of Q fever in livestock in Saudi Arabia. It is, therefore, crucial to consider these factors when interpreting the results of serological studies on the prevalence of *C. burnetii* infection in animal populations.

In this study significantly higher seroprevalence of *C. burnetii* infection was observed in camels, goats, and sheep than cattle. In consent to our findings, previous studies have demonstrated significantly higher seroprevalence of *C. burnetii* infection in camels than in other livestock species in Kenya (Schelling *et al*., 2003), the Sahel region (Muturi *et al.*, 2021), Saudi Arabia (Alkenani *et al*., 2024), UAE (Sheek-Hussein *et al*., 2025) and Ethiopia (Gumi *et al*., 2013). This suggests that camels and small ruminants serve as important reservoir of *C. burnetii* to humans in the area. In contrast, significantly higher seroprevalence was reported in goats than cattle, sheep and camels in Kenya (Mutisya *et al*., 2025).

The variation in seroprevalence among different animal species could be attributed to differences in susceptibility to *C. burnetii* infection, which can vary based on several factors including the immune system, age, sex, and management practices (Eldin *et al*., 2003). Previous studies have revealed variations in seroprevalence among different animal species, where transmission dynamics and environmental factors differ (Dhaka *et al*., 2020, Mutisya *et al*., 2025). The higher seroprevalence in camels may be attributed to several factors. Camels are known to have longer lifespans (~40 years) than other livestock species, and therefore, they may have greater exposure to *C. burnetii* (Hoffman and Valencak, 2020). The possible role of camels in serving as a source of infection due to longer longevity and persistence of infection has been discussed recently (Sheek-Hussein *et al*, 2025). In addition, Borana pastoralists are known for cattle husbandry while camel herding is relatively recent practice. Hence there is poor camel hygiene and management exposing them to infection (Wondimu *et al*., 2023).

The prevalence recorded in sheep and goats is consistent with the results of previous studies conducted in Ethiopia and elsewhere in the world (Knobel *et al*., 2013; Gumi *et al*., 2013; Klaasen *et al*., 2014; Mohabati Mobarez *et al*., 2021; Rahravani *et al*., 2022). The lower

seroprevalence in cattle observed in the present study is also in agreement with the reports of previous studies (Larson *et al*., 2019; Barlozzari *et al*., 2020). Similarly, lower prevalence was reported in cattle from few African countries such as Togo and Chad (Schelling *et al*., 2003; Dean *et al*., 2013). However, higher seroprevalence is reported from other countries by Adesiyun *et al*. (1984) who reported prevalence of 59.8% in dairy cows from Nigeria, Kelly *et al*. (1993) who recorded 39% in cattle and 10% in goats from Zimbabwe and Hussien *et al*. (2017) who reported prevalence of 64.5% in camels and 29.9% in cattle from Sudan.

In our study, we observed a significantly higher seroprevalence of *C. burnetii* infection in adult livestock (OR = 3.23; $P < 0.001$) than in younger livestock. This finding is in agreement with the results of several previous studies (Gumi *et al*., 2013; Larson *et al*., 2019; Mwololo *et al*., 2022; Robi *et al*., 2023). This is supported by previous studies demonstrating a higher prevalence of *C. burnetii* infection in livestock with a longer exposure history (Knobel *et al*., 2013).

The higher seroprevalence of *C. burnetii* infection in older livestock may be attributed to the cumulative exposure to the pathogen over time. Older livestock have a higher likelihood of being exposed to the pathogen through contact with infected livestock or via tick infestation and repeated exposure may increase the likelihood of infection and development of antibodies. It could also be attributed to the pathogen's ability to infect the reproductive tissues of livestock, leading to a higher dissemination and impact in older livestock, which have a longer reproductive history (Celina and Cerný, 2022). The association of anti- *C. burnetii* with age suggests the exposure dynamics might vary depending on environmental factors or husbandry practices and highlight the need for targeted control intervention such as vaccination and biosecurity measures to young livestock than adult livestock.

Our study demonstrated a significant difference in the seroprevalence of *C. burnetii* infection between livestock infested with ticks and those which were tick free. This observation is consistent with the reports of previous studies suggesting the possible role of ticks in the transmission and spread of the bacterium (Körner *et al*., 2020; Celina and Cerný, 2022). Korner and colleague (2021) extensively reviewed the role of ticks in the transmission of *C. burnetii*

including experimental studies. Results of 72 molecular prevalence studies testing more than 25 tick species from 23 European countries supported transmission of *C. burnetii* by ticks. The involvement of ticks in the transmission of *C. burnetii* has also been documented by different scholar (Olivier *et al*., 2021; Celina and Cerny, 2022; Ronald *et al*., 2022). In south western Ethiopia, significant association was observed between seroprevalence of *C. burnetii* infection and age of cattle (Robi *et al*., 2023). Investigators from other countries also revealed that tick infestation was significantly associated with seroprevalence of *C. burnetii* infection in small ruminant populations in Pakistan (Amin *et al*., 2022).

Our study revealed a higher seroprevalence of *C. burnetii* infection in livestock slaughtered at Addis Ababa abattoir. This could be attributed to the fact that Addis Ababa abattoir receives livestock from all corners of the country mainly from the three largest regions where over 70% of the national livestock are located. This observation implies that Addis Ababa abattoir is a high-risk area for the transmission of *C. burnetii* to abattoir workers and the community. Implementation of biosecurity measures such as personal protective equipment is needed. Moreover, there should be screening of livestock before slaughter for zoonotic diseases including *C. burnetii* infection in order to protect the public health (Woldeyohannes *et al*., 2018).

The seroprevalence recorded in this study at abattoirs and pastoral districts is comparable to results of most of the previous studies conducted in various countries of Africa and the world. Although definitive epidemiological information is lacking, *C. burnetii* may result in considerable economic losses and public health impacts (WOAH, 2018). In domestic livestock Q fever has been shown to result in reproductive disorders as described elsewhere in this manuscript. Since linked human study was not carried out in this study to evaluate the public health of *C. burnetii*, it is difficult to explain the public health risk but possible to suggest the threat.

