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Research Article

Detection of *Coxiella burnetii* DNA Targeting the Cytochrome c oxidase subunit 1 (COX1) Gene by RT-qPCR from Addis Ababa, Adama, Modjo Abattoirs, and Pastoral Areas of Oromia, Ethiopia

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Abstract

Background: Coxiella burnetii represents a substantial global concern in terms of public health, veterinary implications, and economic impact. However, data regarding the molecular epidemiology of this pathogen in Ethiopia remains limited. This study aimed to explore Coxiella burnetii from diverse biological samples including milk, urine, vaginal swabs, aborted fetal tissues, blood, and ticks from seropositive animals.

Methods: A total of 4,140 whole blood samples were collected from livestock species at three abattoirs (Addis Ababa, Adama, and Modjo), along with pastoral regions (Mega, Dubuluk, and Yabello districts) between January 2021 to May 2022. Not only from abbatoirs but also 264 samples 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). In addition, 292 ticks were collected from infested livestock during of the biological sample collection. Sera samples were screened by using an indirect enzyme-linked immunosorbent assay (iELISA) for anti C. burnetii antibody. Additionally, buffy coat from iELISA serum positive animals, 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. Georeferencing was performed to accurately delineate the sample locations.

Results: Out of 4,140 sera samples, 777 were found positive with iELISA yielding a seroprevalence of 18.77% (95% CI: 17.59 – 19.99). Furthermore, out of 384 buffy coat samples randomly selected from the iELISA sera positive that were 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. Camels had the highest tick infestation rate, with 143 ticks (49.0% of the total collection) while cattle had the lowest infestation rate (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.

Conclusion: The molecular detection of a single copy COX1 gene showed no positive results even though the serological survey revealed valuable insights into the epidemiology of C. burnetii infections in both abattoirs and pastoral regions of Oromia, Ethiopia. Therefore, further molecular investigations on large scale sample size and area coverage are essential to deepen our understanding of its epidemiological dynamics.

Keywords: *Coxiella burnetii*; COX1 gene; Buffy Coat; RT-qPCR assay; Livestock; Ethiopia.

Introduction

Coxiella burnetii is an important bacterium causing Q fever in humans and coxiellosis in animals posing significant health risks (Oyston and Davies, 2011; Shaw and Voth, 2019). In humans, the disease featured by an acute, self-limiting illness varying from mild to potentially life-threatening (Eldin et al., 2017). In animals, particularly ruminants, coxiellosis primarily affects the reproductive system, often resulting in spontaneous abortions and infertility resulting in considerable losses (Robi et al., 2023). For instance, an outbreak of the disease in the Netherlands between 2007 and 2010 culminated in the culling of over 58,000 livestock (approximately 21%) and impacted

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over 4,000 individuals (Whelan et al., 2011). This event underscores the severe implications of the disease for the agricultural sector and highlights its status as a significant public health crisis (van Asseldonk et al., 2015).

In both humans and animals, C. burnetii infection is primarily transmitted through the inhalation of dried aerosol particles from the parturition material of infected animals (Knobel et al., 2013). Transmission via ticks has also been demonstrated experimentally in animals and more than 40 species of ticks are known to vector C. burnetii (Duron et al., 2015). While there is no definitive evidence that humans can contract Q fever from ticks, it is possible that they play a role in the transmission cycle. Genotyping of C. burnetii isolates from ticks has revealed the occurrence of strains of C. burnetii known to cause severe infection in humans and/or animals (Kumsa et al., 2015).

Since its first report in Africa in 1947, the occurrence of C. burnetii have been studied in several African countries (Dupont et al., 1995). However, sufficient information is not available mainly due to poor diagnostic capacity, lack of required tools and/or expertise, and competing infectious diseases, which are often on the priority list of many African countries (Eldin et al., 2017). Previous research has shown that the prevalence of Q fever exhibits significant variation across sub-Saharan Africa. Higher seroprevalence has been reported in countries with more pastoral land use such as Chad, Egypt, Tanzania, Niger and Kenya (Dupont et al., 1995; Vanderburg et al., 2014; Maina et al., 2016).

