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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY, AND POULTRY HEALTH

MSc THESIS

ONE HEALTH SURVEILLANCE OF ZOONOTIC DISEASES AT THE WILDLIFE–HUMAN–LIVESTOCK INTERFACE IN BORENA ZONE, ETHIOPIA: DETECTION AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* FROM RAW CAMEL MILK

BY

GUYO GELMA GOLO

ADVISOR: OLANA MERERA (DVM, MSc; ASSISTANT PROFESSOR)

A MSc Thesis (ONEH 8632) Submitted to the Department of Microbiology, Parasitology, and Poultry Health for the Degree of Master of Science in One Health Program

JUNE, 2026

BISHOFTU, ETHIOPIA

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As a member of examining board of the final MSc, open defense we certify that we have read and evaluate the thesis prepared by Guyo Gelma Golo

Entitled:

ONE HEALTH SURVEILLANCE OF ZOONOTIC DISEASES AT THE WILDLIFE–HUMAN–LIVESTOCK INTERFACE IN BORENA ZONE, ETHIOPIA: DETECTION AND CHARACTERIZATION OF LISTERIA MONOCYTOGENES

And

We recommend that it be submitted as fulfilling the MSc thesis requirement for the degree of Master of One Health

Chairman	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

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SIGNED DECLARATION SHEET

By my signature below, I declare and affirm that this thesis is my own work. I have prepared, gathered, analyzed, and finished this thesis in accordance with all ethical guidelines for scholarship. Every academic reference in the thesis has been acknowledged with a citation. I certify that every source I used to create this document has been cited and referenced. This thesis has been prepared with every serious precaution to prevent plagiarism.

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LIST OF ABBREVIATIONS

ABM	Agent-Based Model
AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
BHI	Brain Heart Infusion
BLEB	Buffered Listeria Enrichment Broth
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistical Agency
CVMA	College of Veterinary Medicine and Agriculture
EPHI	Ethiopian Public Health Institute
FGD	Focus Group Discussion
ISO	International Organization for Standardization
KAP	Knowledge, Attitude, and Practice
KII	Key Informant Interview
LM	*Listeria monocytogenes*
NMA	National Meteorological Agency
OHHLEP	One Health High-Level Expert Panel
OXA	Oxford Agar
PA	Peasant Association
SIM	Sulfide Indole Motility
STATA	Statistical Software for Data Analysis
TSA	Tryptic Soy Agar
USFDA	United States Food and Drug Administration
CFSAN	Center for Food Safety and Applied Nutrition
WHO	World Health Organization

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ABSTRACT

This study in investigated the prevalence of *Listeria monocytogenes* in raw camel milk and assessed community knowledge, perceptions, and practices related to zoonotic disease transmission at the livestock-human-wildlife interface in Borena Zone, southern Ethiopia. A cross-sectional study was undertaken from November 2025 to May 2026 in Yabello, Elwaye, and Gomole districts to investigate the zoonotic diseases occurrence and transmission at the livestock-human-wildlife interface. Additionally, isolation and characterization of *Listeria monocytogenes* in raw camel milk and milking equipment was done. A total of 411 camel milk related samples were collected from lactating camels and milk handling equipment, while 207 pastoralists participated in a questionnaire survey. Microbiological isolation and identification of *Listeria monocytogenes* standard bacteriological procedures were employed, and antimicrobial susceptibility testing was performed using the disk diffusion method. The overall prevalence of Listeria in camel milk and related samples was 3.89% (95% CI: 2.0–5.7%), with slightly higher contamination at the animal level (4.47%) than in milk handling equipment (3.77%). None of the investigated animal-level factors, including age, herd size, and body condition, showed a statistically significant association with Listeria occurrence ($p > 0.05$). Antimicrobial susceptibility testing revealed high sensitivity of isolates to Gentamicin (100%) and high susceptibility to Chloramphenicol, Ciprofloxacin, Amoxicillin, Doxycycline, Azithromycin, and Meropenem (80%). However, Penicillin and Cefoxitin shows higher resistance against tested isolates 80% and 100%, respectively, with evidence of multidrug resistance among some isolates. The questionnaire survey discovered widespread livestock–wildlife interactions, frequent cross-border livestock movement, and high-risk practices including consumption of raw animal products and lack of quarantine measures. Although community awareness of disease risks was relatively high, risky management and unsafe food consumption behaviors remained common. The findings highlight the presence of *Listeria monocytogenes* in camel milk and emphasize the importance of improved milk hygiene, antimicrobial stewardship, and integrated One Health approaches to mitigate zoonotic disease risks in pastoral production systems.

Keywords: *Borena, Camel, Listeria monocytogenes, Milk, One Health, Wildlife*

1. INTRODUCTION

Zoonotic diseases are estimated to account for 2.5 billion cases of human illness globally annually (Salyer *et al*.,2017). Over 36% of emerging and re-emerging Zoonotic diseases are commonly associated with food-producing animals. (Otte *et al*., 2021). Approximately two-thirds of infectious diseases affecting humans originate from pathogens shared with wildlife or domesticated animals. However, the ecological, evolutionary, social, economic, and epidemiological mechanisms that influence the persistence and emergence of zoonoses remain poorly understood. (Karesh *et al.,* 2012). In recent years, the risk of transmission of these diseases is exacerbated by the interactions among human, animal, and ecosystem dynamics, driven by the exponential growth of human and livestock populations, rapid urbanization, transformative agricultural practices, increased contact between livestock and wildlife due to forest encroachment, habitat destruction, alterations in ecosystems, and the globalization of trade (Jones *et al.,* 2013).

The livestock-wildlife interface refers to the region where communities engaged in livestock production frequently interact, either directly or indirectly, with wild animal populations. The emergence of wildlife diseases is particularly significant; as such diseases can adversely affect not only wild animals but also domesticated species and humans. There has been an increasing concern regarding both emerging and re-emerging diseases, many of which are notably prevalent at the fertile livestock-wildlife interface (Gortázar *et al.,* 2006). Zoonotic diseases originating from wildlife pose a substantial threat to global health among all emerging infectious diseases (Jones *et al.,* 2008). Currently, the risk of disease transmission is exacerbated by the interactions among humans, animals, and ecosystems, driven by the exponential increases in human and livestock populations, rapid urbanization, evolving agricultural practices, closer integration between livestock and wildlife due to forest encroachment, habitat destruction, ecosystem changes, and the globalization of trade (Jones *et al.,* 2013).

The interface between wildlife and livestock diseases occurs not only through direct physical contact but also indirectly through various mediums such as soils, forage, water sources, insect

vectors, and intermediate hosts (Atuman *et al*., 2021). In recent years, the management of livestock, wildlife, and the environment at this interface has presented significant challenges in reconciling development with environmental conservation in Sub-Saharan Africa (Tschopp *et al.,* 2010). Ethiopia faces a significant burden of zoonotic diseases and is home to a large population of low-icome livestock keepers who depend on animals for their livelihoods. However, limited coordination between the human and animal health sectors, together with constrained public health resources, has weakend surveillance systems and reduced the country's ability to respond effectively to emerging public health threats (Pieracci *et al.,* 2016).

To address the intricate interactions among humans, animals, and the environment, the One Health approach has garnered increasing attention within the realms of global health research and policy. The framework of One Health posits that human health, animal health, and ecosystem health are interrelated, necessitating collaborative and multidisciplinary strategies for effective disease prevention and control that engage veterinary, medical, environmental, and public health sectors (Machalaba *et al.,* 2018). Within this context, *Listeria monocytogenes* provides a valuable model organism for investigating zoonotic risks at the livestock human wildlife interface. This bacterium is widely distributed in the environment and can persist in soil, water, vegetation, and animal reservoirs, enabling its circulation across multiple ecological niches. It is the causative agent of Listeriosis, a severe infection that manifests as meningitis, septicemia, abortion, and neonatal infections in humans, and encephalitis, septicemia, and reproductive disorders in livestock. (Jordan and McAuliffe, 2018). Because of its environmental persistence, wide host range, and transmission through contaminated food, water, and environmental sources, *Listeria. monocytogenes* increasingly recognized as a valuable indicator pathogen for studying the eco-epidemiological studies of zoonotic diseases in complex ecosystems. Despite its recognized public health importance and ecological adaptability, comprehensive data on its occurrence, environmental distribution, and transmission dynamics of this pathogen in pastoral ecosystems of southern Ethiopia remain limited. In particular, to the best of current knowledge, no previous studies have investigated the presence or eco-epidemiological characteristics of *L. monocytogenes* in the livestock–human–wildlife interface of the Borena Zone, highlighting a critical knowledge gap that this study seeks to address.

1.1. Problem Statement

The increasing incidence of outbreaks caused by *Listeria monocytogenes* and the associated food recalls highlight the need for closer examination of its contamination in food systems, particularly in milk within African contexts. Milk represents an important dietary component in many African countries and is among the food products most vulnerable to contamination due to production, handling, and storage conditions that often favor microbial proliferation. In pastoral production systems where raw milk consumption is common and cold-chain infrastructure is limited, the risk of contamination may be further amplified. Consequently, a more comprehensive biosafety and risk assessment is warranted (Oluwafemi *et al*., 2023).

Although Listeriosis is generally considered a relatively rare foodborne disease globally, it can lead to severe clinical outcomes such as meningitis, septicemia, abortion, and neonatal infections. Despite its serious health consequences, listeriosis remains largely underrecognized and underreported in many African public health systems, and the true burden of the disease across the continent remains poorly understood (Havelaar *et al*., 2015). In addition, most available studies in Africa focus primarily on food contamination and clinical cases, while the broader ecological and environmental reservoirs of the pathogen receive limited attention.

Pastoral ecosystems present particularly complex settings for the circulation of zoonotic pathogens due to the close interactions among humans, livestock, wildlife, and shared environmental resources. The Borena Zone represents one of the largest pastoral areas in Ethiopia, where livestock production is the primary livelihood and where pastoral communities frequently share grazing lands and water sources with wildlife. These ecological and socio-economic dynamics may facilitate the persistence and transmission of environmentally resilient pathogens such as Listeria monocytogenes, which is capable of surviving in soil, water, vegetation, and animal reservoirs. However, there remains a substantial knowledge gap regarding the eco-epidemiology, environmental distribution, and transmission pathways of this pathogen within pastoral livestock–human–wildlife interface systems.

Despite the ecological conditions that may favor pathogen circulation, little is known about the occurrence and transmission dynamics of *Listeria monocytogenes* in pastoral ecosystems of southern Ethiopia. In particular, no previous studies have investigated the presence or eco-epidemiological characteristics of this pathogen at the livestock–human–wildlife interface in the Borena Zone. Addressing this knowledge gap is essential for improving understanding of zoonotic risks in pastoral systems and for informing integrated surveillance and control strategies within a One Health framework.

The increasing incidence of *Listeria* outbreaks and associated food recalls necessitates a closer examination of *Listeria monocytogenes* contamination, particularly in milk within Africa. Milk is a significant food source in the region and is among the products most susceptible to Listeria contamination due to the conditions surrounding its production and storage, which favor microbial proliferation. Thus, a more comprehensive biosafety assessment is warranted. While global initiatives aimed at preventing contamination and outbreaks are gaining traction, the extent to which these efforts are implemented in Africa remains largely unclear (Oluwafemi *et al.,* 2023). Although listeriosis is typically classified as a rare foodborne illness, it is not prioritized within African public health systems, and its prevalence in the continent is not thoroughly understood (Havelaar *et al.,* 2015). The Borena Zone of Ethiopia presents a unique ecological environment where pastoral communities rely heavily on livestock while coexisting with wildlife species and sharing natural resources. However, there is a paucity of information regarding the eco-epidemiology of zoonotic pathogens in this area (Megersa *et al.,* 2011).

1.2. General Objective

To investigate the zoonotic diseases occurrence and transmission at the livestock-human-wildlife interface in the Borana Zone of Ethiopia, using ecological, socio-economic, and behavioral factors that influence disease transmission. Additionally, *Listeria monocytogenes* was isolated and characterized as an indicator pathogen within a One Health framework.

1.3. Specific Objectives

- To assess the community perception of the influence of ecological factors such as climate variability and land-use change on the transmission dynamics of infectious diseases.
- To assess percieved socio-economic factors, including pastoral mobility and resource competition, contribute to the persistence and spread of infections.
- To evaluate community knowledge, attitudes, and practices (KAP) related to infectious disease prevention and control using participatory epidemiology and survey methods.
- To isolate and characterize *Listeria monocytogenes* in Camel milk, and equipment's used for milk handling in the Borena Zone.

2. LITERATURE REVIEW

2.1. One Health and Eco- Epidemiological Approaches to Zoonotic Diseases

One Health is an emerging concept that seeks to sustainably improve the well-being of humans, animals, and ecosystems, addressing current and emerging health threats at the human–animal–environment interface (WHO, 2021; Mackenzie and Jeggo, 2019). The importance of One Health has increased due to the rising incidence of infectious diseases, many of which are of environmental zoonotic origin. Around 60% of human infections have an animal origin, of which approximately 75% are considered emerging or re-emerging diseases. The remaining 40% either co-evolved with humans or arose from non-zoonotic environmental sources. (Weiss and Sankaran, 2022; Thompson *et al.,* 2024). Health service provision in pastoralist areas typically involves separate interventions for human and livestock health, often disregarding the critical environmental factors upon which both depend for their survival. More recently, however, there have been attempts to integrate human, animal, and environmental health service provision in these areas through the application of One Health approaches. (Griffith *et al.,* 2020a, 2020b; OHHLEP, 2022).

One Health is a collaborative and multi-disciplinary approach that recognizes the interdependent health of humans, animals, plants, and the wider environment. Ethiopia launched its National One Health Strategy in 2018 to improve coordination across sectors, aiming to prevent, detect, and rapidly respond to zoonotic diseases and related health challenges. This is particularly crucial in pastoral areas where communities maintain strong consumption, aesthetic, and economic ties with their livestock. Empirical One Health research can elucidate the risks from zoonotic infections, thereby guiding the development of effective interventions.
(Alemayehu *et al.,* 2021).

Eco-epidemiological thought is recognized as a more suitable and effective approach to epidemiology in the modern world. However, the risk factor paradigm remains the dominant, if not exclusive, focus of training in epidemiology. (Bain and Awah, 2014).