The geographical information clearly showed that infection with *C. burnetii* is widely distributed throughout Ethiopia except the western and north western parts, from livestock were not sampled. The widespread infection in livestock poses a risk to human health. By

utilizing this data, targeted interventions may be designed to mitigate the risk of zoonotic disease transmission, thereby improving public health outcomes. The prevalence was found to be higher in livestock at the Addis Ababa abattoirs than those at the other two abattoirs suggesting the exposure of livestock to the bacterium from all corners of the country. This has important implication for the public health as contact with livestock, their secretion and execration can transmit *C. burnetii*. Both raw and pasteurized were show to harbor *C. burnetii* (Ahmed *et al*., 2025). The wide spread occurrence of exposure to *C. burnetii* in livestock from diversified geographical locations could be attributed to animal husbandry without access to facilities of hygiene regimes.

None of the samples analyzed using COX1 RT-qPCR showed positive results. This observation is consistent with previous reports of Kumsa and his colleagues (2015) from western and central Oromia who did not detect *C. burnetii* DNA in ticks and that of Selmi *et al*. (2019) from Tunisia. This could be due to lower bacterial load in the infected livestock, which cannot be detected by COX1 RT-qPCR. Similarly, about 23 countries in Europe were studied and half of the studies conducted on ticks did not detect *C. burnetii* DNA with clear geographical variation and variability in abundance of tick species (Korner *et al*., 2021). In contrast, several studies carried out elsewhere such as Egypt, Saudi Arabia, and Iran reported *C. burnetii* DNA in 46%, 15.9%, and 10.76%, respectively, of blood samples using molecular techniques (Doosti *et al*., 2014; Mohammed *et al*., 2014; Abdullah *et al*., 2018).

The occurrence of *C. burnetii* has also been previously reported in 6.4% and 10.8% of ticks in Ethiopia and elsewhere in Africa including 5.5% in Uganda, 5.53% in Kenya, 2.5% in rural Western Kenya (Knobel *et al*., 2013; Hornok *et al*., 2014; Kumsa *et al*., 2015; Koka *et al*., 2018; Eneku *et al*., 2024). Hence, geographical location and species of ticks tested could be responsible for the variations in the detection of *Coxiella* DNA in ticks.

Despite the critical importance of milk as a vehicle for transmission of *C. burnetii*, the RT-qPCR analysis revealed no genetic material of the pathogen in any of the milk samples collected. This finding contradicts the reports from other countries where *C. burnetii* has been detected in milk samples with prevalence of 10% in Lebanon, 4% in Hungary, 6.5% in Turkey,

1.4% in Iran, and 22% in Spain (Rahimi *et al*., 2010; Shujat *et al*., 2024). The difference observed could be due to regional variations in *C. burnetii* distribution and infection pressure or due to variation in the sensitivity of the target gene amplified. Other scholars showed that DNA concentration methods should be applied for detection of *C. burnetii* in milk samples due to its low level in milk, the method which is able to detect a single *C. burnetii* pathogen in 1 mL of milk (Willems *et al*., 1994). The absence of positive results in milk samples does not rule out the excretion of the pathogen in milk the population from which the sample is drawn. It rather emphasizes the challenges faced in identifying *C. burnetii* within these populations.

The negative results obtained from molecular analysis of samples from reproductive tract such as vaginal swabs and placenta/aborted fetal material is striking. Given the bacterium's known association with reproductive health, this raises important questions regarding the epidemiology of *C. burnetii* in Ethiopia. The samples were collected from seropositive livestock, which had history of abortion and other reproductive problems. In contrast to our observation, previous authors reported detection of *C. burnetii* DNA in 61.1% of vaginal swabs targeting other gene than COX 1 (Sánchez-Rodríguez *et al*., 2024). This emphasizes the need for further studies to understand the epidemiology of the disease under the Ethiopian context and establish the differential diagnosis of reproductive problems in livestock species. That will enhance our understanding of the disease dynamics in different regions in different livestock species and help to establish localized epidemiology of coxiellosis. In countries with higher seroprevalence of *C. burnetii* infections such as Iran, molecular studies revealed the role of this pathogen in abortions in small ruminants (0.0-21.8%) and cattle (21.7-25%) (Hassani *et al*., 2025).

The absence positive results from aborted materials are particularly in contrast to the evidence in the literature. Previous reports showed that the DNA of *C. burnetii* was detected in aborted samples ranging from 2.7% to 24.7% in Iran, 47.6% in Hungary and 44.4% in Switzerland (Kreizinger *et al*., 2015; Magouras *et al*., 2017; Mohabati Mobarez *et al*., 2021). Hence, the negative findings in aborted fetal tissues observed in this study, highlight the urgent need for alternative target genes and cohort studies to monitor the occurrence of reproductive problems such as abortion. As aborted materials and reproductive discharges are crucial for diagnosing

coxiellosis in livestock, our results should be interpreted with caution. The other possible reason could also be the intermittent excretion of the bacterium from infected livestock. Samples collected during the time when no *C. burnetii* is excreted give negative results. Previous studies revealed that the amount of *C. burnetii* in reproductive fluids/materials decrease to undetectable levels after parturition or abortion (Roset *et al*., 2012). Moreover, it has been shown that PCR methods are not reliable techniques to determine the cause of abortion (Agerholm, 2013).

Urine samples, one of the potential diagnostic media for *C. burnetii*, were analyzed from livestock with history of abortion although none of them were found positive. This contrasts with the results of previous studies from other countries such as the reports of Tozer *et al*. (2014) in Queensland, where 6.7% tested positive and in Saudi Arabia where 23.8% showed positive results (Hussein *et al*., 2015). Although factors including environmental and epidemiological domains influence the presence of the pathogen in urine, the negative results in this study raises important questions regarding the dynamics of *C. burnetii* infection in the study areas. This may reflect differences in the bacterial load, host immune responses, or other local factors influencing the shedding of *C. burnetii*.

The use of molecular methods for diagnosis of *C. burnetii* infection is a sensitive approach. However, the effectiveness PCR based diagnosis can be affected by the timing of sample collection and the presence of PCR inhibitors (Sidstedt *et al*., 2020). Molecular diagnostic methods are generally preferred for early diagnosis before detectable level of antibody develops while low – level infection and chronic infections may not be detected by PCR (Yang and Rothman, 2004). Previously, Schneeberger and colleague (2010) documented that the proportion of qPCR positive samples declines as infection progresses from acute to convalescent phase. Therefore, the negative RT-qPCR results observed in this study in samples collected from livestock does not rule out infection with *C. burnetii*. *Coxiella burnetii* is shed intermittently in vaginal mucus, milk, or feces meaning that the bacterium may not be detected using PCR even if livestock are infected if samples are collected when livestock are not shedding it (Guatteo *et al*., 2007; Rodolakis *et al*., 2007). Shedding can also be influenced by the time of the year (Trachsel *et al*., 2023). As a result, samples collected during the period

when livestock are not shedding the bacterium could lead to false negative results. Hence, in herds or flocks exhibiting reproductive problems such as abortion, infertility, stillbirth, a negative PCR result should not rule out the occurrence of *C. burnetii* infection.