The first report on the evidence of occurrence of C. burnetii infection dates back to 1966 when it was detected in ticks and ruminants (Philip et al., 1996). Subsequently, evidence of Q fever infection in humans was reported in 1990 with 6.5% prevalence in abattoir workers in Addis Ababa (Abebe, 1990). Since then, only few studies had been conducted on C. burnetii in livestock species revealing seroprevalence of 31.6% in cattle, 90% in camels and 54.2% in goats (Gumi et al., 2013). Additionally, evidence of circulation of C. burnetii in ticks collected from pastoral regions reveals the occurrence of different genotypes with two novel genotypes in addition to those known to cause severe diseases in humans, as well as spontaneous abortion in animals (Hornok et al ., 2014; Sulyok et al., 2014; Kumsa et al., 2015).

In Ethiopia, it is hypothesized that many cases of C. burnetii infection remain undiagnosed due to inadequate diagnostic facilities. In other regions globally, various molecular techniques are frequently employed to identify C. burnetii in various sample types, such as milk, blood, serum, ticks, and urine. Various PCR assays targeting different genes have been utilized for molecular diagnosis including COX1 gene (Ashour et al., 2024). Molecular evidence of C. burnetii infection is notably scarce in Ethiopia. However, information obtained from molecular assays is very important in tracing the sources of infection and devising effective prevention and control measures. Thus, the aim of this study was to detect C. burnetii infection in livestock species and ticks collected from various areas of Ethiopia, utilizing RT-qPCR by targeting the COX1 gene.

Material and Methods

Description of Study Area

The study animals primarily came from the Borana pastoral regions, intended for slaughter at abattoirs in Addis Ababa, Adama, and Modjo, as illustrated in Figure 1 located in the southern part of Oromia Regional State, the Borana pastoral area is approximately 575 kilometers south of Addis Ababa. This region comprises thirteen districts and shares its southern border with Kenya. Characterized by a semi-arid to arid climate, the Borana Zone is situated between 4° to 6° N latitude and 36° to 42° E longitude, featuring isolated mountains and valleys. The altitude varies from 1,000 to 1,700 meters above sea level, with mean annual rainfall ranging from 250 to 700 mm. The average annual temperature in this area falls between 19°C and over 25°C. For this study, six districts (Yabelo, Dubuluk, Mega, Boku Luboma, Elweye and Moyale) were chosen based on the history of occurrence of livestock reproductive problems such as abortion. The key livestock species in the region include cattle, goats, sheep, and camels. According to data from the Borana Zone Pastoral Development Office, the livestock population includes 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 in the region was recorded at 1,283,925 (BZFEDO, 2015).

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, followed by additional data collection in January and February 2025. The study involved cattle, sheep, goats, and camels that were slaughtered at abattoirs in Addis Ababa, Adama, and Modjo, mainly sourced from the Borana pastoral areas. The study included indigenous animal breeds of both sexes that were over six months old. In total, 648 samples were collected from these abattoirs and six selected pastoral districts, with the oversight of veterinary professionals. The age of the animals was estimated using a previously established method (Schelling et al., 2003).

Sample Size Determination for Molecular Investigation: The minimum number of animals required for this study was calculated using a formula outlined by Thrusfield (Thrusfield, 2013). In this equation, n denotes the necessary sample size, Pexp represents the anticipated prevalence, and d signifies the absolute precision, as detailed in equation (1).

$$n = \frac{(1.96)^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where n represents the total sample size, Pexp indicates the expected prevalence (50%), and d denotes the absolute precision (0.05) at a 95% confidence interval.

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

However, since our primary objective was to conduct molecular assays on seropositive animals, the total number of sampled animals was increased to 648 to enhance the likelihood and accuracy of detection. A total of 384 samples from the initial collection phase were analysed by RT-qPCR. Additionally, in the second phase of sample collection, 264 samples were collected, including milk, urine, aborted fetal tissues, whole blood, and vaginal swabs. In addition, from both the first and second phases, a total of 292 ticks were collected for

molecular analysis. For each sample, GPS coordinates, location names, and geographical distribution data were carefully documented.