Ecology and disease ecology provide better insights into understanding disease dynamics and outbreak management. The key reason for the increase and expansion of emerging infectious diseases is directly associated with human-driven factors, including deforestation, global climate change, and urbanization. The transmission of pathogens from wild animals to humans is defined as zoonotic spillover. Indeed, between 60% and 75% of human infectious diseases are caused by pathogens that originated in other animal species. Zoonotic spillover refers to the transmission of pathogens from wild animals to humans. The majority of human infectious diseases (60–75%) originate from pathogens that initially circulated in other animal species. (Ellwanger and Chies, 2021). The use of ecological fundamentals with epidemiological modeling approaches provides useful insights for mitigating the exacerbating threats caused by zoonotic spillover. Epidemiological models, including meta-population models, compartmental models, and agent-based models (ABM), together with machine learning models, facilitate valuable data such as disease prediction and improve both ecosystem and public health in terms of disease transmission and control (Saqlain *et al.,* 2025).

The livestock-human-wildlife interface, hereafter referred to as the interface, constitutes a network of ecological and epidemiological interactions occurring between livestock and wildlife within their shared geographical space. (Vicente *et al., 2021*). The development and geographical range of the interface are influenced primarily by forage and water resource distribution, climate change, and human, livestock, and wildlife population dynamics (Madzingira *et al.,* 2025). Understanding the origins of antimicrobial resistance in wildlife is crucial for human health, given the increasing significance of zoonotic diseases and the imperative to predict emerging resistant pathogens. (Radhouani *et al.,* 2014). The design and implementation of such efforts, aiming at human health improvement and poverty alleviation, should be framed into adaptive social-ecological system management perspectives (Gilioli *et al.,* 2014).

2.2. Biology and Characteristics of *Listeria monocytogenes*

Listeria monocytogenes is an intracellular, Gram-positive,non-spore-forming,non-encapsulated, facultative anaerobic, rod-shaped, and psychrotrophic foodborne pathogen that causes listeriosis,

an infection whose outbreaks frequently garner significant attention due to their association with high mortality rates.(Manyi *et al*., 2025). *Listeria monocytogenes* It comprises at least four evolutionary lineages (I, II, III, and IV) with distinct but overlapping ecological niches. Most *L. monocytogenes* isolates primarily belong to lineages I and II, which encompass the serotypes most frequently associated with human clinical cases, including serotype 1/2a (lineage II) and serotypes 1/2b and 4b (lineage I). Lineage II strains are prevalent in food products, are widely distributed in natural and farm environments, and are also frequently isolated from animal listeriosis cases and sporadic human clinical cases. (Orsi *et al.,* 2011).

Listeria is mobile at temperatures of 20-25°C, owing to the presence of peritrichous flagella, and immobile at 37°C. It is catalase-positive, oxidase-negative, and Esculin hydrolysis-positive. During its exponential phase of multiplication, *L. monocytogenes* produces listeriolysin O (hemolysin), a toxin that results in in vitro hemolysis on blood agar. *L. monocytogenes* demonstrates increased resistance to the external environment (Saha *et al.,* 2015). It can survive at a relatively low water activity (aW <0.90), across a broad pH range of 4.6 to 9.5, and tolerates salt concentrations up to 20% (Bucur *et al.,* 2018). These characteristics render it a highly adaptable organism, capable of persisting in a wide range of environmental and food-related niches (NicAogáin and O'Byrne, 2016).

A further factor to consider is the capacity of pathogenic microorganisms to develop resistance to existing antimicrobial agents when forming biofilms. (Mazaheri *et al.,* 2021). The ability of microbes to form biofilms is considered an adaptable trait and a long-established survival mechanism, providing biofilm-producing bacteria with enhanced environmental stability, improved access to nutritional sources, increased metabolic activity and intercellular interactions, greater tolerance to biocides, and a superior capacity for growth in oligotrophic environments, unlike their planktonic counterparts. (Muhammad *et al.,* 2020). In pastoral ecosystems, specific physiological traits enable *Listeria* to survive harsh dry seasons and facilitate continuous reintroduction into livestock populations (Oevermann *et al.,* 2010).

2.3. Epidemiology of *Listeria monocytogenes*

The foodborne pathogen *Listeria monocytogenes* is the causative agent of human listeriosis, a severe disease particularly dangerous for the elderly, pregnant women, and newborns. Although this infection is relatively uncommon, it is often associated with a significant worldwide mortality rate of 20–30%. (Buchanan *et al.*, 2017). *L. monocytogenes* can adapt, survive, and grow across a broad spectrum of environmental stressors prevalent in food production facilities, including temperature fluctuations, extreme pH levels, elevated salinity, ultraviolet radiation, biocides, and heavy metals. Upon exposure to cold stress, *L. monocytogenes* demonstrates a range of responses. (Osek *et al., 2022*). Several investigations have shown that *Listeria monocytogenes* is widely distributed in food processing environments, where it can persist for extended periods due to ineffective cleaning and sanitation (Carpentier and Cerf, 2011; Ferreira *et al.*, 2014; Buchanan *et al.*, 2017). These organic osmolytes are found in high concentrations in various foods, which may promote the survival and growth of *L. monocytogenes* at lower temperatures (Zeisel *et al.*, 2003).

The extended refrigerated storage of ready-to-eat foods, which can facilitate the proliferation of *L. monocytogenes* in products contaminated during production or post-processing; the practice of feeding domestic ruminants silage often contaminated with *L. monocytogenes*; and the dissemination of contaminated sewage treatment byproducts (e.g., biosolids) to agricultural fields and waterways. (Ivanek *et al., 2006*). Listeriosis is a serious foodborne disease with increasing incidence in humans and ruminants. Despite its manifestation as rhombencephalitis in both hosts and the role of ruminants as a reservoir for human-pathogenic *Listeria monocytogenes* (LM) strains, the pathogenesis of listeriosis in ruminants remains underexplored. (Oevermann *et al.*, 210). Owing to their extensive biodiversity, natural environments serve as reservoirs for pathogens novel to domestic animals and humans. Consequently, transmission can occur via direct or indirect contact with wildlife. (Marrana, 2022).

The surveillance efforts in low- and middle-income countries (from which the majority of disease-specific challenges emerge (Phalkey *et al.*, 2015). limited to humans, when over 60% of

the emerging diseases detected between 1940 and 2004 were caused by zoonotic pathogens (Jones *et al*. 2008). Food safety challenges, such as poor hygiene and consumption of raw animal products, increase the risk of contamination in these regions (Uyttendaele *et al.,* 2016). In the Borena zone, frequent livestock movement and interaction with wildlife at water points create favorable conditions for the persistence of this zoonotic pathogen (Grace *et al., 2012*).

2.4 Pathogenesis and Clinical Manifestations of Listeriosis

Listeria is a soil-borne bacterium that causes disease following ingestion by the animal. It is especially associated with cattle eating mouldy silage in winter. If the silage harvester picks up bits of soil during harvesting, there is usually poor fermentation around this, as well as the presence of Listeria (Hernandez and Payeras, 2014). Invasive *L. monocytogenes* infections have been associated with meningitis in adults. Typically, clinical presentation involves symptoms characteristic of subacute bacterial meningitis, such as fever, headache, and neck stiffness, which may evolve over several days. (Schlech, 2019).

In the context of foodborne listeriosis epidemics, meningitis caused by *L. monocytogenes* can also affect apparently healthy individuals across all age groups. In contrast, in sporadic cases, a defect in cell-mediated immune function predisposes subjects to invasive listeriosis. (Carrillo-Esper *et al*., 213). In addition, *L. monocytogenes* can induce rhomboencephalitis in humans and animals, which is mainly described as circling disease. When these features appear, fever, headache, nausea, and vomiting occur early, and signs of meningeal irritation are less commonly observed. Subsequently, multiple abnormalities of cranial nerves develop with associated cerebellar dysfunction, mainly ataxia. (Giménez-Muñoz *et al.,* 2015).

Listeria monocytogenes is the causative agent of human listeriosis, a potentially fatal foodborne infection. Clinical manifestations range from febrile gastroenteritis to severe invasive forms, including sepsis, meningitis, rhombencephalitis, perinatal infections, and abortion (Bongiovanni *et al.,* 2024). In recent years, a rising incidence of listeriosis has been reported in several European countries. This increase primarily reflects a higher incidence of bacteraemic listeriosis

among individuals aged 65 years and older, and shows no correlation with geography, gender, ethnicity, socioeconomic factors, or infectious serotypes. (Allerberger and Wagner,2010).
Although relatively rare, foodborne listeriosis is a serious disease with high fatality rates (20%–30%) compared with other foodborne microbial pathogens. (FAO, 2004). In developing countries, the burden is exacerbated by the common practice of consuming raw products in proximity to livestock settlements (Tola, 2024). Adopting a one health perspective is essential for understanding how the pathogen moves between environmental reservoirs and human populations (Grace *et al.,* 2012).

2.5. Environmental Reservoirs of *Listeria monocytogenes*

Listeria monocytogenes is a ubiquitous bacterium that can be isolated from soil, water, and feed. It has been demonstrated that the bacteria (Linke *et al.,* 2014). can survive in the environment for at least 8 weeks (Dhama *et al.,* 2015). Transfer of *Listeria innocua* from soil fertilized with contaminated compost or irrigated with contaminated water to the edible parts of lettuce grown on these soils, and its survival in lettuce and soil under field conditions. (Oliveira *et al.,* 2011). *L. monocytogenes* is transmitted to consumers primarily via contaminated ready-to-eat foods.
(Allerberger and Wagner, 2010; Todd and Notermans, 2011).

The presence and potential persistence of Listeria spp in food processing facilities are often caused by environmental recontamination at the farm or plant level (GelbíčoVá and KaRpíšKoVá.,2012). Animal feces and the broader farm environment represent another significant source of contamination, as livestock can shed the bacterium as asymptomatic carriers (Hellström *et al*,2008). The environment Listeriathrives in silage or other preserved forages that do not reach the appropriate pH level during the fermentation phase; pH <4.5 inhibits growth of this potentially harmful bacterium (Quiroz *et al*., 2018. Poor waste management and the accumulation of manure further facilitate the persistence of the pathogen on farm equipment and surfaces (Carpentier and Cerf, 2011).

Listeria monocytogenes is a facultative intracellular pathogen asymptomatically harbored by various animals and shed in their feces. The prevalence and characteristics of *L. monocytogenes* isolated from livestock, wildlife, and potential human sources of contamination were investigated. (Lyautey *et al.*, 2007). In pastoral regions like Borena, the interaction between domestic animal and wildlife is high due to shared rangelands and water sources (Grace *et al.*, 2012). Understanding these wildlife environmental dynamics is vital for identifying sources of contamination that prevent spillover in to the human food chain (Ivanek *et al.*, 2006).

2.6. Transmission Pathways of *Listeria Monocytogenes*

Listeriosis is a serious invasive disease primarily affecting pregnant women, neonates, and immunocompromised adults. The causative organism, *Listeria monocytogenes*, is primarily transmitted to humans through contaminated foods. This pathogen presents a significant challenge to control due to its ability to thrive in diverse environments, including refrigerated conditions, which are typically considered safe for food preservation. (Ribeiro *et al.*, 2023; Belias *et al.*, 2024). In pastoral communities, the widespread consumption of raw milk significantly contributes to meeting protein and micronutrient requirements. However, this practice carries inherent health risks stemming from inadequate hygienic practices during milk handling and consumption. In pastoral communities, raw milk is widely consumed and substantially contributes to the community's protein and micronutrient requirements. However, this practice entails health risks stemming from suboptimal hygienic practices during milk handling and consumption. (Sadler *et al.*, 2009; Elhadi *et al.*, 2015).

The pathogen is widely distributed in the environment and can be transmitted through several pathways (Swaminathan and Gerner-Smidt, 2007). Environmental reservoirs, encompassing soil, water, and biofilms, are recognized as critical to the pathogen's persistence and transmission. The interplay of climate change, agricultural practices, and industrial processes further exacerbates the complexity of *L. monocytogenes* control, thereby necessitating cross-disciplinary approaches. (Ryzhova *et al.*, 2025). Livestock animals may also carry the pathogen asymptomatically, leading to contamination of raw milk and meat products during milking, slaughtering, or

processing. Consequently, animal-derived foods represent an important source of human infection when proper hygiene and food safety measures are not implemented (Ribeiro *et al.*, 2023). Listeria *monocytogenes* is one of the few pathogens capable of crossing the placental barrier and causing significant harm to the fetus, leading to spontaneous abortion, stillbirth, preterm labor, and disseminated neonatal infection, even despite antibiotic treatment. (Lamond *et al.*, 2018).

2.7. *Listeria monocytogenes* in Livestock Production Systems

Listeria. monocytogenes can infect various livestock species, including cattle, sheep, goats, and pigs. (Bagatella *et al.*, 2021). *L. monocytogenes* is present in the faeces and feedstuffs of cattle and other ruminant livestock, and can persist on farms through biofilm formation on surfaces. (Obaidat *et al*, 2020). In farming, affected animals can shed the pathogen in their faeces, facilitating bacterial circulation. (Kotzamanidis *et al*, 2019). Pathogens in manure applied to agricultural land can be disseminated via leaching, surface runoff, contamination of water sources, and contamination of crops. Therefore, it is crucial to understand the persistence of multiple pathogens in manure and soil environments at the farm scale, and the potential for crops cultivated under these conditions to serve as a transfer route for zoonotic pathogens. (Black *et al.*, 2021). Poorly fermented silage facilitates the survival and multiplication of *L. monocytogenes*, the causative agent of listeriosis and the pathogen associated with silage disease" or "circling disease in ruminants. (Gezali *et al*, 2016). Animals consuming contaminated silage may develop clinical listeriosis or become asymptomatic carriers capable of shedding the organism into the farm environment. This contamination cycle increases the likelihood of transmission among animals and contamination of livestock products (Dreyer *et al*, 2016).

Listeria monocytogenes present in livestock environments may contaminate animal-derived food products such as raw milk and meat during milking, slaughtering, or processing, thereby posing a significant food safety risk and contributing to human listeriosis outbreaks (Jordan and McAuliffe, 2018). Listeriosis presents a significant health challenge to cattle, sheep, and goats, manifesting as abortions, septicemia, and meningoencephalitis. Ruminants are key reservoirs for

Listeria, thereby facilitating zoonotic transmission. In addition to animal health impacts, listeriosis results in economic losses due to decreased productivity, reproductive failure, treatment costs, and animal mortality Effective control of *L. monocytogenes* in livestock production systems requires proper silage fermentation, improved manure management, farm hygiene, and routine microbiological monitoring to reduce environmental contamination and prevent transmission to animals and humans (Koncurat *et al.,* 2024).