Limitations of the Study

Our study was carried out on livestock brought for slaughter. Healthy, young, and mostly male livestock were slaughtered across the abattoirs selected. Therefore, the study livestock did not represent the source populations. The pastoral livestock were also sampled at a single point in time (cross-sectional) to collect the samples and associated data. As a result, it was impossible to discern whether the antibody detected is due to current infection. Repeated sampling is warranted for accurate assessment of active infections.

We used an indirect ELISA to test the presence of antibodies against *C. burnetii* and could not distinguish between historical exposure and active infections. Additionally, serological tests have been shown to suffer from cross-reaction with antibodies against bacteria such as *Bartonella* spp. and *Chlamydia* spp. (van der Hoek *et al*., 2013). Finally, we used a COX1 gene based molecular method for detection of *C. burnetii* in biological samples collected from livestock and in ticks. The primers and other reagents were provided by volunteers as there was financial shortage to purchase the primers and reagents. As a result, we could not use primers targeting more sensitive multicopy genes.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The current study reported the seroprevalence of *C. burnetii* (18.77%; 777 out of 4140 samples) in four domestic livestock species. Serological evidence of infection with *C. burnetii* in livestock species was widespread in the country. The seroprevalence was significantly higher in camels, sheep and goats than in cattle. Potential risk factors were identified including age and sex of livestock, and tick infestation, which are significantly associated with *C. burnetii* infection. Adult livestock showed a higher seroprevalence of *C. burnetii* infection than younger livestock, indicating an age-related increase in the risk of exposure to the bacterium.

Furthermore, our study highlights the significant association between tick infestation and serological evidence of *C. burnetii* infection, emphasizing the importance of ticks in the transmission and spread of the bacterium. Our findings highlight the importance of *C. burnetii* in livestock, which can pose serious public health risks since domestic livestock are the primary reservoirs of the bacterium.

Molecular analysis of biological samples collected from livestock as well as ticks did not give positive results. Hence, it is difficult to rule out the possible involvement of *C. burnetii* in reproductive disorders in livestock. It is crucial to use multi-copy genes to clarify the potential role of *C. burnetii* in reproductive health issues.

6.2. Recommendations

Therefore, based on the present results the following recommendations were forwarded;

- ✓ Isolation and identification of *C. burntii* from livestock with reproductive problems should be carried out.
- ✓ Further epidemiological studies are warranted to elucidate the transmission dynamics of *C. burnetii* in animal populations including the routes of infection in different hosts

- ✓ There should be linked study to estimate the prevalence of infection in humans associated with animal populations
- ✓ Further molecular epidemiological study is necessary to better understand and characterize *C. burnetii* strains circulating in Ethiopia including their pathogenicity for vectors and wild hosts
- ✓ Awareness creation among livestock owning communities is needed regarding the impact of the disease, its modes of transmission, risk factors, and methods of prevention
- ✓ Surveillance and monitoring of infection with *C. burnetii* is recommended

PORTFOLIO OF SCIENTIFIC PUBLICATIONS

The following articles were produced during my doctoral candidacy at Adama Science and Technology University (ASTU):

I. Publications Arising from this Dissertation Research

These manuscripts directly represent the findings of the current PhD research project on *Coxiella burnetii*:

- Milkesa, A., Rufael, T., Kinfe, G., Belaineh, R., Bulbula, A., Cho, D., Naimuddin, M., Sori, T., & Dinka, H. (2024). A sero-epidemiological analysis of *Coxiella burnetii* infection and its risk factors in livestock from Addis Ababa, Adama, and Modjo abattoirs and pastoral areas of Oromia, Ethiopia. *PLoS Neglected Tropical Diseases*, *18*(7), e0012287. https://doi.org/10.1371/journal.pntd.0012287

- Milkesa, A., Rufael, T., Sori, H., Aliy, A., Guyasa, C., Ashagrie, T., Ishag, H. Z. A., Belaineh, R., Belete, M., & Dinka, H. (2025, November). Detection of *Coxiella burnetii* DNA targeting the Cytochrome c oxidase subunit 1 (COX1) gene by RT-qPCR in Ethiopia. *Journal of Veterinary Medicine and Animal Health*. (in press)

II. Other Peer-Reviewed Publications (During Doctoral Program)

These manuscripts were completed during the coursework and independent study phases of my PhD program:

- Milkesa, A., Dinka, H., Belaineh, R., & Aliy, A. (2021). Research Note: Molecular surveillance of Avian Paramyxovirus type-1 in nonvaccinated village chickens in Central Rift Valley of Oromia, Ethiopia. *Poultry Science*, *100*(4), 101004. https://doi.org/10.1016/j.psj.2021.01.027

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APPENDICES

Appendix 1. Supplementary Figures

Figures 25 through 32 provide an essential visual record of the detailed field and abattoir procedures used for collecting all diagnostic samples, including blood, milk, aborted materials, vaginal swabs, urine, and ticks, across the Borana Zone districts and the Addis Ababa, Adama, and Modjo slaughterhouse facilities.

Figure 25. Presents a visual record detailing the standardized field procedure for blood sample collection from various animal species within the Borana Zone study area.

Figure 26. Presents the visual documentation of blood sample collection from livestock carried out at the Modjo Slaughterhouse Facility.

Figure 27. Documents the blood sample collection activities specifically conducted on livestock at the Adama Abattoir facility.

Figure 28. Provides a visual record detailing the precise procedure for blood sample collection from livestock at the Addis Ababa Slaughterhouse Facility.

Figure 29. Presents a visual record detailing the precise field procedure and aseptic techniques utilized for milk sample collection from livestock in the Borana Zone.

Figure 30. Documents the field procedure for the collection of aborted materials (specifically fetal tissues and placentae) and the corresponding vaginal swab specimens from livestock in the Borana Districts.

Figure 31. Details the visual record of the field procedures specifically employed for the collection of urine samples from livestock in the Borana Pastoral Districts.

Figure 32. Presents a visual record detailing the standardized field procedure for tick sample collection from livestock across the Borana Districts and slaughterhouse facilities.