Sampling Technique, Sample Collection, and Processing

Sample Collection:

Collection of Blood Samples: A total of 4,208 whole blood samples were collected, consisting of 4,140 samples from the first phase for iELISA analysis and 68 samples from the second phase for molecular analysis. Additionally, 452 buffy coat samples were obtained for further molecular analysis from all study animals. To maintain the integrity of the samples, appropriate restraint techniques were employed to minimize animal movement during the collection process. The puncture site on the jugular vein was cleansed to ensure sterility. Blood was collected in a 10 mL volume using a tube containing EDTA as an anticoagulant. Each sample was labeled with specific identification code. Following collection, the blood was centrifuged to separate the serum from the red cell layer. The buffy coat was utilized for the molecular detection of C. burnetii, while the serum was reserved for antibody detection through the ELISA technique. When immediate shipping was not feasible, samples were either refrigerated or stored at -20°C in the regional laboratory. During transportation to the Animal Health Institute (AHI), the samples were kept in ice cold chain to preserve their integrity.

Collection of Milk Samples: A total of 71 milk samples were collected from lactating animals, including 11 cows, 15 goats, 37 camels, and 8 sheep. The udder were washed and dried and the teats were disinfected with 70% alcohol. 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 were 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. Cryotubes were stored at -20°C. The frozen specimens were transferred to AHI and processed for DNA extraction and PCR detection of C. burnetii specific DNA fragments.

Collection of Placental Samples: Aseptically, a total of 4 aborted fetal tissues were collected from 2 goats and 2 cattle. The placentae from aborted foetuses were collected in labelled sterile universal bottles and preserved on ice and were stored at 4°C prior to processing.

Collection of Vaginal Swab Samples: A total of 62 vaginal swabs were collected aseptically from 18 goats, 13 cattle, 23 camels, and 8 sheep. Each swab sample was placed in a labelled sterile cryogenic vial containing preservative and stored at -20°C.

Collection of Urine Samples: A total of 59 urine samples were collected from female camels in clean and sterile labelled bottles. 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).

Ticks Sample Collection: Ticks were collected from study animals following proper restraint techniques to ensure animal and personnel safety. 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 animals included in the study and pooled per host species resulting in 30 pools. Each pool contained a maximum of 10 ticks for a maximum of 292 individual ticks.

Post-collection, hands and the affected skin areas were sanitized using soap and water or 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 Animal Health Institute's parasitology laboratory for further identification and analysis.

Sample Transport, Storage and Disposal:

The samples collected were transported to Animal Health Institute (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.

Laboratory Procedures

Serological Analysis:

The blood samples collected were screened for the presence of specific anti – C. burnetii antibodies using iELISA (Switzerland AG, CH3097, Liebefeld-Bern, Switzerland) following manufacturer's instructions at the Animal Health Institute (AHI) Immunology Laboratories.

Molecular Analysis:

DNA Extraction: DNA was extracted from all (940) 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 the Animal Health Institute (AHI) Molecular biology and Genomics laboratories. The eluted bacterial DNAs were preserved at −80°C for subsequent analysis.

Real –Time quantitative Polymerase Chain Reaction (RT-qPCR) Analysis: The extracted DNA underwent analysis using quantitative real-time polymerase chain reaction (RT-qPCR), focusing on the COX1 gene of Coxiella burnetii, utilizing an Applied Biosystems 7500 Fast real-time quantitative PCR (RT-qPCR) system (Applied Biosystems, USA). The following primers were employed: Forward Primer (Cox-F) - 5'-GTC TTA AGG TGG GCT GCG TG-3' and Reverse Primer (Cox-R) - 5'-CCC CGA ATC TCA TTG ATC AGC-3'. A specific probe, Cox-TM (5' FAM-AGC GAA CCA TTG GTA TCG GAC GTT TAT GG-TAMRA -3'), was also incorporated into the reaction.

The reaction mixture totaled 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.

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 cycling conditions for the COX1 gene assay were set to 40 cycles, comprising a 3-minute denaturation step at 95°C, followed by a 3-second annealing phase at 95°C, and a 30-second extension at 60°C.

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).

Morphological Identification of Ticks

Cleaning and Preparing Tick Samples: 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.

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 these chemicals. Waste disposal was conducted in accordance with the biosafety guidelines established by the Animal Health Institute (AHI) to ensure safety and compliance.