2.8. Role of Wildlife in the Epidemiology of *Listeria monocytogenes*

Wildlife plays an important ecological role in the maintenance and dissemination of *Listeria monocytogenes*, a zoonotic foodborne pathogen widely distributed in natural environments (Lourenco *et al.,* 2022) Numerous studies have reported the presence of *L. monocytogenes* in a variety of wild animal species, including mammals, birds, and reptiles. These animals often carry the bacterium asymptomatically and shed it through feces, thereby contributing to environmental contamination of soil, water, and vegetation Such contamination can subsequently affect livestock, crops, and food processing environments, creating pathways for transmission to humans through the food chain (Schoder *et al.,* 2022).

Wild birds are considered particularly important in the epidemiology of *L. monocytogenes* due to their wide geographic movement and ability to disseminate pathogens over long distances. Several studies have reported *L. monocytogenes* in fecal samples of birds such as pigeons, gulls, sparrows, and crows, indicating that these species can act as reservoirs and vectors of the bacterium. The strains isolated from wild birds have sometimes shown genetic similarity to strains detected in food products and human infections, suggesting a potential link between wildlife and foodborne transmission (Hellström *et al.,* 2008).

In addition to birds, many wild mammals, including deer, rodents, foxes, and wild boars, have been identified as carriers of *L. monocytogenes*. These animals may contaminate agricultural environments directly through fecal deposition or indirectly through contamination of water sources used for irrigation or livestock production Recent genomic studies have demonstrated

that *L. monocytogenes* strains isolated from wildlife share genetic relationships with strains detected in food processing environments and food products, highlighting the potential role of wildlife as reservoirs introducing pathogenic and persistent strains into the food production chain (Chiaverini *et al.,* 2026).

The wildlife livestock environment interface is therefore a critical component in the epidemiology of *L. monocytogenes* (Schoder *et al.,* 2023). Increasing interactions between wildlife, livestock, and human activities driven by land-use change, agricultural expansion, and habitat encroachment may facilitate cross species transmission of the pathogen Consequently, surveillance of wildlife populations and integration of wildlife data into One Health frameworks are essential for improving the understanding and control of *L. monocytogenes* transmission dynamics and reducing risks to public health (Wareth and Neubauer, 2025).

2.9. Zoonotic Risk at the Livestock Human Wildlife Interface

Climate change has emerged as a significant factor influencing the ecology and epidemiology of zoonotic diseases. Elevated temperatures, altered rainfall patterns, and the increasing frequency of extreme weather events are reshaping the distribution, transmission, and virulence of pathogens circulating at the human-livestock-wildlife interface (Reginald, 2024). These environmental shifts are particularly detrimental to pastoral systems, which are highly exposed and exhibit limited ecological resilience and adaptive capacity (Wang *et al.,* 2025). Persistent droughts have precipitated a scarcity of feed and water, leading to livestock starvation and mortality, and thereby jeopardizing the livelihoods of pastoralists (Mengistu, 2016). Moreover, declines in milk production and diminishing market prices have further contributed to adverse socio-economic impacts (Tamene *et al.,* 2023).

Borana pastoralists have customarily employed several long-term adaptation strategies, including the diversification of livelihood sources, strategic livestock mobility to access forage and water resources, and the diversification of herd composition to leverage varying drought tolerance. (Sintayehu *et al.,* 2025). Common practices such as traditional husbandry, poor management

practices, mixing of wild and farm animals, and unrestricted movement and cohabitation of pastoralists with their animals are thought to facilitate the spread of zoonotic diseases. Additionally, consuming unpasteurized milk, handling aborted fetuses with bare hands, and improper disposal of birth or aborted materials (e.g., by throwing them in fields) are significant contributing factors (Belay, 2006; Desta,2015). Humans and animals can become asymptomatic carriers of *Listeria monocytogenes* and introduce the pathogen into their environment with their feces. In turn, this environmental contamination can become the source of food- and feed-borne illnesses in humans and animals, with the food production chain representing a continuum between the farm environment and human populations susceptible to listeriosis (Schoder *et al.*, 2022).

In order to evaluate this risk, a One Health approach is necessary since it takes into consideration the intricate socio-ecological interaction (Karesha *et al*, 2012). Due to population mobility and a lack of integrated reporting across sectors, traditional surveillance frequently fails in remote locations (Zinsstag *et al.,* 2011). Researchers can determine how environmental stressors raise the contact rate between species by looking at the interface (Jones *et al*, 2023).

2.10. Antimicrobial Resistance in *Listeria monocytogenes*

Antimicrobial resistance in *L. monocytogenes* is influenced by both innate and acquired mechanisms. Intrinsic resistance is indicated by decreased β-lactam binding to penicillin-binding proteins (PBPs), but acquired resistance is associated with genes regulated by plasmids and transposons, such as tet(M), erm(B), and aadA (Moura *et al.,* 2024). Currently, *Listeria* species do not frequently exhibit multidrug resistance. However, *Listeria* species possess the capacity to rapidly acquire resistance to antimicrobial drugs, a characteristic that poses an emerging and escalating threat to human and animal health, as has been observed with other clinically significant pathogens. (Luque Sastre *et al.,* 2018). The pathogenicity of certain bacterial species, such as *Listeria monocytogenes*, is contingent upon not only their virulence but also adaptive mechanisms facilitating their persistence and proliferation in challenging environments, including biofilm formation and antibiotic resistance. (Naves *et al* 2008; Matereke and Okoh,

2020). Some strains of *Listeria monocytogenes* are capable of forming robust biofilms on different surfaces in food processing plants (Ripolles-Avila *et al*, 2019). persisting in these environments, because to the substantial tolerance sessile cells exhibit towards disinfectants employed during facility sanitization. (Finn *et al*, 2023).

Furthermore, the use of sub-lethal concentrations of disinfectants can be counterproductive, as low doses of biocides are linked to increased tolerance to these substances, resistance to antibiotics, and an enhanced bacterial capacity to form biofilm. (Rodríguez-Melcón *et al.,* 2021). Antimicrobial Resistance (AMR) is influenced by factors such as human practices (antimicrobial overuse and misuse, insufficient infection deterrence, and lack of awareness);animal-related practices (widespread antibiotic use in livestock and aquaculture, transmission through food chains and direct contact); environmental factors (release of antibiotics and resistant bacteria into water bodies, soil, and waste systems); and wildlife-related factors (transmission through contact with wildlife and habitat encroachment) (Endale *et al* .,2023).

Antimicrobial resistance's rise. *Listeria monocytogenes* is a prime example of how environment, animal, and human health are intertwined. Antibiotic misuse in livestock production leads to resistance that may spread across the ecosystem and food chain (Getaneh *et al.,* 2025). Even in the absence of selective pressure, biofilm persistence and environmental pollution contribute to the maintenance of resistance strains (Tarawneh *et al.,* 2022). To reduce the dangers associated with antimicrobial resistance (AMR), a one-health approach that incorporates environmental, public health, and veterinary surveillance is crucial (Singh *et al.,* 2024). This strategy supports the prudent use of antibiotics, improves Ethiopia's food safety regulations, and fortifies laboratory capability (Woldu, 2024).

2.11. Occurrence of *Listeria monocytogenes* in Ethiopia and Africa

Listeriosis is a serious foodborne disease that can cause severe illness and death in high-risk groups, such as pregnant women, newborns, the elderly, and immunocompromised individuals (Koopmans et al., 2023). The disease is caused by *Listeria* species, with *L. monocytogenes* being the main pathogenic species that affects both animals and humans (Cheng *et al*., 2020; Chlebicz *et al*., 2018). In Ethiopian foods, *Listeria* contamination rates range from 14.3% to 62% in raw meats, dairy products, and vegetables (Molla *et al*., 2004). Notably, the highest prevalence has been observed in raw beef (62%) and ice cream (43%) samples (Tola, 2024).

In African public health systems, *Listeria monocytogenes* is a pathogen of relatively low priority. However, the largest listeriosis outbreak recorded to date occurred in Africa in 2018 (Sibanda *et al., 2023*). This discrepancy between the historically low incidence and the occurrence of an outbreak of such magnitude in Africa likely reflects deficiencies in food safety systems and an insufficient understanding of the risk factors influencing *L. monocytogenes* transmission dynamics within African food value chains (Jaffee *et al.,* 2020).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from November 16, 2025 to May 26, 2026 in three selected district of Borena Zone, namely Yaballo, Elwayye, and Gomole districts of the Borena Zone, Oromia Region, Ethiopia (Figure 1). Geographically, the study area is located at latitudes of 3°30′–6°30′N and longitudes 37°30′–39°30′E an area of approximately 95,000 km2. The zonal town is 575 km away from Addis Ababa (the capital city of Ethiopia) (Bekele *et al*. 2020). According to the National Meteorological Agency (NMA) of Ethiopia, the climate is characterized by a mean annual temperature of 19 °C and with a mean maximum and minimum temperature of 24.6 °C and 12.96 °C, respectively. In general, the warmest period of the year is from March to May, while the lowest annual minimum temperatures occur between the months of November and January (NMA (National Meteorological Agency). 2007; Worku *et al*.,2022). The area is semi-arid with highly variable rainfall ranging between 500 and 900 mm per annum (Ng'ang'a *et al*.,2016). The rainfall has a bimodal distribution, with long rains occurring between March and June and short rains occurring between August and October The elevation ranges from 1000 m above sea level on the plains to 1500 m in the highlands (Solomon *et al*. 2007). The livestock population of Borana zone is estimated at 602,593 cattle, 420,512 sheep, 657,479 goats, and 65,075 donkeys. The Zone has 88,174 camels, accounting for 30% of the Region's camel population (CSA, 2021). In the Borana pastoral area, livestock production system is predominantly based on pastoral and agro-pastoral systems, in which indigenous animals are allowed to graze freely on natural pasture that composed mainly of grasses, forbs, and browses such as tree leaves, shrubs, and pods during the daytime and kept in open enclosure or shaded areas during the night. The dynamic nature of this system is marked by keeping diverse livestock species with seasonal movement of herds. Breeding females are commonly kept for milk production and form the largest proportion of pastoral herds (Tolera and Abebe, 2007).

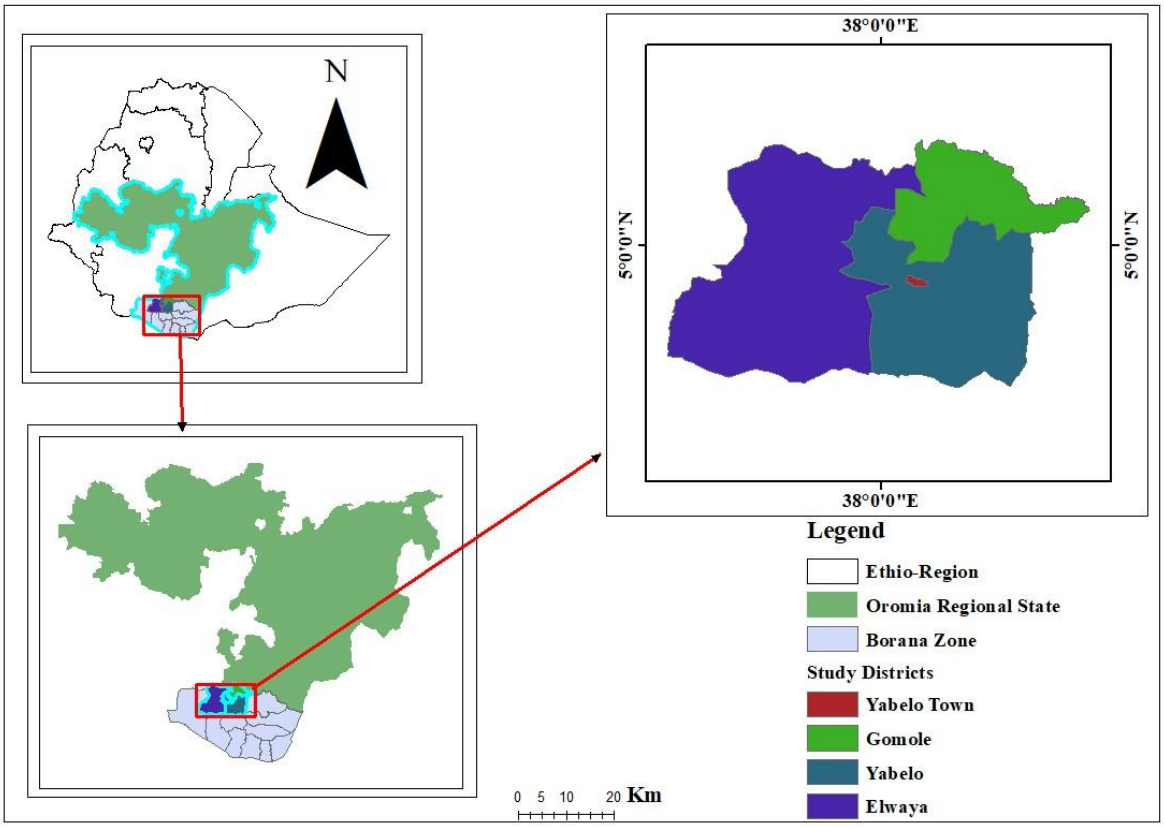

Figure 1. Map of the study area (ArcGIS 10.8, 2026)

3.2. Study Populations

3.2.1. Study animal population

The animal study population consisted of lactating camels randomly selected from Yaballo districts of the Borena Zone, Oromia Region, Southern Ethiopia. Raw camel milk samples were collected directly from the udders of lactating camels, as well as from household milk containers and camel milk markets. Yabello covers an area of 1,286.99 km² and comprises 14 kebeles. The district has an estimated camel population of approximately 56,551 heads (Legesse *et al*., 2023).

3.2.2. Human Study Population

The human study population comprised pastoralists living around wildlife–human–livestock interface in the selected districts of Borena Zone, Oromia Region, Southern Ethiopia. Participants included livestock owners, household members involved in animal husbandry, milk handlers, and camel milk sellers who had regular contact with livestock and wildlife or their products. A structured questionnaire was administered to selected respondents during field sample collection to obtain information on socio-demographic characteristics, livestock management practices, interactions between humans, livestock, and wildlife, awareness of zoonotic diseases, disease occurrence, risk behaviors, and preventive measures relevant to One Health surveillance.

3.3. Study Design

A cross-sectional study design was employed from October, 2025 to May, 2026 in the Borena Zone, including Yabello District, to comprehensively investigate the transmission dynamics of infectious diseases within a One Health framework. The study integrated ecological, socio-economic, microbiological, and behavioral components to address the specified objectives. Ecological factors, including climate variability and land-use changes were assessed and community-level observations to evaluate their influence on disease transmission patterns. Socio-economic drivers such as pastoral mobility, herd management practices, and resource competition were examined through structured questionnaires to understand their role in the persistence and spread of infections in pastoral systems.