Appendix 2. Indirect ELISA Protocol for the Serological Detection of *C. burnetii* Antibodies in Livestock.

This appendix details the reagents, materials, procedure, and data interpretation for the detection of antibodies against *Coxiella burnetii* (the causative agent of Q Fever) in ruminant samples (serum, plasma, or milk) using a commercially available indirect Enzyme-Linked Immunosorbent Assay (iELISA) kit.

2.1. Principle of the Indirect ELISA Assay for *C. burnetii*

The iELISA kit utilizes microtiter plates pre-coated with an inactive *C. burnetii* antigen. The test is a simple, sensitive, and specific method for antibody detection.

1. **Antigen-Antibody Binding-** Diluted test samples (serum, plasma, or milk) and controls are incubated in the wells. Any IgG antibodies specific to *C. burnetii* present in the sample bind to the coated antigen, forming an antigen-antibody complex on the plate well surface. Unbound material is removed via washing.

2. **Conjugate Binding-** A peroxidase-labeled anti-ruminant IgG conjugate is added. This conjugate binds to the species-specific IgG antibodies that are already complexed with the *C. burnetii* antigen. Unbound conjugate is removed via a secondary washing step.

3. **Color Development and Detection-** A 3,3',5,5'-Tetramethylbenzidine (TMB) substrate is added, which reacts with the bound peroxidase enzyme to produce a blue color. The reaction is stopped by adding a Stop Solution, changing the color from blue to yellow. The intensity of the yellow color is measured photometrically at a wavelength of 450 nm, where the optical density (OD450) is directly proportional to the amount of *C. burnetii* specific antibody present in the sample.

2.2. Reagents and Consumables for Indirect ELISA

2.2.1. Reagents Supplied in the Kit

No.	Reagent	Quantity	Description	Storage
1	*C. burnetii* Antigen Coated Plate	2 Plates	Microtiter plate pre-coated with inactive antigen.	2∘C−8∘C
2	Positive Control (PC)	1×0.4 mL	Serum control with known anti-*C. burnetii* antibodies.	2∘C−8∘C
3	Negative Control (NC)	1×0.4 mL	Serum control known to be free of anti-*C. burnetii* antibodies.	2∘C−8∘C
4	Conjugate	1×24 mL	Peroxidase-labeled anti-ruminant IgG solution.	2∘C−8∘C
5	TMB Substrate N∘12	1×20 mL	Chromogenic substrate.	2∘C−8∘C
6	Stop Solution N∘3	1×20 mL	Acid solution to terminate the enzymatic reaction.	2∘C−8∘C
7	Wash Concentrate (10X)	1×100 mL	Buffer concentrate for preparing wash solution.	2∘C−8∘C

2.2.2. Consumables and Equipment

- Precision micropipettes (10 µL,100 µL,300 µL)
- Disposable pipette tips
- Graduated cylinder (for Wash Solution preparation)
- 96-well microplate reader (equipped with 450 nm filter)
- Microplate washer or multi-channel pipette
- Distilled or deionized water
- Microplate covers/sealers

- Vortex mixer or equivalent
- Microplate shaker (optional)
- Humid chamber (optional, for maintaining incubation environment)

2.3. Assay Preparation

2.3.1. Preparation of Wash Solution

1. Bring the 10X Wash Concentrate to room temperature (18∘C−26∘C) and mix thoroughly to ensure dissolution of any precipitated salts.
2. Dilute the 10X Wash Concentrate 1/10 with distilled or deionized water prior to use.

Wash Solution=1 part Wash Concentrate+9 parts Water

3. The diluted Wash Solution can be stored for up to one week at 2∘C−8∘C.

2.3.2. Preparation of Samples and Controls

1. All serum samples, the Positive Control (PC), and the Negative Control (NC) must be prediluted 1/400 using the prepared Wash Solution in separate tubes.

2.4. Test Procedure

All steps should be performed at room temperature (18∘C−26∘C) unless otherwise specified.

Step	Action	Volume	Incubation	T	Notes
1	Plate Setup	-	-	2∘C−8∘C	Record sample positions. If using partial plates, remove only necessary wells and immediately reseal remaining wells with desiccant.
2	Dispense Negative Control (NC)	100µL	-	-	Add diluted NC to duplicate wells.

3	Dispense Positive Control (PC)	100µL	-	-	Add diluted PC to duplicate wells.
4	Dispense Samples	100µL	-	-	Add diluted samples to appropriate wells.
5	Mix	-	-	-	Gently tap the plates or use a microplate shaker to mix the well contents.
6	Incubate (Primary)	-	60 minutes(±5 min)	37∘C	Cover the microplate and ensure it is tightly sealed to prevent evaporation.
7	Wash	≈300µL/well	3 times	-	Remove solution, then wash each well 3 times with the prepared Wash Solution. Tap dry on absorbent paper.
8	Dispense Conjugate	100µL	30 minutes	37∘C	Add Conjugate to all wells and incubate.
9	Wash (Secondary)	≈300µL/well	3 times	-	Repeat the washing step (Step 7). Tap dry.
10	Dispense Substrate (TMB)	100µL	15 minutes	18∘C−26∘C (RT)	Add TMB Substrate to all wells and incubate in the dark.
11	Dispense Stop Solution	100µL	-	-	Add Stop Solution to all wells to terminate the reaction.
12	Read Results	-	-	-	Read the OD value immediately using a photometer at 450 nm.

2.5. Calculation and Interpretation

2.5.1. Calculation of Control Mean OD (X)

The mean OD for the Negative (NCX) and Positive (PCX) controls is calculated as the average of the duplicate wells (where A(450) denotes the OD reading at 450 nm):

NCX=2NC1A(450)+NC2A(450)

PCX=2PC1A(450)+PC2A(450)

2.5.2. Validity Criteria for Controls

The assay run is considered valid if the following conditions are met:

Control Parameter	Threshold	Status
NCX	≤0.500	Valid if ≤0.500
PCX	≤2.500	Valid if ≤2.500
PCX−NCX	≥0.300	Valid if ≥0.300

2.5.3. Sample-to-Positive Ratio (S/P%)

The relative antibody level for each sample is expressed as the Sample-to-Positive ratio (S/P%):

S/P%=100×PCX−NCXSample A(450)−NCX

2.5.4. Interpretation of Results

The S/P% value determines the serological status of the tested animal:

S/P% Value	**Interpretation**
S/P%<30%	Negative (Neg)
30%≤S/P%<40%	Suspect (Sus)
S/P%≥40%	Positive (Pos)

2.6. Summary of ELISA Run and Results

2.6.1. ELISA Run Validation Template

Parameter	Details	Control Status	Validity Criteria	Interpretation
Date	22.03.24	PCX≤2.5	PCX=3.502	Invalid
Analyzed By	AM & GK	NCX≤0.5	NCX=0.101	Valid
Sampling Area	ILRI - Mojo Abt	PCX−NCX≥0.3	PCX−NCX=3.402	Valid
Species	Shoat	Kit Batch	M401	06-40669-04
Laboratory	NAHDIC / NAI	Filter	450 nm	
OD Results		No. Positive	10	No. Negative

2.6.2. Raw Optical Density (OD450) Values

The table below shows the raw OD450 values obtained from the microplate reader for each well.