Tick Sample Identification Procedure: Tick samples were identified utilizing established taxonomic keys, specifically those outlined by Walker et al. (2003) for African ticks, along with other relevant regional references. Identification was conducted using camera-equipped stereomicroscopes, such as the Olympus SZ models, 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.

Geo-referencing (Mapping)

Throughout the study period, samples were collected and analyzed using thematic density maps generated with QGIS 10.0. These maps visually represented both the distribution of the samples collected and the locations of those that tested negative for C. burnetii. One thematic map was created to show the distribution of the samples: indicating area with geographical coordinate's serologically-positive samples identified by using iELISA and RT-qPCR negative samples.

Ethics Statement

The sampling of animals was conducted in accordance with the approved experimental protocols and guidelines set forth by the Animal Research Scientific and Ethics Review Committee (ARSERC) at the Animal Health Institute (AHI), the Institutional Animal Care and Use Committee (IACUC), and Adama Science and Technology University (ASTU). This study was carried out after obtaining ethical clearance with Reference number: RECSoANS/BIO/11/2021.

Results

Detection of C. Burnetii Using RT-qPCR in 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 on molecular analysis. The results of serological and molecular analysis are given in Table 1. In addition, the result of molecular analysis is depicted in Figure 2.

Detection of C. Burnetii Using Real-Time qPCR in Biological Samples

Table 2 and Figure 3 present the results of molecular analysis using Real-Time qPCR on various biological samples collected from livestock species from pastoral areas. Out of a total of 264 samples from cattle, camels, goats, and sheep analyzed, none of them showed positive results to C. burnetii COX1 gene.

Prevalence of Tick Infestation and Species Composition

A total of 292 ticks were collected from the study animals and identified, representing three genera: Amblyomma, Hyalomma, and Rhipicephalus. Tick infestation rates varied significantly among the different host species (Table 3). 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