Community knowledge, attitudes, and practices (KAP) related to infectious disease prevention and control were evaluated using questionnaire-based surveys administered to camel owners, community members, and animal health personnel. Concurrently, a laboratory-based investigation was conducted to isolate and characterize *Listeria monocytogenes* from raw camel milk, and equipment used for milk handling. Representative raw camel milk samples (10–25 mL) were aseptically collected from the udder of lactating camels, household storage containers,

and local milk markets, while swab samples were obtained from milk handling equipment. All samples were collected using sterile techniques, appropriately labeled with relevant metadata, and transported under cold chain conditions (4 °C) in iceboxes to the Yabello Regional Veterinary Laboratory. Samples were processed within 4–6 hours of arrival, or were stored at 4 °C and processed within 24 hours, following standard bacteriological procedures (ISO 11290:2022).

3.4. Sampling Techniques, Sample Collection and Transportation

A multistage sampling technique was employed to select study units at different administrative levels. In the first stage, Borena zone was selected purposively based on its high potential for the target livestock (camel, sheep, goats, and cattle) and the presence of relevant wildlife especially associated with presence of national Borena Park. In the second stage, woredas within the selected zones were also chosen purposively considering factors such as accessibility, livestock production potential, the density of livestock populations and informal information about mixing of livestock with wild live animals. In the third stage, Peasant Associations (PAs) or kebeles were selected using random sampling methods to ensure representation of different production systems such as mixed farming and pastoral systems. In the fourth stage, households owning the target animal species were selected from the chosen PAs using systematic sampling techniques based on an available sampling frame to minimize selection bias. Finally, for the Listeria isolation and characterization objective, individual animals were selected from the sampled households from one district (Yabello district) using purposive and random sampling methods depending on the study objectives, such as lactating she camels relevant to the objective of the current research.

All samples were collected aseptically using sterile swabs for milking and milk handling equipment, and sterile containers for milk samples following standard microbiological procedures. Each sample was labeled with a unique identification code and accompanied by relevant data such as location of collection, sample type, and animal related variables such as age, parity and body condition. Immediately after collection, samples were placed in sterile

containers and kept in insulated cool boxes containing ice packs to maintain a temperature of approximately 4°C. The samples were then transported to the microbiology laboratory of Yabello Regional Veterinary Laboratory within 24 hours for primary bacteriological analysis and isolation. During transportation, strict cold-chain procedures were maintained to preserve sample integrity and prevent bacterial overgrowth or contamination. Upon arrival at the laboratory, samples were processed according to standard bacteriological techniques for the isolation and identification of the Listeria pathogen (ISO 11290:2022). Then Secondary bacteriological identification and antimicrobial susceptibility test was done at Microbiology Laboratory of College of Veterinary Medicine and Agriculture (CVMA), Addis Ababa University (AAU).

3.5. Sample Size Determination

The sample size *for isolation and characterization of Listeria* was determined using the formula proposed by Thrusfield *(*2018) by assuming 5% precision, 95% level of confidence interval and 50% expected prevalence of *Listeria* species, as there are no previous studies on Isolation of *L. monocytogenes* from raw camel milk in the study area.

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

Where: n= the required sample size, P exp = expected prevalence and d= desired absolute precision. Based on assumptions, the minimum sample size calculated is 207. To increase the precision of the study, the minimum sample size was increased by 7% and a total of 411 microbiological samples were collected and analyzed. The sample size of 411 were distributed in all selected district and villages, and local milk market based on availability of lactating camels, transportation and the availability camel milk during sampling period.

The sample size for the knowledge component of this study was determined using the single population proportion formula, assuming a 95% confidence level (Z = 1.96) and a margin of error of 5%. The proportion of adequate knowledge (p = 14.28%) was taken from a previous study conducted among pastoral communities in the Borena area of Oromia region, Ethiopia.

Substituting these values into the formula yielded an initial sample size of 188 participants. To account for potential non-response or incomplete data, a 10% contingency was added, resulting in a final sample size of 207 participants for the knowledge component.

3.6. Isolation and Identification of *Listeria monocytogenes*

Qualitative detection of *L. monocytogenes* from raw camel milk and milking equipment was performed by isolation and Identification using the standard procedures described by United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (USFDA-CFSAN) method. Briefly, a 25 mL of each milk sample was homogenized in 225 mL (1:9) of Listeria Enrichment broth (BLEB) (HiMedia, India) and incubated at 30 °C for 24 hours. Then, a loop full of aliquots was then streaked aseptically onto esculin based Listeria selective agar, known as Oxford agar (OXA) for esculin hydrolysis to obtain typical Listeria colonies. The plates were then incubated at 37°C for 24 hours. Listeria positive samples were indicated by growth of typical colonies that appear black with halos on OXA medium. Presumptive Listeria colonies were streaked onto the Brain Heart Infusion (BHI) agar and incubated at 37°C for 24 hours or until sufficient growth was obtained. Listeria isolates growth on BHI agar were then cryopreserved and stored at -80°C in BHI broth supplemented with 20% glycerol. After transportation via cargo, the isolates were refreshed on OXA medium. Following that, up to five colonies suspected as *L. monocytogenes* were taken from each petri dish and inoculated into Tryptic Soy Agar-Yeast Extract (Himedia, M1214) agar (TSAye) for purification and incubated at 30 °C for 24 h. The colonies were confirmed by biochemical tests. The catalase positive, and oxidase negative colonies that exhibited umbrella-like growth in Sulfide Indole Motility medium (SIM) were determined to be Listeria spp. Further identification and confirmation were carried out by using serial of biochemical tests.

3.6.1. Biochemical tests

Catalase test: The catalase activity was determined by adding a drop of 3% hydrogen peroxide on clean glass slide and placing small amount of suspected colony growth into it. The positive

results were indicated by immediate bubble formation, indicating breakdown of hydrogen peroxide.

Oxidase test: The oxidase activity was tested using filter paper method. The suspected listeria colonies were smeared on the filter paper moistened with 1% tetraethyl phenylenediamine dihydrochloride solution (oxidase reagent). The reaction was observed for 40-60 seconds. The absence of a color change to dark purple or blue was recorded as a negative result, consistent with the genus Listeria.

Indole test: To assess indole production from the amino acid tryptophan, the isolates were inoculated into tryptone broth (or SIM medium) and incubated at 35°C for 48 hours. Following incubation, 0.5 mL of Kovac's reagent (p-dimethylaminobenzaldehyde) was added to each tube. The absence of a red ring and the persistence of the reagent's original yellow color indicated a negative indole reaction, confirming the lack of tryptophanase activity, which is characteristic of the genus Listeria.

Methyl red test: The Methyl Red test was employed to detect the production of stable acid end products from glucose fermentation. Isolates were inoculated into 5 mL of MR-VP broth and incubated at 35°C for 48 hours. Following incubation, five drops of methyl red indicator (0.02%) were added to the broth. A positive reaction was defined by the immediate appearance of a stable red color, indicating a pH of 4.4 or lower.

Voges-Proskuer test: The VP test was performed to determine the ability of the isolates to produce acetoin (acetylmethylcarbinol) via the butylene glycol pathway. To 2 mL of the 48-hcurrent MR-VP broth culture, 0.6 mL of 5% alpha-naphthol (Barritt's Reagent A) and 0.2 mL of 40% KOH (Barritt's Reagent B) were added. The tubes were shaken vigorously and allowed to sit for 15 to 30 minutes. The development of a pink-to-crimson red color at the surface of the medium was recorded as a positive result.

Triple sugar iron (TSI) test: The fermentation profile and hydrogen sulfide (H2S) production were evaluated using TSI agar slants. The medium was inoculated by stabbing the butt and

streaking the slant, followed by incubation at 37°C for 24 hours. Results were recorded based on the color change of the phenol red indicator in both the slant and the butt, as well as the presence of blackening (indicating H2S production) or cracks in the medium (indicating gas production).

Urease test: Urease is a constitutively expressed enzyme that hydrolyzes urea to carbon dioxide and ammonia. A heavy inoculum from a 24-hcurrent pure culture was inoculated to the broth. The tube was gently shaken to suspend the bacteria. The tubes were incubated at 37°Cfor 24 to 48 hours. Observed the broth for a color change.

Nitrate utilization test: Isolates were inoculated in peptone nitrate broth and incubated at 37°C. 0.5 mL of α-naphthylamine reagent and sulphanilic acid reagent was added. Development of red colcurrent was observed.

Motility test: The motility of Listeria species was determined using semi-solid motility test medium (SIM medium) incubated at 25°C for up to 48 hours. Listeria species are motile and exhibita typical umbrella-like growth pattern. Listeria spp. are slim, short rods with slight rotating or tumbling motility when examined under a phase-contrast microscope using 0.85% saline for suspending medium and oil immersion.

Hemolysis test: The hemolytic activity was determined using 5% Horse Blood Agar. *L. monocytogenes* was produced narrow zone of Beta-hemolysis around the colony. To further differentiate it from other species.

Carbohydrate fermentation tests: The tests were conducted using purple carbohydrate broth base containing 0.5% of different sugars, such as dextrose, maltose, esculin, rhamnose, mannitol, and xylose. Tubes were incubated at 35°C for 7 days, and acid production were indicated by a change for color from purple to yellow. *L. monocytogenes* typically ferments dextrose, maltose, esculin, and rhamnose but not mannitol or xylose.

3.7. Antimicrobial Susceptibility Test

An antimicrobial susceptibility test was carried out for isolates of *L. monocytogenes* using the disc diffusion method on Mueller-Hinton Agar. The common standard antimicrobial agents including Penicillin G, Amoxicillin, Chloramphenicol, Tetracycline. Ciprofloxacin, High-Level Gentamicin, Cefoxitin, Doxycycline, Azithromycin and Meropenem were evaluated. The agar plate antibiotic disk diffusion method (Kirby Bauer technique) was used for antimicrobial resistance testing, ensuring that it meets 0.5 McFarland turbidity standard. Approximately 3 to 5 pure colonies were taken from Tryptone Soya Yeast Extract Agar, suspended in Muller Hinton broth, and incubated at 37°C for 1-2 hours. The suspension was checked for slight formation of turbidity. It was then be spreaded onto Mueller-Hinton agar plates using a sterile cotton swab. The swab was used to evenly coat the entire agar surface. Once the inoculum has dried, various antimicrobial-impregnated disks were placed on the plates' surface, which were incubated aerobically at 37 °C for 24 hours. After incubation, each plate was observed under an indirect light source from a lamp to assess the development of inhibition zones around the disks. The inhibition zones were measured using a sliding caliper to the nearest millimeter. The measured inhibition zones were compared with a reference table to classify them as susceptible, intermediate, or resistant according to criteria set by the Clinical and Laboratory Standards Institute (CLSI, 2025). The control strain (*S. aureus* (ATCC 25923)) were sourced from the Ethiopian Public Health Institute (EPHI) in Addis Ababa, and it was served as a quality control on each testing date and with every batch of antibiotic disks used.

3.8. Quality Control

The questionnaire and observation checklist were pretested on 5% of the study sample size, outside the study areas, using face-to-face interviews to ensure clarity and practicality of the questionnaire. For maintaining data quality, all reagents and media were subjected to quality control using standard bacterial strains. To prevent cross contamination, standard microbiological procedures were strictly followed during the experiments. Aseptic technique was used for sampling, handling and testing. Throughout the identification process, positive and

negative strains were used to ensure the reliability of results. *L. monocytogenes* ATCC19115 was used as the positive control, while *L. innocua* ATCC33090 was used as the negative control. The techniques were kept consistent throughout the study to minimize errors, using sterile materials, flames, and refrigeration. All procedures during sample collection and microbiological analysis were conducted following standard protocols. An autoclave was used to sterilize the medium and equipment at 121°C for 15 minutes. Furthermore, 70% ethyl alcohol was used for disinfection.

3.9. Data Management and Analysis

All field and laboratory data collected at different stage were recorded and entered into Microsoft Excel spread sheet, checked and coded and then analyzed using STATA statistical software (StataCorp, LLC College Station, Texas). Descriptive statistics like frequency, mean, and percentage were used to express the findings. Chi-square test were performed to determine significance differences in occurrence of *L. monocytogenes* in sample sources and to check association between *L. monocytogenes* contamination and individual hygienic practices. Logistic regression analysis was performed to identify independent predictors of *L. monocytogenes* contamination while controlling for potential confounding factors such as milk handler experience, education, and milk storage practices. In all the cases, 95% confidence level and 0.05 absolute precision errors were considered.

3.10. Ethical Considerations

Ethical clearance was obtained from the College of Veterinary Medicine and Agriculture, Addis Ababa University by the research ethical committee. **Ref. No: VM/ERC/08/106/18/2026** (Annex 3).

4. RESULTS

4.1. Questionnaire Survey Results

4.1.1. Socio-Demographic Characteristics of the Study Participants

In this study, 207 people were participated. Over half about 60% were men, and 40% were women. Most participants were elderly (63%), followed by those in the early stages of old age (19%), middle-aged adults (17%), and very few young people (just under 1%). When it came to marital status, the majority were married (74%), while 20% were widowed, 4% divorced, and only about 1% had never married. Education levels were very low: over 80% could not read or write, and only about 18% had completed primary school or higher. Participants came mainly from Yabello district (just over half), with the rest split evenly between Elwaye and Gomole (about 27% each). In terms of how people made a living, pastoralism (livestock herding) dominated over 90% relied on it, while only about 8% practiced agropastoralism (farming combined with herding). Most households were semi-mobile (64%), meaning they moved seasonally with their animals, while about 36% were settled. 54%, 39% and 7% of tudy participant's household sizes were generally large, medium-sized, and small respectively. This shows that large, extended family structures are common in the area (Table 1).

Table 1. Distribution of socio-demographic characteristics among study respondents

Variable	Category	Observation	Percentage (%)
Sex	Male	124	59.90
	Female	83	40.10
Age	Youth	2	0.97
	Early Elders	35	19.32
	Middle aged	40	16.61
	Elder	130	62.80
Marital status	Single	3	1.45
	Married	154	74.4
	Divorced	8	3.86
	Widowed	42	20.29
Literacy level	Can't read and write	169	81.64
	Primary School and above	38	18.36
District	Elwaye	51	26.64
	Gomole	51	26.64
	Yabello	105	50.72
Primary Livelihood	Agropastoral	17	8.21
	Pastoral	190	91.79
Household Mobility	Semi mobile	133	64.25
	Settler	74	35.75
Household size	Small size	14	6.76
	Medium size	81	39.13
	Large size	112	54.11

4.1.2. Community Perceptions assessment of major factors contributing to zoonotic disease occurrence

Respondents ranked drought as the most significant perceived driver of zoonotic disease occurrence (84.5%), followed closely by wildlife (81.6%) and livestock density (81.2%). Market activity (65.7%) and population growth (63.3%) were moderately important, while ecological change (52.2%) and conflict (44.4%) were considered least influential. The findings suggest that the community perceives environmental and ecological factors particularly drought and wildlife interactions as more critical drivers of zoonotic disease risk than socioeconomic or conflict-related issues.