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	NC2	0.081	1.297	0.301	1.296	0.41	0.464	0.718	0.495	0.33	0.176
B	PC1	PC2	0.716	0.968	0.287	0.469	2.04	0.156	0.139	0.161	0.958	0.113
C	1.588	0.076	0.089	3.034	0.399	0.608	2.95	0.265	0.236	0.185	0.267	0.175
D	0.081	0.361	0.119	0.085	0.357	3.171	0.81	0.434	1.075	0.236	0.174	0.323
E	0.076	0.192	0.092	0.229	0.562	0.318	0.41	1.355	1.549	0.138	0.152	0.438
F	1.052	0.841	0.111	1.345	0.6	0.877	0.41	0.246	0.13	0.138	0.437	0.165
G	0.15	2.665	0.083	0.081	0.772	0.549	2.71	0.195	0.144	0.312	0.176	0.186
H	3.233	0.426	0.069	0.089	0.568	0.213	0.77	0.252	0.405	0.087	0.776	0.166

2.6.3. Sample-to-Positive Ratio (S/P%) Calculations

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	–	–	-0.573	35.18	5.894	35.15	9.22	10.69	18.154	11.6	6.747	2.22
B	–	–	18.09	25.5	5.483	10.83	56.9	1.63	1.1319	1.78	25.21	0.37
C	100.91	-0.72	-0.338	86.24	8.776	14.92	83.7	4.84	3.9835	2.48	4.895	2.19
D	99.089	7.658	0.544	-0.46	7.541	90.27	20.8	9.8	28.649	3.98	2.161	6.54
E	-0.7203	2.69	-0.25	3.78	13.57	6.39	9.04	36.88	42.584	1.1	1.514	9.92
F	27.973	21.77	0.309	36.59	14.68	22.83	9.1	4.28	0.8673	1.1	9.893	1.9
G	1.4552	75.39	-0.514	-0.57	0.029	13.19	76.7	2.78	1.2788	6.22	2.22	2.51
H	92.092	9.569	-0.926	-0.34	13.74	3.31	19.8	4.45	8.9519	-0.4	19.86	1.93

2.6.4. Serological Interpretation

The final serological status based on the S/P% values:

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	–	–	Neg	Sus	Neg	Sus	Neg	Neg	Neg	Neg	Neg	Neg
B	–	–	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
C	Pos	Neg	Neg	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
D	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg
E	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Sus	Pos	Neg	Neg	Neg
F	Neg	Neg	Neg	Sus	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
G	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
H	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

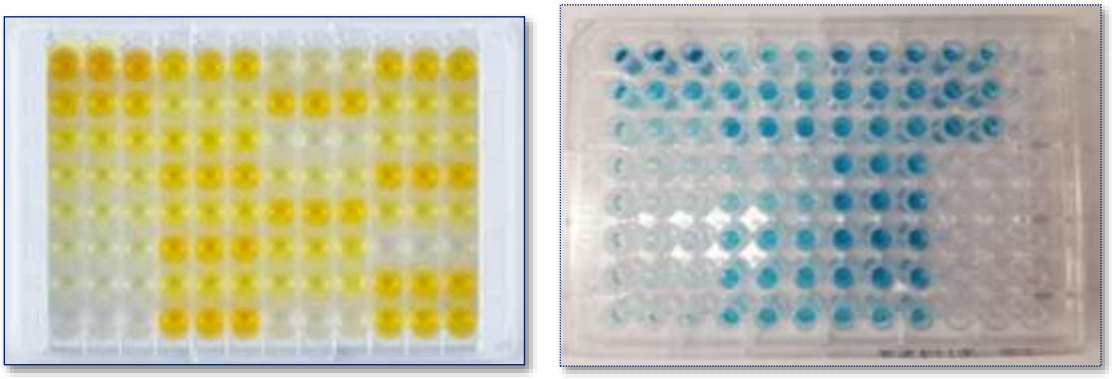


Figure 33. Visual Confirmation of *C. burnetii* Seropositivity via Indirect ELISA. The development of a blue color in the microtiter wells, transitioning from the initial yellow substrate, serves as the positive qualitative result for *Coxiella burnetii* antibodies.