Table 1: 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

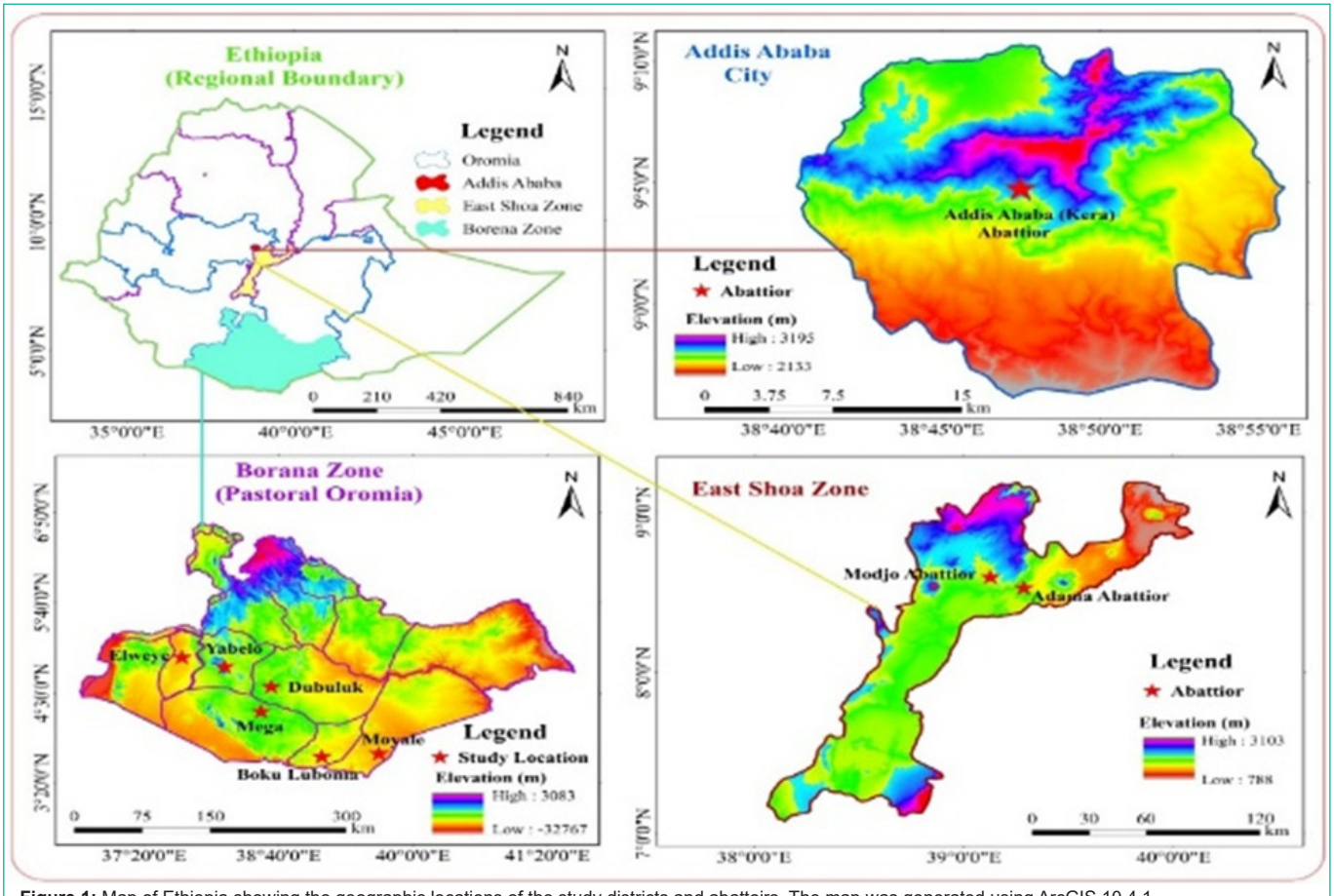

Figure 1: Map of Ethiopia showing the geographic locations of the study districts and abattoirs. The map was generated using ArcGIS 10.4.1.

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.

Tick Species Identified by Stereomicroscopy

The species of ticks identified by stereomicroscopy are shown in Figure 4.

Findings from Quantitative Real-Time qPCR on Tick Samples

Table 2: Results of analysis of biological samples collected from domestic livestock from pastoral area using Real-Time 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

Table 3: 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 lipidium	4 (2.8%)	20 (25.3%)	15 (31.9%)	5 (21.7%)	44
Hyalomma dromedarii	30 (21.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	30
Rhipicephalus evertsi	7 (4.9%)	16 (20.3%)	9 (19.1%)	4 (17.4%)	36
Amblyomma variegatum	7 (4.9%)	20 (25.3%)	10 (21.3%)	8 (34.8%)	45
Amblyomma cohaerens	4 (2.8%)	8 (10.1%)	2 (4.3%)	1 (4.3%)	15
Amblyomma gemma	15 (10.5%)	4 (5.1%)	2 (4.3%)	1 (4.3%)	22
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**

Table 4 and Figure 5 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.

Geospatial Mapping of Molecular Detection for C. Burnetii

Figure 6 illustrates the geo-referencing results for all study sites. This includes the initial sampling areas of Adama, Modjo, and Addis Ababa Abattoirs, as well as the Borana pastoral area including Mega, Dubuluk, Yabello, Elweye, Boku Luboma, and Moyale.