(Table 2).

Table 2. Respondents' ranking of key environmental and socio-economic factors for zoonotic disease distribution and transmission.

Ranks	Factors	Observation (%)
1th choice	Drought	175 (84.5)
2st choice	Livestock density	168 (81.2)
3rd choice	Wild life	169 (81.6)
4th choice	Market activity	136 (65.7)
5th choice	Population Growth	131(63.3)
6th choice	Ecological Change	108 (52.2)
7th choice	Conflict	92 (44.4)

4.1.3. Community Observations of Wildlife Presence and Interaction with Livestock

When asked about wildlife species commonly seen near homesteads or grazing areas, the vast majority of respondents (90.3%) identified a wide range of animals including zebra, hyena, birds of prey, monkeys, reptiles, bats, and rodents. Only 4.3% and 5.3% reported smaller combinations of these species. This indicates that wildlife diversity is well recognized by the community. Regarding livestock–wildlife interactions, nearly all participants (99.5%) reported that livestock and wildlife meet daily at grazing areas. Furthermore, all respondents (100%) stated that they always meet at water points. These findings suggest frequent and regular contact between domestic animals and wildlife across shared resources. Finally, when asked whether they had ever observed disease signs in wildlife, the overwhelming majority (99.5%) answered yes, while only 0.5% said no. This highlights a strong community awareness of illness in wild animals. Overall, the community perceives frequent wildlife presence and daily livestock–wildlife contact at grazing and water points, alongside observable disease signs in wildlife, as potentially significant factors in zoonotic disease occurrence in the study area. (Table 3).

Table 3. Wildlife presence, livestock–wildlife interaction, and disease observation in the study area

Questions	Answer Category	Frequency (%)
What are common wildlife seen near homestead/grazing areas?	Hyena, monkeys, Birds of prey, Rodents, Bats, Reptiles Zebra	9 (4.3)
	Hyena, Zebra, monkeys, Birds of prey, Rodents, Bats, Reptiles	11(5.3)
	Zebra, Hyena, Birds of prey, monkeys, Reptiles, Bats, Rodents	187 (90.3)
How often do livestock and wildlife meet at grazing areas?	Daily	206 (99.5)
	Sometimes	1 (0.5)
How often do they meet at water points?	Always	207 (100)
Have you ever observed disease signs in wildlife?	No	1 (0.5)
	Yes	206 (99.5)

4.1.4. Community Perceptions of Cross-Border Disease Transmission and Contact Pathways at the Livestock Wildlife Interface

The results show that the majority of respondents (97.6%) believe that major transboundary livestock diseases such as PPR, FMD, and CBPP are introduced through cross-border animal movement, highlighting strong community awareness of transboundary disease risks. Most respondents (98.1%) indicated that animals are only sometimes inspected before mixing during trade, suggesting limited veterinary control in livestock markets. Regarding direct contact mechanisms, 91.78% reported that livestock grazing in areas recently used by wildlife or wildlife grazing in livestock areas is the most common interaction, while fewer respondents mentioned wildlife feeding near homesteads (3.86%) or simultaneous sharing of water points (3.86%). After livestock restocking programs, the majority (93.7%) perceived that multiple diseases particularly PPR, CCPP/CBPP, FMD, lumpy skin disease, brucellosis, and anthrax commonly spread. Concerning specific transmission pathways, respondents most frequently identified grazing near wildlife conservation areas (41.06%) and wildlife entering livestock enclosures at night (32.36%), followed by sharing water points with wildlife (17.87%), while mixing of animals at

markets (1.4%) and introduction of animals from other herds after drought (7.24%) were less commonly reported. Overall, the findings highlight that cross-border movement, wildlife–livestock interactions, and grazing near protected areas are perceived as key drivers of livestock disease transmission in the study area (Table 4).

Table 4. Respondents' assessment of disease introduction, contact mechanisms, and transmission pathways between livestock and wildlife

Questions	**Answer Category**	**Frequency (%)**
What diseases do you think come through cross-border movement?	PPR CBPP FMD	5 (2.4)
	PPR FMD CBPP	202 (97.6)
Do traders inspect animals before mixing?	Always	4 (1.9)
	Sometimes	203 (98.1)
Have you seen any of the following direct contact mechanisms?	Wildlife feeding on household waste near homesteads	8 (3.86)
	Livestock grazing in areas recently used by wildlife and Wildlife grazing inside livestock grazing areas	190 (91.78)
	Sharing the same water points simultaneously	8 (3.86)
Which disease do you think most commonly spreads after livestock restocking (e.g., by NGOs)?	PPR Brucellosis CCPP/CBPP FMD	2 (1.0)
	PPR FMD CCPP/CBPP Lumpy Skin Disease Brucellosis Anthrax	4 (1.9)
	PPR CCPP/CBPP FMD Brucellosis Lumpy Skin Disease Anthrax	4 (1.9)
	PPR CCPP/CBPP FMD Anthrax Brucellosis Lumpy Skin Disease	3 (1.4)
	PPR CCPP/CBPP FMD Lumpy Skin Disease Brucellosis Anthrax	194 (93.7)
Which specific contact pathways do you believe cause disease spread ?	Sharing water points with wildlife	37 (17.87)
	Mixing of animals at livestock markets	3 (1.4)
	Introduction of animals borrowed or received from other local herds after drought	15 (7.24)
	Grazing near wildlife conservation or protected areas	85 (41.06)
	Wildlife entering livestock enclosures at night	67 (32.36)

4.1.5. Community Practices and Perceptions Related to Livestock Movement, Disease Management, and Zoonotic Risk

Nearly all respondents (99%) said they had seen new diseases appear after moving their livestock, suggesting that people strongly link animal movement to the spread of illness. When herds travel long distances, they frequently mix with animals from other districts and even neighboring countries, meaning there's plenty of contact between different herds across borders. Interestingly, while most people (99%) reported that they don't immediately mix returning animals with their main herd, formal quarantine practices are almost unheard of (99.5% don't use them). At the same time, participation in cross-border livestock markets is very high (99%), which adds another layer of disease risk.

When it comes to prevention, the most common measure people mentioned was vaccination (around 54%). Fewer talked about isolating sick animals (25%) or properly disposing of carcasses (13%). Very few recognized the importance of avoiding shared water sources (just 7%), and hardly anyone (less than 1%) mentioned using protective gear like gloves or masks.

As for treatment, just over half of the respondents (52%) said they isolate and treat sick animals using traditional methods. Another 28% rely solely on traditional treatments, while only a small fraction (8%) get help from a veterinarian. Carcass disposal practices are largely unsafe about 94% leave dead animals out in the open for scavengers to eat. And risky eating habits are universal: every single respondent (100%) reported consuming raw milk, raw meat, and even products from sick animals. None used any protective equipment when handling animals. Taken together, these findings paint a clear picture: high-risk practices in livestock management, disease prevention, and food consumption are widespread, and they may be fueling the spread of zoonotic diseases at the critical intersection where humans, livestock, and wildlife meet (Table 5).

Table 5. Respondents' practices on livestock movement, disease prevention, and food safety behaviors

Questions	Answer Category	Frequency
Experience new diseases afterward	No	2 (1.0)
	Yes	205 (99.0)
When livestock move long distances, do they?	Mix with herds from other Woredas Mix with herds from Kenya Pass through wildlife corridors	16 (7.7)
Mix them immediately with their herd	Yes	2 (1)
	No	205 (99)
Observe (quarantine)	Yes	1 (0.5)
	No	206 (99.5)
Do you participate in cross-border livestock markets (Kenya, Somalia)	No	2 (1)
	Yes	205 (99)
Which measures prevent livestock/wildlife–human infections	Vaccination	111 (53.62)
	Quarantine of sick animals	52 (25.12)
	Proper carcass disposal	27 (13.04)
	Avoiding shared water points	15 (7.24)
	Wearing gloves during animal handling	2 (0.96)
How do you handle sick animals	Call vet	17 (8.21)
	Isolate	13 (6.28)
	Isolate and Treat traditionally	108 (52.17)
	Treat traditionally	57 (27.53)
	Treat traditionally, Isolate and Call vet	12 (5.79)
Carcass disposal of dead animals	Given to scavengers Open field	7 (3.4)
	Open field	6 (2.9)
	Open field (Given to scavengers)	194 (93.7)
Consumption of raw milk	Always	207 (100)
Consumption of raw meat	Yes	207 (100)
Consumption of meat and milk from sick animals	Yes	207 (100)
Use of protective equipment during handling	None	207 ()

4.1.6. Community Attitudes Toward Disease Prevention, Land Use Change, and Treatment Preferences

The findings reveal a remarkably positive attitude toward disease prevention. Nearly everyone (99.5%) strongly agreed that vaccinating animals is essential for stopping disease outbreaks only a tiny fraction (0.5%) simply agreed without strong conviction. Even more striking, every single respondent (100%) strongly agreed that changes in land use like new roads, farms, and settlements increase disease risk. This shows a high level of awareness in the community about how environmental changes can trigger disease emergence. When it comes to treating sick animals, the vast majority (99.5%) said they use both traditional and modern veterinary medicine together. Only a very small number (0.5%) rely on traditional treatments alone. Taken together, these results paint a picture of a community that is well aware of how to prevent diseases and understand environmental risks but in practice, they continue to blend traditional and modern approaches when treating their animals (Table 6).

Table 6. Assessment of respondents' attitudes toward livestock disease prevention and environmental risk factors

Questions	**Answer Category**	**Frequency**	**%**
Vaccination of animals is important to prevent outbreaks	Agree	1	0.5%
	Strongly agree	206	99.5%
Land-use cover (roads, farms, settlements) increases disease risk	Strongly agree	207	100.0%
Preference for disease treatment methods	Traditional Medicine	1	0.5%
	Both combined	206	99.5%

4.1.7. Community Knowledge, Risk Perception, and Ecological Drivers of Livestock–Wildlife Interface Diseases

The results indicate a very high level of awareness and perceived risk regarding livestock–wildlife interactions and disease transmission. Almost all respondents (97.6%) demonstrated good knowledge of major livestock diseases, including anthrax, brucellosis, CCPP/CBPP, FMD, rabies, TB, and listeriosis. All respondents (100%) reported that new invasive plant species (e.g., Prosopis) have altered grazing patterns, and similarly, all perceived wildlife–livestock contact as very risky for disease transmission. Despite this awareness, all respondents (100%) still agreed that it is acceptable for livestock to graze inside wildlife habitats, and reported engaging in practices that increase interface risk, including borrowing grazing land and water, entering wildlife habitats, cutting trees/charcoal production, mixing herds, selling distressed animals, and using unsafe water sources (Table 7).

Table 7. Assessment of respondents' knowledge, risk perception, and ecological drivers of livestock–wildlife interface diseases

Questions	Response Category	Frequency	%
Which diseases do you know	Anthrax, Brucellosis, CCPP/CBPP, Foot and Mouth disease, Rabies, TB, Listeriosis	2	1.0%
	Anthrax Brucellosis CCPP/CBPP Foot and Mouth Rabies Listeriosis	202	97.6%
	Brucellosis TB Foot and Mouth Anthrax Rabies CCPP/CBPP	3	1.4%
Have new invasive species (e.g., Prosopis) changed grazing patterns	Yes	207	100.0%
How risky do you believe wildlife–livestock contact is for spreading infectious diseases to livestock or humans	very risky	207	100.0%
It is acceptable for livestock to graze inside wildlife habitats	Strongly agree	207	100.0%
Borrowing grazing land/water	Yes	207	100.0%

Entering wildlife habitats for grazing	Yes	207	100.0%
Cutting trees/charcoal production	Yes	207	100.0%
Mixing herds with neighbors	Yes	207	100.0%
Selling distressed animals	Yes	207	100.0%
Using unsafe water sources	Yes	207	100.0%
Which of the following disease hotspot locations do you use	Dida Hara grazing system Water points shared with wildlife	42	20.3%
	Teltelle bushlands Water points shared with wildlife	2	1.0%
	Water points shared with wildlife	163	78.7%
Have you seen wildlife carcasses near grazing areas or water points recently	No	171	82.6%
	Yes	36	17.4%
At which places do you most commonly observe livestock and wildlife meeting	Grazing hotspots	2	1.0%
	Grazing hotspots Water points	203	98.1%
	Water points Grazing hotspots Night enclosures	1	.5%
	Water points Market areas	1	.5%
Which wildlife species most often come close to livestock	Zebra	207	100.0%
Has climate change forced wildlife closer to settlements	Yes	207	100.0%
Which season has the highest risk of interface contact	Long dry season	207	100.0%

4.1.8. Perceived Ecological and Socio-Economic Drivers of Livestock and Wildlife Interface Changes

The results show that respondents strongly associate environmental and livelihood changes with livestock–wildlife interactions and disease dynamics. The most frequently reported ecological change affecting livestock and wildlife was the dry season (60.4%), followed by prolonged dry season (38.6%), indicating that increasing aridity and pasture scarcity are major concerns in the area. Only a very small proportion (0.5% each) mentioned prolonged drought and generalized rain shortage as key drivers (Table 8).

Table 8. Perceived Ecological and Socio-Economic Drivers of Livestock and Wildlife Interface Changes

Questionnaire	Response	Frequency	Percentage
What is the biggest ecological or socio-economic change affecting livestock and wildlife today	Prolonged dry season that leads to lack of grazing pasture and clean water	1	.5%
	Dry Season	125	60.4%
	Prolonged dry season	80	38.6%
	shortage of rain and Drought season	1	.5%
	Mobility	50	24.2%

4.2. Detection of *L. monocytogenes* and other Listeria species in raw camel milk samples

4.2.1. Overall prevalence of Listeria

Out of the total 411 camel milk related samples analyzed, the overall prevalence of Listeria was 3.89% (95% CI: 0.02–0.057), indicating that the true contamination level in the sampled population is likely between 2% and 5.7%. At the animal level, 344 samples were examined and 4.47% were positive (95% CI: 0.006–0.095), suggesting slightly lower contamination at the production level. At the market equipment level, 67 samples were analyzed with a prevalence of 4.48% (95% CI: 0.017–0.058), indicating that contamination may also occur through equipment used during milk handling, storage, or marketing. Overall, these findings demonstrate a low but noteworthy presence of Listeria in camel milk and associated market equipment, highlighting potential contamination risks along both the production and marketing (Table 9).