2.7. Visual Record of the Serology Laboratory Workflow- Sample Preparation and i-ELISA Testing for *C. burnetii*.

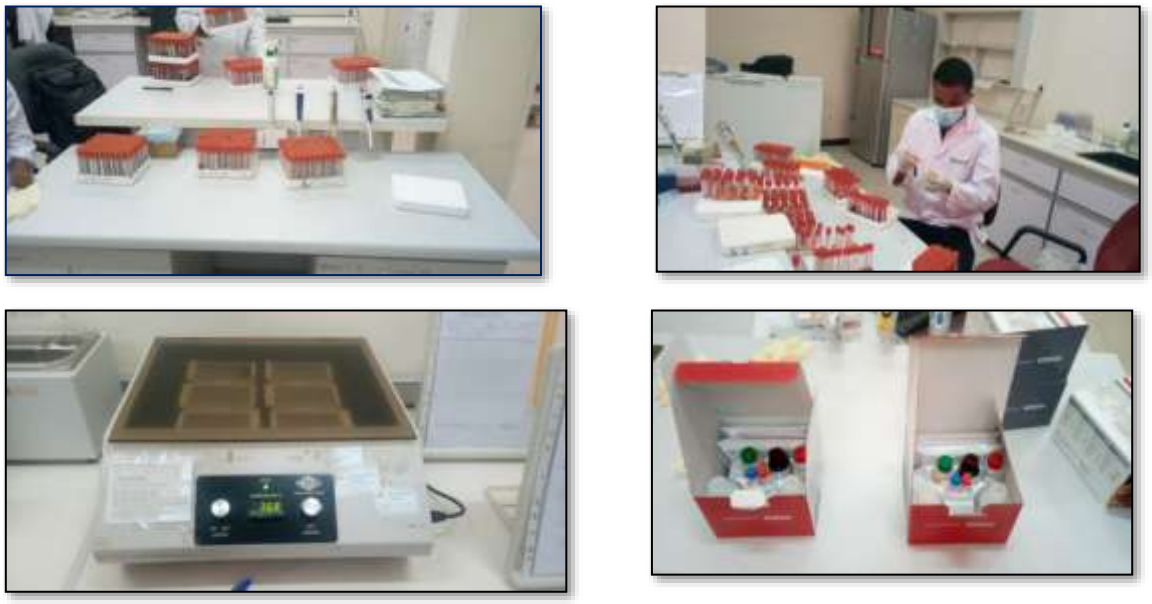


Figure 34. Provides a photographic documentation of the key laboratory procedures, specifically illustrating the sample preparation and the execution steps of the Indirect ELISA used for serological testing.

Appendix 3. Detailed step-by-step protocol used for Genomic DNA extraction from samples utilizing the commercial QIAamp DNA Mini Kit.

Protocol 3.1. DNA Purification from Blood or Body Fluids (Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge.

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- Use carrier DNA if the sample contains <10,000 genome equivalents
- 200 µl of whole blood yields 3–12 µg of DNA. Preparation of buffy coat is recommended if a higher yield is required

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Heat a water bath or heating block to 56°C for use in step 4.
- Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

1. Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.

2. Add 200 µl sample to the microcentrifuge tube. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 106 lymphocytes in 200 µl PBS.

If the sample volume is less than 200 µl, add the appropriate volume of PBS. QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Note: It is possible to add QIAGEN Protease (or proteinase K) to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. If the sample volume is larger than 200 µl, increase the amount of QIAGEN Protease (or proteinase K) and Buffer AL proportionally; for example, a 400 µl sample will require 40 µl QIAGEN Protease (or proteinase K) and 400 µl Buffer AL. If sample volumes larger than 400 µl are required, use of QIAamp DNA Blood Midi or Maxi Kits is recommended; these can process up to 2 ml or up to 10 ml of sample, respectively.

Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

4. Incubate at 56°C for 10 min.

DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA.

5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

If the sample volume is greater than 200 µl, increase the amount of ethanol proportionally; for example, a 400 µl sample will require 400 µl of ethanol.

7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.*

Close each spin column to avoid aerosol formation during centrifugation. Centrifugation is performed at 6000 x g (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 µl.

9. Blood or Body Fluid Spin Protocol Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

10. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

11. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

Incubating the QIAamp Mini spin column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

A second elution step with a further 200 µl Buffer AE will increase yields by up to 15%.

Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 µg of DNA, elution in 50 µl Buffer AE or water is recommended. Eluting with 2 x 100 µl instead of 1 x 200 µl does not increase elution efficiency.

For long-term storage of DNA, eluting in Buffer AE and storing at–20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

A 200 µl sample of whole human blood (approximately 5 x 106 leukocytes/ml) typically yields 6 µg of DNA in 200 µl water (30 ng/µl) with an A260 /A280 ratio of 1.7–1.9.

Protocol 3.2- DNA Purification from Tissues (QIAamp DNA Mini Kit)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from tissues using the QIAamp DNA Mini Kit.

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- Use carrier DNA if the sample contains <10,000 genome equivalents.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size.
- Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR. If RNA-free genomic DNA is required, include the RNase A digest, as described in step 5a of the protocol.

Things to do before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- Heat 2 water baths or heating blocks: one to 56°C for use in step 3, and one to 70°C for use in step 5.
- Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C.

Procedure

1. Excise the tissue sample or remove it from storage. Determine the amount of tissue. Do not use more than 25 mg (10 mg spleen).

Weighing tissue is the most accurate way to determine the amount.

If DNA is prepared from spleen tissue, no more than 10 mg should be used.

The yield of DNA will depend on both the amount and the type of tissue processed.

1 mg of tissue will yield approximately 0.2–1.2 µg of DNA.

2. Cut up(step 2a), grind (step 2b), or mechanically disrupt (step 2c) the tissue sample. The QIAamp procedure requires no mechanical disruption of the tissue sample, but lysis time will be reduced if the sample is ground in liquid nitrogen (step 2b) or mechanically homogenized (step 2c) in advance.

2a. Cut up to 25 mg of tissue (up to 10 mg spleen) into small pieces. Place in a 1.5 ml microcentrifuge tube, and add 180 µl of Buffer ATL. Proceed with step 3. It is important to cut the tissue into small pieces to decrease lysis time. 2 ml microcentrifuge tubes may be better suited for lysis.

2b. Place up to 25mgoftissue (10 mg spleen) in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into 1.5 ml microcentrifuge tube. Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw, and add 180 µl of Buffer ATL. Proceed with step 3.

2c. Add up to 25 mg of tissue (10 mg spleen) to a 1.5 ml microcentrifuge tube containing no more than 80 µl PBS. Homogenize the sample using the TissueRuptor or equivalent rotor–stator homogenizer. Add 100 µl Buffer ATL, and proceed with step 3. Some tissues require undiluted Buffer ATL for complete lysis. In this case, grinding in liquid nitrogen is recommended. Samples cannot be homogenized directly in Buffer ATL, which contains detergent.

3. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform. Note: Proteinase K must be used. QIAGEN Protease has reduced activity in the presence of Buffer ATL. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. Lysis overnight is possible and does not influence the preparation. To ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.

4. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

5. If RNA-free genomic DNA is required, follow step 5a. Otherwise, follow step 5b. Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

5a. First add 4 µl RNase A(100mg/ml), mix bypulse-vortexing for 15 s, and incubate for 2minatroomtemperature(15–25°C). Brieflycentrifugethe1.5mlmicrocentrifuge tube to remove drops from inside the lid before adding 200 µl Buffer AL to the sample. Mix again by pulse-

vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. Awhite precipitate may form on addition of Buffer AL. In most cases it will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

5b. Add200µlBuffer AL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL, which in most cases will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

6. Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

It is essential that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the QIAamp Mini spin column. This precipitate does not interfere with the QIAamp procedure or with any subsequent application.