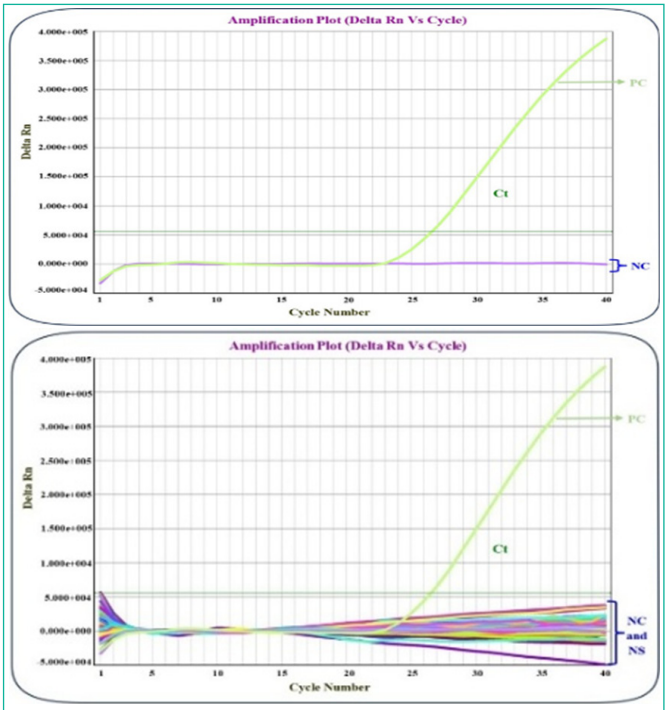

Figure 2: The result graphs generated from the Real-Time quantitative PCR analysis. A) RT-qPCR amplification curve displays the positive and negative controls. B) RT-qPCR amplification curve, both the positive and negative controls are shown alongside the samples. This comprehensive depiction underscores the validity of the Real-Time qPCR procedures *Coxiella burnetii*. Where NC and PC represent the negative and positive controls, respectively. NS indicates a negative sample, and Ct stands for cycle threshold.

Discussion

The close interaction between humans and animals 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). There are widespread occurrence of abortion and infertility in domestic ruminant population in the country, where cases of endocarditis have also been reported in humans (Moges et al., 2015; Alemayehu et al., 2021). 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. This study analyzed samples from both abattoirs and pastoral areas of Ethiopia spanning wider geographical areas. Additionally, diverse biological materials including milk, urine, vaginal swabs, aborted fetal tissues, and blood were tested for detection of C. burnetii DNA.

None of the samples analyzed using COX1 real-time PCR showed positive results. This observation is consistent with previous reports of Kumsa and colleague, who did not detect C. burnetii DNA in ticks and that of Selmi et al. (Kumsa et al., 2015; Selmi et al., 2019). This could be due to lower bacterial load in the infected animals, which cannot be detected by COX1 RT-qPCR. In contrast, our observation is lower than the reports made elsewhere such as Egypt, Saudi Arabia, and Iran where C. burnetii was detected 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% ticks in Ethiopia and elsewhere in Africa including 5.5%

Table 4: 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

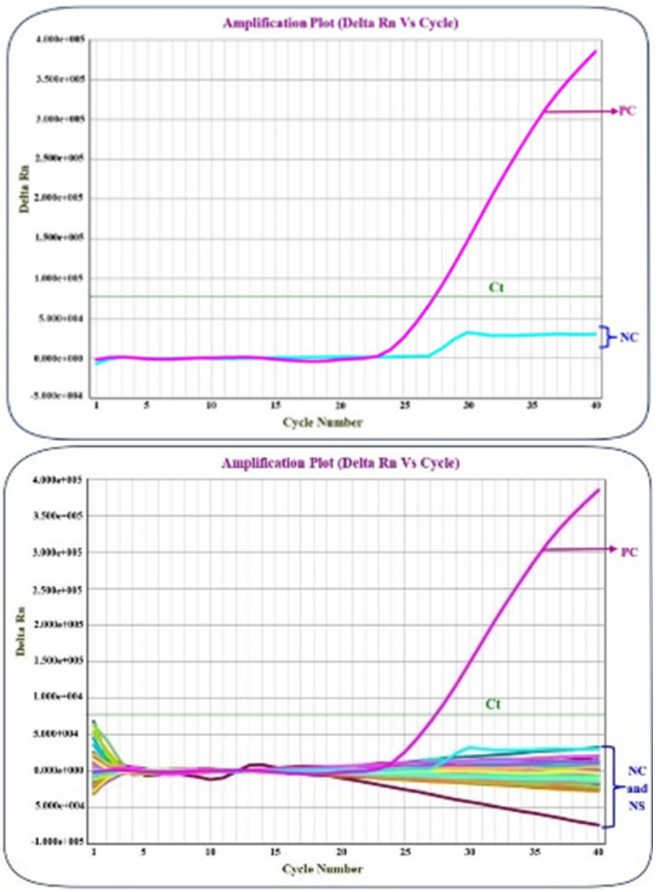

Figure 3: The result graphs generated from Real-Time qPCR analysis on biological samples collected from cattle, camels, sheep and goats from Borana pastoral areas. A) RT-qPCR amplification curve displays the positive and negative controls used in the assay. B) RT-qPCR amplification curve presents both 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.

Figure 4: Ticks Negative for *C. burnetii*. This figure shows images of ixodid (hard) ticks that tested negative for the presence of *Coxiella burnetii*. The ticks were collected from cattle, sheep, goats, and camels at three abattoirs and six districts in the pastoral Oromia region of Ethiopia.