Table 9. Prevalence of *L. monocytogenes* isolation

Variable	Observation	%	SD	95% CI
Animal level	344	4.47	.025	0.006- 0.095
Market Equipment's	67	4.48	0.01	0.017- 0.05.80
Overall	411	3.89	0.0095	0.02- 0.057

NB: %: Prevalence, CI: Confidence interval, SD: Standard Error

4.2.2. Association of Selected Animal-Level Risk Factors with Listeria *Positivity in Camel Milk*

The association result showed no statistically significant association between the tested animal-level factors and Listeria occurrence. Although 13 positive cases were detected among adult camels (339 samples) and none among old camels (5 samples), the difference was not significant ($\chi^2 = 0.65$, $p = 0.20$). Similarly, herd size and body condition were not significantly associated with Listeria positivity ($p = 0.28$ and $p = 0.41$, respectively), despite slightly higher positives in medium-sized herds and animals with medium body condition. Overall, these findings suggest that the studied animal-level factors did not significantly influence the occurrence of Listeria in camel milk (Table 10).

Table 10. Association of listeria isolation from lactating camels with intended variables

Variable	Category	Observation	Positives	P Value	Chi^2-Square
Age	Adult	339	13	0.2	0.65
	Old	5	0		
Herd size	Low	92	1	0.28	2.54
	Medium	183	9		
	Large	69	3		
Body Condition	Medium	209	9	0.41	0.52
	Good	135	4		

4.3. Antimicrobial Susceptibility Test Results

4.3.1. Mono-drug susceptibility test result

Antimicrobial susceptibility testing showed that all isolates (100%) were susceptible to high-level gentamicin (HLG-120). High susceptibility (80%) was observed for chloramphenicol (C-30), ciprofloxacin (CIP-5), amoxicillin (AX-10), doxycycline (DO-30), azithromycin (AZM-30), and meropenem (MEM-10). Moderate susceptibility was observed for tetracycline (TE-30) at 60%, while intermediate responses were observed for chloramphenicol, ciprofloxacin, amoxicillin, and tetracycline. High resistance was recorded for penicillin (80%) and cefoxitin (100%) (Table 11).

Table 11. Antimicrobial susceptibility and efficacy pattern of *Listeria monocytogenes* isolates (N=5)

No	Antimicrobial Disk	Content	Number of isolates (%)		
			Resistant	**Intermediate**	**Susceptible**
1	P-10	10 µg	6 (50%)		6 (50%)
2	AX 10	10 µg		1 (8.33%)	11 (91.67%)
3	C30	30 µg		1 (8.33%)	11 (91.67%)
4	TE 30	30 µg		2 (16.67%)	9 (83.33%)
5	CIP5	5 µg		2 (16.67%)	9 (83.33%)
6	HLG 120	120 µg			12 (100%
7	FOX 10	10 µg	10 (83.33%)		2 (16.67%)
8	DO-30	30 µg	1 (8.33%)	1 (8.33%)	9 (83.33%)
9	AZM-30	30 IU	1 (8.33%)		11 (91.67%)
10	MEROPENEM	30 µg	2 (16.67%)	1 (8.33%)	8 (75%)

4.3.2. Multi-drug susceptibility test result

Multidrug resistance patterns among the isolates showed that three isolates (41.67%) were resistant to two antibiotics (P-10 and FOX-10), while one isolate (8.33%) exhibited resistance to three antibiotics (P-10, FOX-11, and AZM-30) (Table 12).

Table 12. Multi-drug resistance (MDR) pattern of *L. monocytogenes* isolates (N=5)

No	Number of drug-resistant	Pattern	Frequency of isolates (%)
1	3	P-10, FOX 11, AZM 30	1 (25%)

5. DISCUSSION

5.1. Socio-demographic characteristics of study participants

Current study respondents' demographic profile largely reflects the pastoral character of the Borena Zone. The majority of respondents are male (59.9%). This pattern is common in pastoral communities across Ethiopia, where males typically take responsibility for livestock management and are more visible in markets and communal gatherings (Bekele *et al*., 2020). Similarly, elderly individuals are over half (62.8%). In Borena society, older household heads are traditionally the custodians of livestock decisions. The predominance of elderly participants in the current study may therefore reflect their long experience with livestock diseases, environmental change, and wildlife interactions. Youth comprises (0.97%), most likely points to the well-documented trend of young people leaving pastoral areas for urban centers, a shift that has accelerated in the Borena region in recent years (Tamene *et al*., 2023).

The high proportion of respondents who could not read or write (81.6%) is consistent with other studies conducted in Ethiopian pastoral areas where limited access to education is common due to mobility, geographic isolation, and livelihood constraints (Alemu *et al*., 2023; Angassa and Oba, 2010). People who cannot read find it difficult to engage with written health information, follow treatment instructions, or understand the routes by which zoonotic diseases spread. Studies across pastoral Ethiopia have shown that communities with limited formal education are less likely to participate in vaccination campaigns or to adopt modern veterinary practices, relying instead on traditional remedies passed down through generations (Pieracci *et al*., 2016). This supports the design of One Health intervention in the area, pushing communicators toward oral, visual, and community based channels rather than written materials.

The livelihoods data reinforce just how deeply embedded pastoralism is: 91.79% of respondents depended on livestock keeping, and nearly two-thirds (64.25%) described their households as

semi-mobile. This mobility, while central to the pastoral adaptation strategy, has well-recognized implications for disease dynamics (Grace *et al*., 2012).

5.2. Community perceptions of zoonotic disease occurrence risk factors

Respondents reported about the conditions that make disease outbreaks more likely with drought (84.5%) and wildlife interaction (81.6%) ranking as first and second risk factors, respectively. This perception is well-founded: during droughts, livestock and wildlife are pushed together around shrinking water bodies and disappearing pasture. Closer multi-species contact accelerates the exchange of pathogens across species barriers (Megersa *et al*., 2011). Similar observations have been reported from pastoral communities elsewhere, where communities have come to associate dry spells with episodes of animal and human illness (Wang *et al*., 2025).

High livestock density came third (81.2%), reflecting an awareness that crowded animals are more vulnerable to disease. Market activities (65.7%) and population growth (63.3%) were also widely mentioned, suggesting that community members understand how the movement of animals and people can spread infection across distances. This is consistent with reported cases in the Horn of Africa where livestock markets have served as nodes for the amplification and spread of diseases, including brucellosis, foot-and-mouth disease, and contagious caprine pleuropneumonia (Tschopp *et al*., 2010).

The ecological change scored somewhat lower (52.2%) as a perceived risk, even though habitat transformation clearly plays a role in shaping disease dynamics. This may simply reflect the fact that slow, cumulative environmental processes are harder to connect mentally to health outcomes than dramatic, visible events like a drought or a wildlife incursion into a village. When respondents were asked directly, every respondent (100%) confirmed that invasive plants such as *Prosopis juliflora* had displaced traditional grazing areas and forced new movement patterns. The awareness is there; it just may not yet be framed in terms of disease risk.

5.3. Livestock Wildlife Interface Dynamics and Disease Transmission

The frequency of livestock-wildlife contact reported by respondents is high. Daily contact at grazing sites was reported by 99.5% of respondents, and all participants (100%) claimed their animals shared water points with wildlife year-round. From a disease transmission context, this level of interface is deeply concerning. Water points are ideal environments for the survival and accumulation of pathogens in the environment, including *Listeria monocytogenes, Brucella species,* and *Mycobacterium bovis*. Shared use by multiple species effectively creates a common transmission pathway for all of them (Atuman *et al*., 2021).

Nearly all respondents (99.5%) had personally observed sick wildlife. This finding indicates that wildlife populations potentially serve as active reservoirs for circulating pathogens. This aligns with a report from across Sub-Saharan Africa linking wildlife reservoirs to recurring waves of zoonotic disease in neighboring livestock and human communities (Jones *et al*., 2008). The zebras were singled out by all respondents as the wildlife most frequently encountered around livestock. Equids are well-known to concentrate at water sources and, depending on the pathogen, can act as maintenance or amplifying hosts.

Most of the respondents were in the cross-border livestock trade (99.0%). Conversely, the total absence of veterinary inspection (98.1%) reported inspections as only occasional and formal quarantine on return (99.5% reported none). This indicates a scenario where animal movement becomes a reliable vehicle for disease spread rather than a contained, monitored activity. This is not unique to Borena, but given the volume of trade and the intensity of the wildlife interface in this zone, the cumulative risk is considerable.

Therefore, two specific contact scenarios identified in current findings deserve targeted attention. The first is grazing adjacent to wildlife conservation areas, reported by 41.06% of respondents. The second is wildlife entering livestock enclosures at night (32.36%), which represents a more intimate form of contact than daytime grazing encounters. It occurs in confined spaces and may involve direct exposure to wildlife secretions, feces, or carcasses. Both scenarios offer existing

entry points for risk reduction, whether through improved enclosure design, communal deterrence measures, or renegotiation of grazing boundaries near protected areas.

5.4. Community Knowledge, Attitudes, and Practices Related to Disease Prevention

Current findings revealed that the wide gap between what people know and what they do. Disease awareness was high with 97.6% of respondents demonstrating good knowledge. However, the same community reported 100% rates of consuming raw milk, raw meat, and products from visibly sick animals, all without any protective equipment. This paradox, where high knowledge coexists with high-risk behavior, is not unusual in pastoral settings. This supported by report from other areas in communities of Ethiopia (Alemayehu *et al*., 2021). It indicates that the barriers to safe behavior are not primarily informational but structural, cultural, and economic.

Raw camel milk is highly concerned. In Borena communities, it is far more than a food item. It carries cultural meaning, economic value, and social significance, and it is shared as an expression of hospitality and solidarity (Bekele *et al*., 2021). Asking people to stop consuming it raw camel milk is, in practice, asking them to disrupt a set of relationships and meanings that go well beyond nutrition (Sadler and Catley, 2009). A more realistic public health approach would focus on making simple, low-cost heat treatment feasible at household and market level, rather than expecting wholesale behavioral change. Camel milk consumed after brief boiling is far safer. This change can be made without dismantling the social practices surrounding its production and sharing.

The gap regarding veterinary services between attitude and action is equally important. Almost all respondents (99.5%) said they strongly believed vaccination was important for their animals, yet only 8.21% had actually contacted a veterinarian when animals diseased. Some respondents turned to traditional treatments (27.53%) or combined traditional with modern approaches (52.17%). This is not evidence of ignorance or indifference; it is evidence of a system that does not reach people where they are. Distance, cost, and inconsistent availability of veterinary

services are well-documented barriers in remote pastoral zones. No high health education will overcome them without structural improvements in service delivery like mobile veterinary clinics.

The practice of leaving dead animals in open fields, reported by 93.7% of respondents. This adds another layer of risk. Carcasses are sources of environmental contamination. The scavengers that feed on them, including hyenas can travel large distances across the landscape, can mechanically spread pathogens to new locations and new hosts. *Listeria monocytogenes* in particular is known to persist in such environments and can be disseminated widely through carrion feeding wildlife (Lourenco *et al*., 2022). Safe burial or designated disposal sites for livestock carcasses may be a simple but high-impact intervention.

5.5. Ecological and Socio-Economic Drivers of Livestock-Wildlife Interface Changes

Current findings demonstrated that the dry season and prolonged drought were identified as the leading ecological drivers intensifying contact between livestock and wildlife at 60.4% and 38.6%, respectively. This finding makes intuitive sense in a semi-arid landscape where water and pasture are the scarce resources that all species compete for. When those resources shrink, the buffer zones that normally separate livestock from wildlife disappear. Then, both groups converge on the same patches of remaining vegetation and surface water. Under these conditions, the likelihood of disease transmission between species rises substantially, and animal stress-induced immune suppression may further increase susceptibility on both sides (Reginald, 2024).

The common perception that climate change is driving wildlife closer to human settlements (100%), and that the long dry season is the riskiest time of year for interface-related disease (100%), gives disease surveillance programs a clear temporal target. Rather than maintaining uniform surveillance intensity throughout the year, a risk-stratified approach that intensifies monitoring and preventive activities during the dry season could be both more effective and

more efficient. Building this kind of adaptive, seasonally aware surveillance into pastoral health systems is an important challenge for One Health planning in the Borena Zone.

5.6. Cross-Border Livestock Movement and Disease Transmission

The majority of respondents (97.6%) believed that transboundary livestock diseases such as PPR, FMD, and CBPP are introduced through cross-border animal movement. This perception is consistent with numerous studies demonstrating that uncontrolled livestock movement is a major driver of transboundary animal disease transmission (FAO, 2020). Pastoral communities often move animals across borders in search of pasture and water, particularly during drought periods. Such movements increase the likelihood of mixing animals from different regions and countries, facilitating pathogen spread. Additionally, the study found that livestock markets often lack proper veterinary inspection before animals are mixed.

Weak veterinary surveillance systems and limited biosecurity measures in livestock markets have been identified as major factors contributing to disease dissemination in pastoral regions (Grace *et al*., 2015). These findings reinforce the need for coordinated regional disease control strategies involving multiple sectors and countries under the One Health framework.

5.7. Livestock Management Practices and Zoonotic Risk Behaviors

Several management practices identified in this study may increase the risk of zoonotic disease transmission. Nearly all respondents reported experiencing new diseases following livestock movement, emphasizing the epidemiological importance of herd mobility. Despite this awareness, formal quarantine practices were almost nonexistent. Lack of quarantine facilities, veterinary services, and infrastructure may explain the absence of biosecurity measures in pastoral production systems.

Another notable finding was the widespread reliance on traditional treatment methods for sick animals. Ethno veterinary medicine is widely practiced in pastoral communities because it is

culturally accepted and readily available (McCorkle *et al*., 1996). However, exclusive reliance on traditional treatment without proper diagnosis may delay disease detection and control.

Carcass disposal practices also pose potential health risks. The majority of respondents reported leaving dead animals in open fields where scavengers can access them. Improper carcass disposal can contaminate the environment and facilitate pathogen transmission to wildlife, livestock, and humans (WHO, 2017). These findings highlight the interconnected nature of human, animal, and environmental health in pastoral ecosystems, emphasizing the importance of integrated disease control strategies.