Do not use alcohols other than ethanol since this may result in reduced yields.

7. Carefully apply the mixture from step 6 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.*

Close each spin column to avoid aerosol formation during centrifugation. It is essential to apply all of the precipitate to the QIAamp Mini spin column. Centrifugation is performed at 6000 x g (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

10. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

11. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAampMinispincolumnandadd200µlBufferAEordistilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

12. Repeat step 11.

A 5 min incubation of the QIAamp Mini spin column loaded with Buffer AE or water, before centrifugation, generally increases DNA yield.

A third elution step with a further 200 µl Buffer AE will increase yields by up to 15%. Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

Eluting with 4 x 100 µl instead of 2 x 200 µl does not increase elution efficiency. For long-term storage of DNA, eluting in Buffer AE and placing at–20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Yields of DNA will depend both on the amount and the type of tissue processed. 25 mg of tissue will yield approximately 10–30 µg of DNA in 400 µl of water (25–75 ng/µl), with an A260/A280 ratio of 1.7–1.9.

3.3 Protocol- DNA Purification from Swabs (Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from swabs using a microcentrifuge.

Important points before starting

- Due to the increased volume of Buffer AL that is required for the swab protocol, fewer preparations can be performed.
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman Bioscience, www.whatman.com), cotton, and DACRON (Daigger, Puritan® applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company, www.hwppuritan.com, or from Hain Diagnostika, Swab Spin Protocol www.hain-diagnostika.de).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Prepare a 56°C water bath for use in step 3.
- Equilibrate Buffer AE or distilled water to room temperature (15–25°C) for elution in step 10.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

1. Place swab in a 2mlmicrocentrifuge tube. Add 400 µl (cotton and DACRON swab) or 600 µl (Omni Swab) PBS to the sample. The Omni Swab is ejected into the microcentrifuge tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors. QIAamp Mini spin columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

2. Add 20 µl QIAGEN Protease stock solution (or proteinase K) and 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) Buffer AL to the sample. Mix immediately by vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly. Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

3. Incubate at 56°C for 10 min. Briefly centrifuge to remove drops from inside the lid.

4. Add 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) ethanol (96–100%) to the sample, and mixagainbyvortexing. Briefly centrifuge to remove drops from inside the lid.

5. Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate. * Close each spin column to avoid aerosol formation during centrifugation.

6. Repeat step 5 by applying up to 700 µl of the remaining mixture from step 4 to the QIAamp Mini spin column.

7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

9. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAampMinispincolumnandadd150µlBufferAEordistilled water. Incubate at roomtemperature(15–25°C) for1min, andthencentrifugeat6000xg(8000rpm) for 1 min.

Elution with 100 µl buffer increases the final DNA concentration in the eluate significantly, but may slightly reduce the overall DNA yield. Elution with volumes of less than 100 µl is not recommended as the overall DNA yield decreases dramatically.

A second elution step with the same 150 µl eluate containing the DNA will increase yield significantly. However, this is not recommended when using the eluate for PCR.

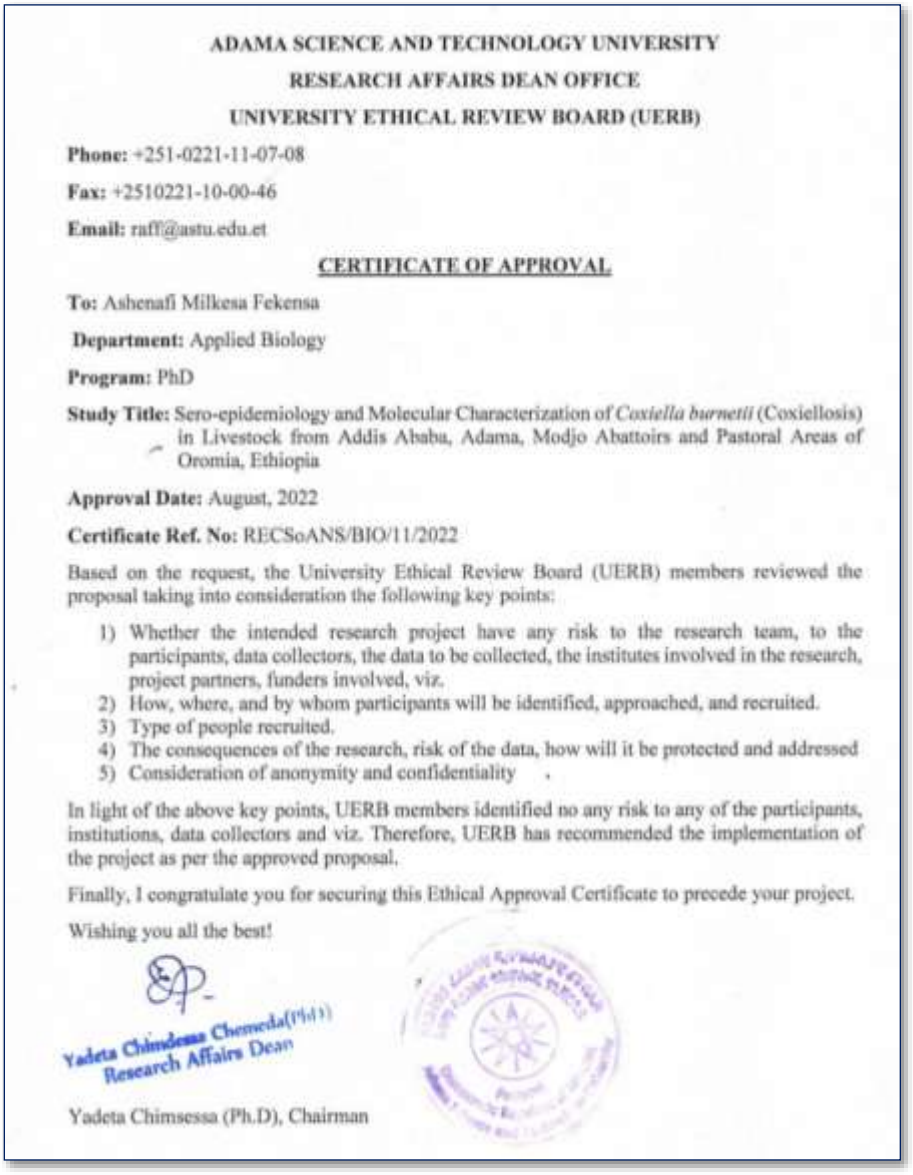
Appendix 4. Visual record detailing the molecular laboratory workflow, specifically illustrating the preparation of the master mix and the individual samples prior to running the Real-Time PCR (RT-qPCR) assay.