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).

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 with 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

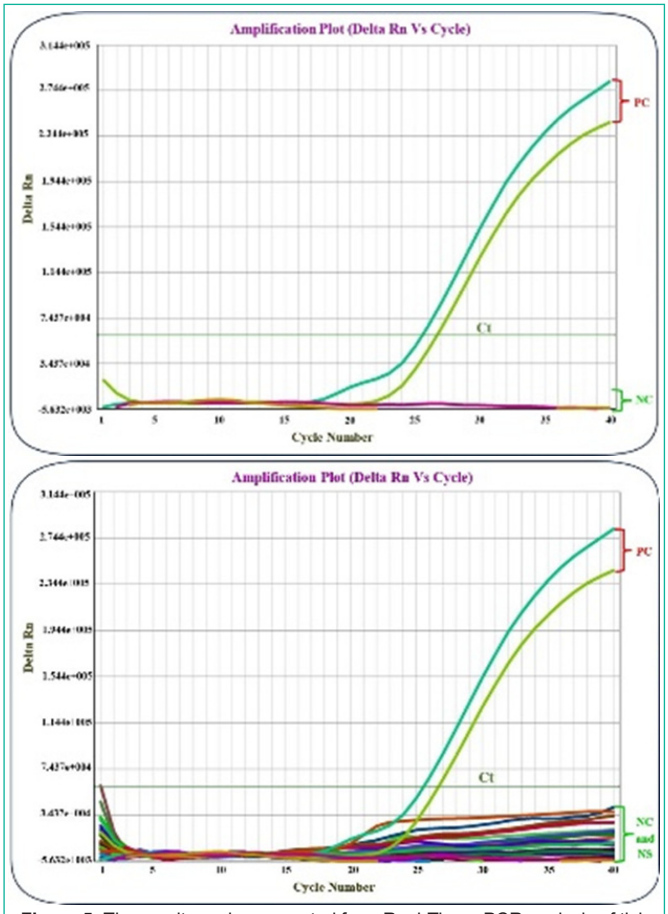

Figure 5: The result graphs generated from Real-Time qPCR analysis of tick samples. A) RT-qPCR amplification curve of this figure shows the positive and negative controls used in the analysis. B) RT-qPCR amplification curve presents the results for the tick samples analyzed along with the positive and negative controls. Where NC and PC represent the negative and positive controls, respectively. NS indicates a negative sample, and Ct stands for cycle threshold.

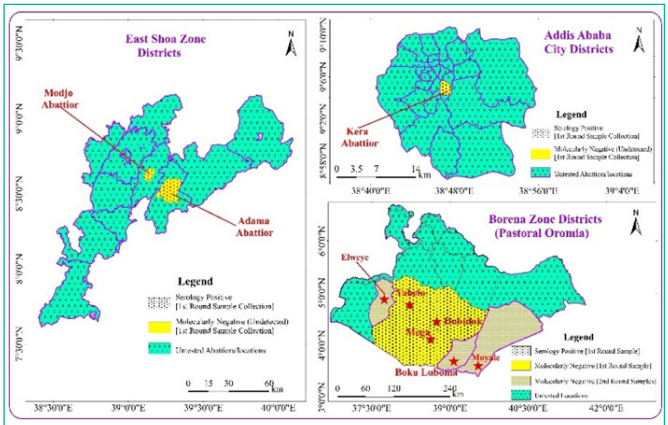

Figure 6: Geo-Referencing of samples collected including their geographical coordinates. It visually maps the locations of the sampling sites, which encompass various abattoirs and pastoral regions in Ethiopia. The figure provides a clear representation of the distribution of the collected samples, facilitating an understanding of the geographic context of the molecular detection of *C. burnetii*.

regional variations in C. burnetii distribution and infection pressure or due to variation in the sensitivity of the PCR methods used. 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 a single C. burnetii particle in 1 ml milk (Willems et al., 1994). The absence of positive results in milk samples does not rule out the occurrence of the pathogen in 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 samples from reproductive tract such as vaginal swabs and placenta/aborted 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 animals, 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 molecular methods 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.