5.8. Isolation of *Listeria monocytogenes* in Camel Milk

The overall prevalence of *Listeria* contamination across sampled camel milk and associated equipment was 3.89% (95% CI: 2.0-5.7%), with animal-level prevalence at 4.47% and equipment-level contamination at 3.77%. This finding indicates that raw camel milk in the Borena pastoral system can serve as a vehicle for Listeria contamination and therefore poses a public health risk, particularly in communities where raw milk consumption is common (Amenu *et al*., 2020; Bekele *et al*., 2021). This is important because camel milk in Borena is widely consumed without heat treatment and is handled under traditional conditions with limited cooling and poor sanitary control (Worku *et al*., 2014; Bekele *et al*., 2021).

Similar studies on Listeria in raw camel milk are scarce in Ethiopia. (Adugna *et al*., 2013) isolated Listeria species from camel milk along the Eastern Ethiopian value chain, although the result was reported as 4.1% of all bacterial isolates rather than as sample-level prevalence. *L. monocytogenes* prevalence observed in the present study was lower than 1.08% reported from Egypt (Abeer *et al*., 2012), whereas study in Algeria reported 14.28% prevalence (Lezzoum-Atek *et al*., 2023). These differences suggest that Listeria occurrence in camel milk is strongly production system dependent and likely influenced by variation in sample size, hygienic conditions, laboratory methods, and the stage of the milk chain sampled.

Compared with Ethiopian bovine milk studies, the prevalence of *L. monocytogenes* recorded in the present study was lower compared with (Wodajo *et al*., 2016), who reported 1.2% *L. monocytogenes* in raw cow milk. In contrast, other studies reported higher prevalence. (Seyoum *et al*., 2015) reported 18.6% Listeria species which is higher than the present findings. (Borena *et al*., 2022) reported 7.03% Listeria species prevalence and 1.82% *L. monocytogenes* prevalence in cow-level raw milk, while bulk tank milk from the same study showed much higher prevalences of *L. monocytogenes* and other Listeria species 15.15% and 9.09%, respectively. (Hassen *et al*., 2025) reported 4.7% *L. monocytogenes* and 12.2% overall Listeria prevalence, with highest prevalence of Listeria species. at 15.2 % in raw milk. Likewise, (Hawaz *et al*., 2023) reported 28.75% Listeria species prevalence and 7.08% *L. monocytogenes* prevalence in raw cow milk. (Girma and Abebe, 2018) reported 8.84% *L. monocytogenes* prevalence in raw bovine milk. Kemal *et al*. (2024) also reported 12.8% and 14% *L. monocytogenes* prevalence in raw bovine milk from dairy farm and from vendors respectively from eastern Ethiopia. These differences suggest that contamination is shaped by host species, production ecology, sample type, degree of pooling, value chain stage, and laboratory method.

The most important finding of the present study was the significantly higher prevalence in pooled container milk (4.48%) than in udder milk (4.47%). This finding agrees with (Borena *et al*., 2022), (Hassen *et al*. 2022), (Kemal *et al*., 2024), and (Girma and Abebe, 2018). In camel milk systems, (Adugna *et al*., 2013), (Abera *et al*., 2016) and (Bekele *et al*., 2025) reported increase in microbial contamination increased from production points to collection points and open markets. The study from Kenya that conducted by (Kaindi *et al*., 2011) and (Mwangi *et al*., 2016) also reported the same idea in microbial contamination increase across value chain. These might be due to occurrence and amplification of contamination during pooling, transfer, transport, storage, and low container hygiene, along the value chain.

The absence of statistically significant associations between *Listeria* positivity and animal level characteristics such as age, herd size, or body condition ($p > 0.05$) is consistent with the idea that contamination in this setting is primarily environmentally driven rather than animal-driven. This

reinforces for focusing interventions on hygiene and handling practices rather than on identifying and segregating specific animals.

5.9. Antimicrobial Resistance Patterns of *Listeria monocytogenes* Isolates

The antimicrobial susceptibility profile of the *L. monocytogenes* isolates showed a mixed pattern to all 10 tested antimicrobial disks. All isolates were susceptible to HLG-120, and most were susceptible to C-30, CIP-5, AX-10, DO-30, AZM-30, and MEM-10. In contrast, all isolates were resistant to FOX-30, and 80% were resistant to P-10. This general pattern of heterogeneity agrees with earlier Ethiopian studies showing that susceptibility to some agents coexists with marked resistance to others. (Hawaz *et al*., 2023) found complete susceptibility of *L. monocytogenes* from raw cow milk to gentamicin, vancomycin, and sulfamethoxazole, but high resistance to nalidixic acid and erythromycin. (Borena *et al*., 2022) also reported complete susceptibility to gentamicin and norfloxacin, alongside high resistance to oxacillin, amoxicillin, vancomycin, and ampicillin. (Tola, 2024) concluded from national evidence that antimicrobial resistance among Ethiopian Listeria isolates is common but highly variable between studies and sample types. The agreement across studies regarding good susceptibility to gentamicin is notable and suggests that this agent continues to retain activity against many dairy associated isolates.

The multidrug resistance finding is one of the most important results of the present study. All five *L. monocytogenes* isolates were resistant to two or more antimicrobial agents, giving an overall multidrug resistance proportion of 100%, with a mean MDR index of 0.24. The most common resistance pattern was combined resistance to P-10 and FOX-30. Although the number of isolates was small, this result is still concerning because it indicates that resistant strains are present in the camel milk chain in the study area. Similar findings have been reported from Ethiopian dairy studies. (Borena *et al*., 2022) reported multidrug resistance in 81.82% of *L. monocytogenes* isolates from milk and milk products, while (Hawaz *et al*., 2023) found multidrug resistance in 94.1% of isolates from raw cow milk. (Belayneh *et al*., 2025) further estimated that antimicrobial resistance among pathogenic bacteria from raw milk and milk products in Ethiopia is high overall, with multidrug resistant isolates accounting for a substantial

share. These parallels suggest that multidrug resistance in foodborne milk isolates is not an isolated event, but part of a broader problem in the Ethiopian dairy sector.

In the Borena context, these findings revealed the need for antimicrobial stewardship that is practical and grounded in the realities of pastoral life. Farmers who combine traditional and modern treatments (52.17%) may be using antibiotics at sub therapeutic doses without veterinary guidance, which is a known driver of resistance selection. Incorporating guidance on appropriate antibiotic use into the work of veterinary extension officers, and improving access to proper veterinary diagnosis before treatment, would be steps toward reducing the resistance burden in this system (Woldu, 2024).

6. CONCLUSION AND RECOMMENDATIONS

This study revealed the presence of *Listeria monocytogenes* in raw camel milk and milk handling equipment in pastoral areas of Borena Zone, indicating a potential public health concern. Although the overall prevalence was relatively low, contamination along the milk value chain suggests possible exposure risks for pastoral communities that traditionally consume raw milk. The absence of significant associations between animal-level factors and Listeria occurrence indicates that contamination may be influenced more by environmental and handling practices rather than individual animal characteristics. Antimicrobial susceptibility testing revealed encouraging sensitivity to several commonly used antibiotics; however, the observed resistance to Penicillin and Cefoxitin and the presence of multidrug-resistant isolates raise concerns about emerging antimicrobial resistance. Furthermore, the questionnaire survey shown extensive livestock–wildlife interactions, frequent animal movement, lack of quarantine practices, and widespread consumption of raw animal products. Despite high awareness of disease risks, these practices create favorable conditions for pathogen transmission at the human–livestock–wildlife interface. Therefore, improving milk hygiene practices, strengthening veterinary services, and promoting One Health–based disease control strategies are essential to safeguard both animal and public health in pastoral production systems. Based on above conclusion the following recommendations were forwarded.

- Strengthen community awareness and public health education on of zoonotic disease between Livestock-wildlife-human triangle.
- Improve disease control measures at livestock–wildlife interface
- Promote antimicrobial stewardship and responsible drug use
- Implement livestock movement management and cross-border animal health control
- Adopt an integrated one health approach between human health, animal health, wildlife conservation agencies.
- Community awareness about possible transmission of *L. monocytogenes* from consumption of raw camel milk and safe milk handling method.
- Support further research and evidence generation regarding priority zoonotic diseases.

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8. ANNEXES

Annex 1. Questionnaire and Consent form

SECTION 1. Informed Consent

Greeting and Purpose

Good morning/afternoon. My name is _____. I am conducting a study on livestock–human–wildlife disease dynamics in Borana Zone. Ycurrent household was selected to participate. Ycurrent participation is voluntary, and all information is confidential.

Consent

1. Do you agree to participate?

   ☐ Yes, *if yes, continue to the next question*

   ☐ No (STOP interview)

SECTION 2. Sociodemographic and Household Livelihood Information

1. **Respondent Code: _____**
2. **Sex:** Male ☐ Female ☐
3. **Age (years): _____**
4. **Marital Status:** Married ☐ Single ☐ Widowed ☐ Divorced ☐
5. **Education Level:** ☐ No formal education ☐ Primary ☐ Secondary ☐ College/TVET ☐ University
6. **District (Woreda): _____**
7. **Kebele: _____**
8. **Primary Livelihood:**

   ☐ Pastoral

   ☐ Agro-pastoral

   ☐ Crop farming

   ☐ Trade/business

Other (specify) _____

9. Household Mobility Pattern:

☐ Fully mobile pastoralist

☐ Semi-mobile

☐ Settled

10 Household Size: _____

11. Livestock Ownership (Number):

Cattle _____

Goats _____

Sheep _____

Camels _____

Equines _____

12. Herding Responsibility:

☐ Self

☐ Spouse

☐ Children

☐ Hired herder

SECTION 3. Wildlife Presence and Interface Contact

1.Common wildlife seen near homestead/grazing areas **(**select all)

☐ Warthog
☐ Buffalo

☐ Antelopes
☐ Zebra
☐ Hyena
☐ Lion
☐ Baboons/monkeys
☐ Birds of prey
☐ Leopard
☐ Black backed jackal
☐ Rodents
☐ Bats
☐ Reptiles
Other _____

2. How often do livestock and wildlife meet at grazing areas?

☐ Daily
☐ Weekly
☐ Monthly
☐ Rarely/Never

3. How often do they meet at water points?

☐ Daily
☐ Weekly
☐ Monthly
☐ Rarely

4. Have you ever observed disease signs in wildlife?

☐ Yes
☐ No

SECTION 4. Knowledge –Infectious Diseases at the Interface

A. Disease Knowledge

1. Which specific contact pathways do you believe cause disease spread?
☐ Sharing water points with wildlife
☐ Grazing near wildlife conservation or protected areas
☐ Predators/scavengers feeding on carcasses near homesteads
☐ Wildlife entering cattle camps at night
☐ Wildlife entering livestock enclosures at night
☐ Mixing of animals at livestock markets
☐ Introduction of animals borrowed or received from other local herds after drought
☐ Introduction of animals provided through NGO or government restocking programs
☐ Informal or illegal cross-border livestock movement
☐ Direct human contact with sick or dead animals

2. Have you seen any of the following direct contact mechanisms?

☐ Livestock and wildlife grazing in the same pasture at the same time
☐ Livestock grazing in areas recently used by wildlife
☐ Wildlife grazing inside livestock grazing areas
☐ Sharing the same water points simultaneously
☐ Wildlife drinking from cattle troughs
☐ Livestock following wildlife trails to grazing or water
☐ Wildlife resting in livestock enclosures or near night camps
☐ Wildlife feeding on household waste near homesteads
☐ Hyenas or other scavengers feeding on livestock carcasses
☐ Livestock eating grass possibly contaminated with wildlife feces
☐ Wildlife licking salt from livestock salt licks
☐ Wildlife approaching mineral/salt lick sites used by livestock

3. Which diseases do you know?

☐ Anthrax
☐ Brucellosis
☐ TB
☐ CCPP/CBPP

☐ Foot and Mouth
☐ Trypanosomiasis
☐ Rabies
Other _____

4. Which disease do you think most commonly spreads after livestock restocking (e.g., by NGOs)?
☐ PPR
☐ CCPP/CBPP
☐ FMD
☐ Lumpy Skin Disease
☐ Rift Valley
☐ Brucellosis
☐ Anthrax
☐ Q Fever
☐ Not sure

B. Knowledge (k) of ecological/environmental drivers

1. In ycurrent opinion, how can recurrent drought affect the health of humans, livestock, or wildlife?
☐ Forcing livestock to migrate further
☐ Increases mixing of herds at limited water points
☐ Bringing wildlife closer to settlements
☐ Weakens animals' immunity
☐ Leads to bush encroachment and clearing
☐ other

2. Do you think changes in climate or land use (e.g., deforestation, bush clearing, expansion of farmland) affect wildlife movement patterns?

☐ Yes
☐ No
☐ Not sure

3. Have new invasive species (e.g., Prosopis) changed grazing patterns ?

☐ Yes
☐ No

C. Knowledge of preventive measures

1. Which measures prevent livestock/wildlife–human infections?

☐ Vaccination
☐ Quarantine of sick animals
☐ Avoiding shared water points
☐ Wearing gloves during animal handling

☐ Proper carcass disposal

SECTION 5. Attitudes (A)

1.How risky do you believe wildlife–livestock contact is for spreading infectious diseases to livestock or humans?

☐ Very risky
☐ Somewhat risky
☐ Not risky
☐ Not sure

2 Land-use cover (roads, farms, settlements) increases disease risk?

☐ Strong Agree
☐ Agree
☐ Neutral
☐ Disagree
☐ Strong Disagree

3. It is acceptable for livestock to graze inside wildlife habitats?

☐ Strong Agree
☐ Agree
☐ Neutral
☐Disagree
☐Strong Disagree

4.Vaccination of animals is important to prevent outbreaks

☐ Strong Agree
☐ Agree
☐ Neutral
☐ Disagree
☐ Strong Disagree

5.Who is responsible for disease control?

☐ Community
☐ Government
☐ Veterinary staff
☐ Wildlife authorities
☐ All above

6. Preference for disease treatment methods

☐ Traditional medicine
☐ Modern veterinary care
☐ Both combined

SECTION 6. Practices (P)

A. Mobility and Contact Behavior

1. When livestock move long distances, do they
☐ Mix with herds from Kenya
☐ Mix with herds from other Woredas
☐ Pass through wildlife corridors
☐ Sleep near dried riverbeds used by wildlife

2. Do you camp (fora) near wildlife routes during migration?

☐ Always
☐ Sometimes
☐ Never

B. Cultural/Traditional Practices Affecting Disease Spread

1. Does the community practice?

☐ Traditional blessing rituals using animal products

☐ Slaughtering weakened animals during drought

☐ Sharing udder washing containers between households

☐ Raw blood consumption

☐ Home slaughter without inspection

2.When NGOs provide restocking animals, do communities?