Figure 35. Provides a visual record detailing the aseptic preparation and precise pipetting of the reagents required to construct the RT-qPCR Master Mix.

Figure 36. Presents a visual record detailing the aseptic transfer and accurate loading of the DNA template into the microplate wells for subsequent RT-qPCR analysis.

Figure 37. Provides a visual record of the RT-qPCR instrument during operation and displays the corresponding real-time fluorescence data (amplification plots) used for the detection and interpretation of results.

Appendix 5. Presents the official Ethics Statement, which includes the approval letter issued by the Adama Science and Technology University Research Committee authorizing the study.



ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

RESEARCH AFFAIRS DEAN OFFICE

UNIVERSITY ETHICAL REVIEW BOARD (UERB)

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CERTIFICATE OF APPROVAL

To: Ashenafi Milkesa Fekensa

Department: Applied Biology

Program: PhD

Study Title: Sero-epidemiology and Molecular Characterization of *Coxiella burnetii* (Coxiellosis) in Livestock from Addis Ababa, Adama, Modjo Abattoirs and Pastoral Areas of Oromia, Ethiopia

Approval Date: August, 2022

Certificate Ref. No: RECSoANS/BIO/11/2022

Based on the request, the University Ethical Review Board (UERB) members reviewed the proposal taking into consideration the following key points:

1) Whether the intended research project have any risk to the research team, to the participants, data collectors, the data to be collected, the institutes involved in the research, project partners, funders involved, viz.
2) How, where, and by whom participants will be identified, approached, and recruited.
3) Type of people recruited.
4) The consequences of the research, risk of the data, how will it be protected and addressed
5) Consideration of anonymity and confidentiality .

In light of the above key points, UERB members identified no any risk to any of the participants, institutions, data collectors and viz. Therefore, UERB has recommended the implementation of the project as per the approved proposal.

Finally, I congratulate you for securing this Ethical Approval Certificate to precede your project.

Wishing you all the best!

Yadeta Chimdessa Chemeda(PhD)
Research Affairs Dean

Yadeta Chimsessa (Ph.D), Chairman

Figure 38. Presents the photographic documentation of the official institutional approval letter that granted ethical clearance for the entire research protocol.

Appendix 6. Presents the comprehensive list of peer-reviewed journal publications that have resulted directly from the Doctoral Research Dissertation**.**

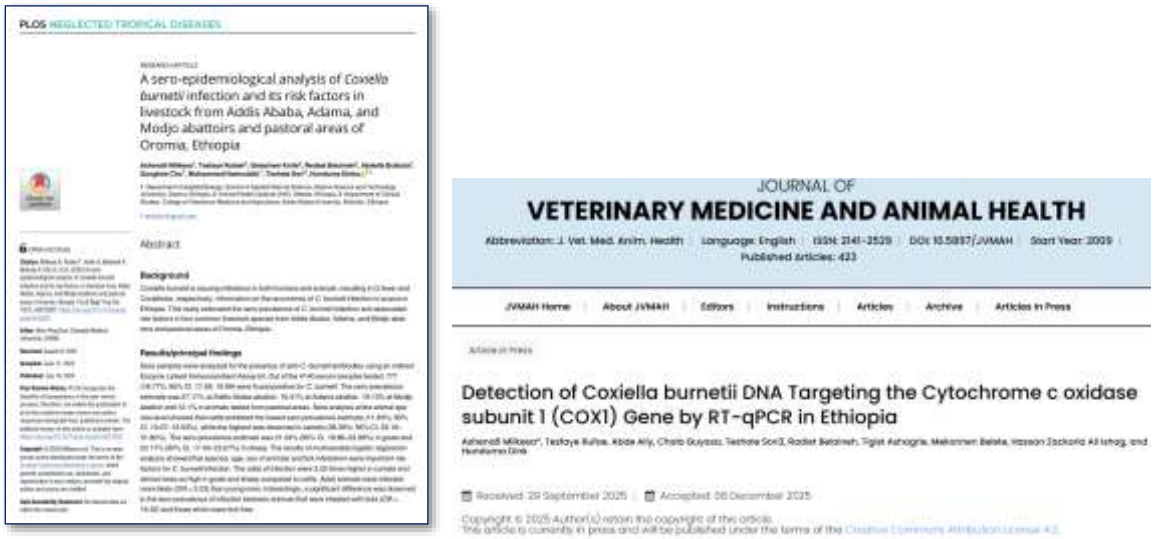

Figure 39. The photographic documentation of the compiled catalogue containing the list of peer-reviewed journal publications resulting from the research work.

Appendix 7. Presents the official documentation of the Academic Integrity Check, specifically showing the Turnitin Similarity Report generated for the Doctoral Dissertation.

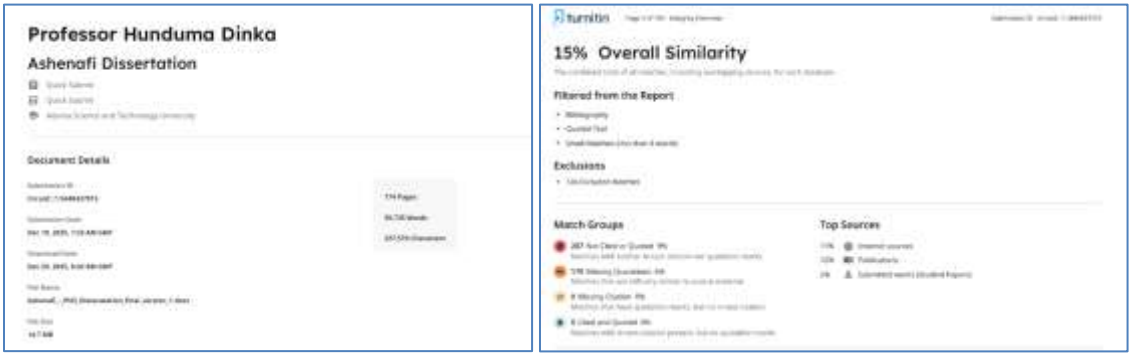

Figure 40. A copy of the Turnitin Similarity Report, which serves as documentation of the academic integrity check and indicates the result of the plagiarism check performed on the Doctoral Dissertation.