Urine samples, one of the potential diagnostic media for C. burnetii, were analyzed from animals with history of abortion although none of them was found positive. This contrasts with the results of previous studies from other countries of the world such as the reports of Tozer et al. in Queensland, where 6.7% tested positive and in Saudi Arabia where 23.8% showed positive results (Tozer et al., 2014; 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. 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. Therefore, the negative 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 animals are infected if samples are collected when animals are not shedding it (Guatteo et al., 2007; Rodolakis et al., 2007). Shedding can also be influenced by the time of the year. As a result, samples collected during the period when animals 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.

In addition, the use of COX1 gene based PCR, which is regarded as less sensitive compared to multiple-copy gene targets may have contributed to the false negative results (Ashour et al., 2024). This limitation may explain the negative results in our study, suggesting a need for the use of more sensitive molecular methods having higher sensitivity and specificity. The occurrence of C. burnetii infection in the area can be supported by serological evidence reported in this study and previous ones.

Conclusion

This study reveals a notable seroprevalence of C. burnetii infection in livestock species, recorded at 18.77% (777 out of 4140 samples). While these findings shed light on the exposure of livestock to C. burnetii, the negative results observed on molecular analysis of biological samples collected from animals and ticks precludes the establishment of involvement of the bacterium in reproductive disorders. Consequently, it is essential for future research to integrate PCR techniques targeting multi-copy genes with serological assays. This multifaceted approach will be instrumental in further clarifying the potential role of C. burnetii in reproductive health issues, ultimately enhancing our understanding and management of this pathogen in livestock populations.

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Author Contributions

Conceptualization: The foundational ideas for this study were developed collaboratively by Ashenafi Milkesa, Teshale Sori, Tesfaye Rufael, and Hunduma Dinka.

Sample Collection: The collection of samples was efficiently carried out by Ashenafi Milkesa, Chala Guyasa, and Mekonnen Belete.

Data Curation: Ashenafi Milkesa, Teshale Sori, Tesfaye Rufael, Chala Guyasa, Abde Aliy, Tigist Ashagrie, and Hunduma Dinka meticulously organized and managed the data.

Formal Analysis: The data analysis was conducted rigorously by Ashenafi Milkesa and Teshale Sori.

Funding Acquisition: The financial support for this research was secured by Ashenafi Milkesa, Abde Aliy, Redeat Belaineh, Tesfaye Rufael, and Hunduma Dinka.

Investigation: The investigative efforts were spearheaded by Ashenafi Milkesa, Abde Aliy, Chala Guyasa, and Tigist Ashagrie.

Methodology: The methodological framework for the study was designed by Ashenafi Milkesa, Abde Aliy, Chala Guyasa, and Hunduma Dinka.

Project Administration: Oversight and administration of the project were managed by Ashenafi Milkesa, Abde Aliy, Redeat Belaineh, Tesfaye Rufael, and Hunduma Dinka.

Resources: Essential resources were provided by Ashenafi Milkesa, Abde Aliy, Hassan Zackaria Ali Ishag, Redeat Belaineh, and Tesfaye Rufael.

Software: The software tools utilized in this research were handled by Ashenafi Milkesa, Teshale Sori, and Hunduma Dinka.

Supervision: The research was supervised by a dedicated team of Ashenafi Milkesa, Teshale Sori, Abde Aliy, Chala Guyasa, Redeat Belaineh, Tesfaye Rufael, and Hunduma Dinka.

Validation: The validation of results was ensured by Ashenafi Milkesa, Abde Aliy, Chala Guyasa, Redeat Belaineh, Tesfaye Rufael, and Hunduma Dinka.

Visualization: The visual representation of the data was crafted by Ashenafi Milkesa, Teshale Sori, Abde Aliy, Chala Guyasa, Tesfaye Rufael, and Hunduma Dinka.

Writing – Original Draft: The initial manuscript was penned by Ashenafi Milkesa, Teshale Sori, Tesfaye Rufael, and Hunduma Dinka.

Writing – Review & Editing: The refinement and editing of the manuscript involved contributions from Ashenafi Milkesa, Teshale Sori, Tesfaye Rufael, Abde Aliy, Chala Guyasa, Hassan Zackaria Ali Ishag, and Hunduma Dinka.

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