Check health status? ☐ Yes ☐ No

Mix them immediately with their herd? ☐ Yes ☐ No

Observe quarantine? ☐ Yes ☐ No

Experience new diseases afterward? ☐ Yes ☐ No

C. Market and Movement Practices

1.Do you participate in cross-border livestock markets (e.g., Kenya, Somalia)?

☐ Yes
☐ No

2. What diseases do you think come through cross-border movement?

☐ PPR
☐ FMD
☐ CBPP
☐ Trypanosomiasis
☐ Not sure

3. Do traders inspect animals before mixing?

☐ Always

☐ Sometimes
☐ Never

4. At markets, do livestock?

☐ Share watering troughs
☐ Mix from multiple regions
☐ Get exposed to wildlife droppings (e.g., birds)
☐ Are transported on the same vehicle with other animals

D. Animal Health Practices

1. Vaccination frequency?

☐ Regular annually
☐ Occasionally
☐ Never

2. Deworming frequency?

☐ Regular
☐ Rare
☐ Never

3. How do you handle sick animals?

☐ Isolate
☐ Treat traditionally
☐ Call vet
☐ Slaughter/sell

4. Carcass disposal of dead animals?

☐ Open field
☐ Burial
☐ Burning
☐ Given to scavengers

E. Food Safety Practices

1. Consumption of raw milk?

☐ Always
☐ Sometimes
☐ Never

2. Consumption of raw meat?

☐ Yes
☐ No

3. Consumption of meat from sick animals?

☐ Yes
☐ No

SECTION 7. Preventive Measures (Field-Level)

1. Use of protective equipment during handling?

☐ Gloves
☐ Boots
☐ Mask
☐ None

2. Reporting unusual livestock deaths?

☐ Always
☐ Sometimes
☐ Never

3. How often disinfect ycurrent barns/tools?

☐ Regularly
☐ Occasionally
☐ Never

SECTION 8. Perceived Drivers of Disease (Ranking)

Rank from 1 = most important to 7 = least important:

Driver	Rank (1–7)
Population growth	___
Livestock density	___
Wildlife contact	___
Ecological change	___
Market activity	___
Drought	___
Conflict	___

SECTION 9. Coping Strategies During Drought/Stress

1. Borrowing grazing land/water?

   ☐ Yes
   ☐ No

2. Entering wildlife habitats for grazing?

   ☐ Yes
   ☐ No

3. Cutting trees/charcoal production?

   ☐ Yes
   ☐ No

4. Mixing herds with neighbours?

   ☐ Yes
   ☐ No

5. Selling distressed animals?

   ☐ Yes
   ☐ No

6. Using unsafe water sources?

☐ Yes
☐ No

7. Risky wildlife encounters during scarcity

Describe: _____

SECTION 10. Spatial/Geolocation Data

1. GPS location (auto-captured)
2. Map sketch of grazing routes:

☐ Primary dry-season route
☐ Primary wet-season route
☐ Both

3. Which of the following disease hotspot locations do you use? (Select all)
☐ Dida Hara grazing system
☐ Malbe plains
☐ Teltelle bushlands
☐ Web valley grazing corridor
☐ Cross-border dry-season grazing zones
☐ Water points shared with wildlife

4. Areas you consider high-risk for disease spread?
☐ Livestock market centers
☐ Boreholes shared with wildlife
☐ Salt-lick areas used by both wildlife and livestock
☐ Wildlife migration corridors
☐ Refuge areas during drought
☐ Communal night enclosures (fora camps)

5. Have you seen wildlife carcasses near grazing areas or water points recently?

☐Yes
☐ No

SECTION 11. Interface Contact Mapping

1. At which places do you most commonly observe livestock and wildlife meeting?
☐ Water points
☐ Salt licks
☐ River valleys
☐ Grazing hotspots
☐ Market areas
☐ Night enclosures

2. Which wildlife species most often come close to livestock?
☐ Hyena
☐ Zebra
☐ Warthog
☐ Antelope
☐ Lion
☐ Buffalo
☐ Birds
☐ other

3. Has climate change forced wildlife closer to settlements?

☐ Yes
☐ No
☐ Not sure

4. Which season has the highest risk of interface contact?
☐ Long dry season
☐ Short rains
☐ Long rains
☐ Drought emergencies

SECTION 12. Open Narrative (Qualitative)

1. What is the biggest ecological or socio-economic change affecting livestock and wildlife today?
2. What is the main cause of increasing diseases in ycurrent community?
3. Describe a recent experience where wildlife and livestock interacted or a disease event occurred.

4. What do you recommend as solutions to reduce disease spread at the interface?

Annex 2. Camera captured during

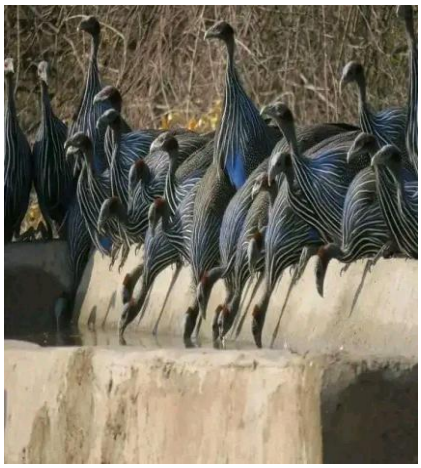
a. Travelling to study sites

b. Field Interviewing

i. NGO, Vétérinaires Sans Frontières Germany(VSF)

ii. Yabelo Regional Laboratory

iii. Borana National Park officer KI

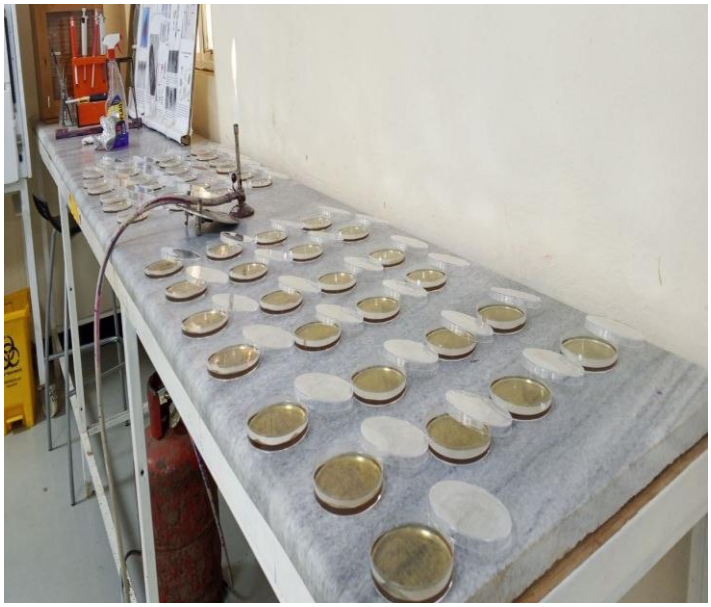




iv. Camera captured from *Borana National Park Sarite Block*

v. Borana District Office of agriculture

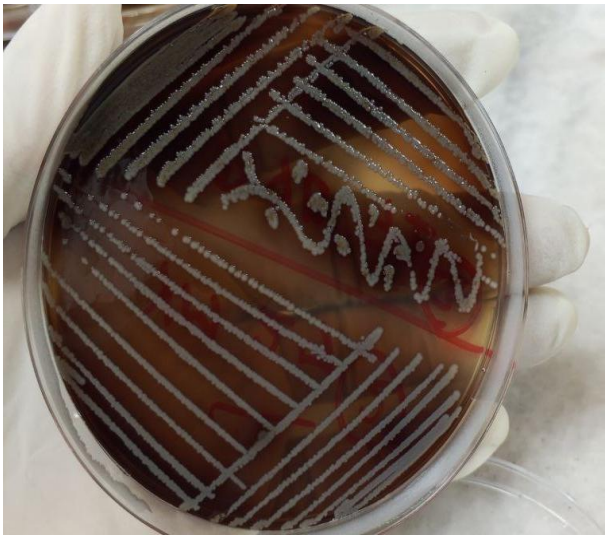
c. Key Informant/Focus Group Discussion

d. Milking and milk sample collection



e. Bacteriological Media Preparation for Listeria Culturing

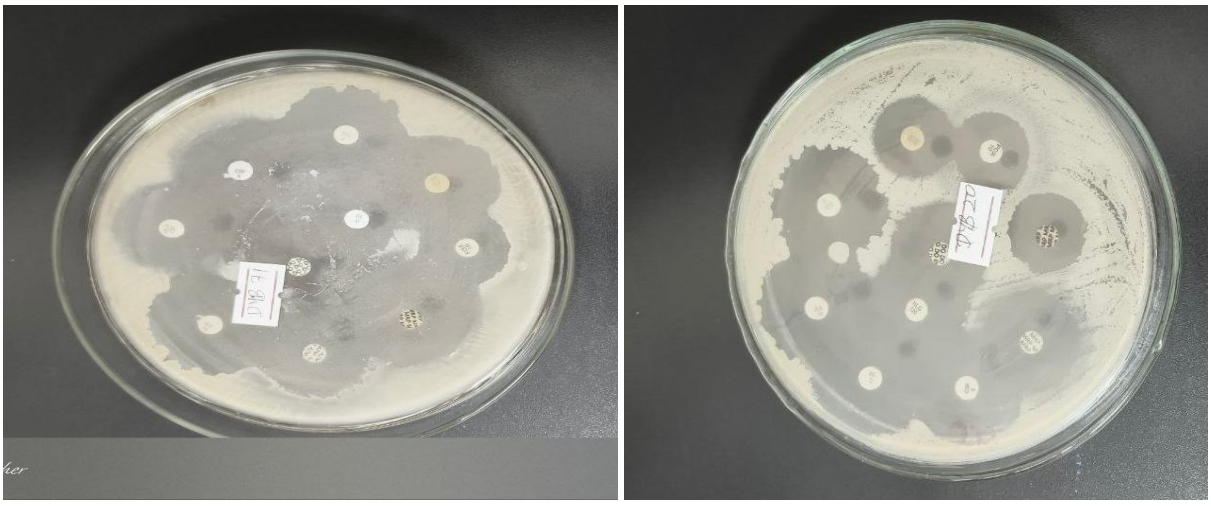
f. Inoculation and culturing

g. media preparation and colony morphology characterization

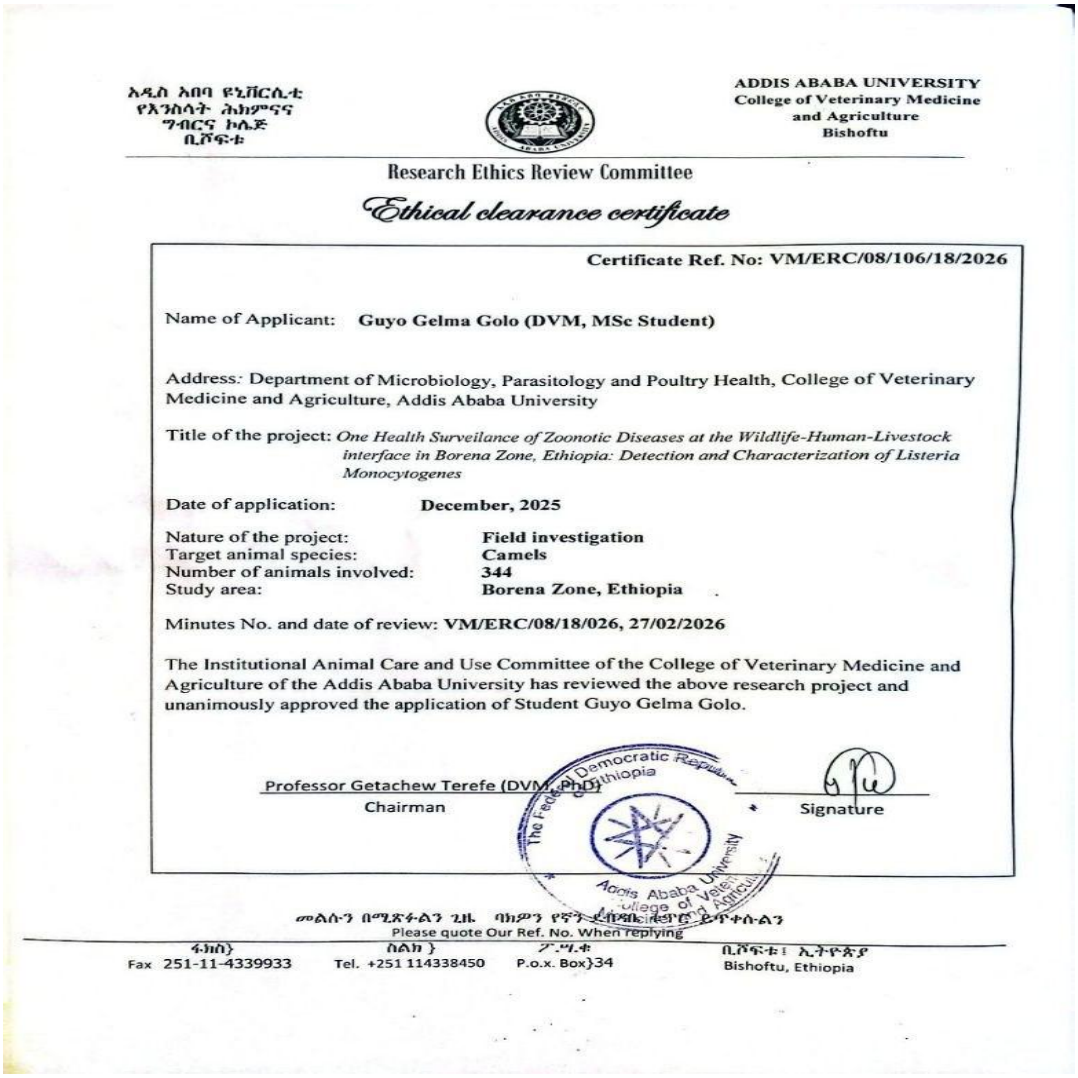
h. Biochemical Testing

i. biochemical test and gram staining



j. Disk diffusion antimicrobial resistance testing

Annex 3. Ethical Clearance



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
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Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/08/106/18/2026

Name of Applicant: **Guyo Gelma Golo (DVM, MSc Student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *One Health Surveilance of Zoonotic Diseases at the Wildlife-Human-Livestock interface in Borena Zone, Ethiopia: Detection and Characterization of Listeria Monocytogenes*

Date of application: **December, 2025**

Nature of the project: **Field investigation**
Target animal species: **Camels**
Number of animals involved: **344**
Study area: **Borena Zone, Ethiopia**

Minutes No. and date of review: **VM/ERC/08/18/026, 27/02/2026**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Guyo Gelma Golo.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

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