SEROPREVALENCE OF HUMAN *BRUCELLA* ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG PATIENTS SEEKING MEDICAL ATTENTION IN SELECTED HEALTH FACILITIES IN WESTERN PROVINCE OF ZAMBIA

BY

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Dissertation is submitted as Partial fulfilment of the requirements for the award of the Degree of Master of Science in One Health Analytical Epidemiology of the University of Zambia.

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DECLARATION

I, ARMAND MAYINDU MAMBOTE, do hereby declare that the contents of the dissertation being submitted herein are my original work, and they have not been previously submitted to any university for the award of a degree or any other qualification.

Signature---------- Date----------

APPROVAL

This dissertation submitted by ARMAND MAYINDU MAMBOTE is approved as fulfilling the requirements for the award of the degree of MASTERS OF SCIENCE IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY of the University of Zambia.

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ABSTRACT

Brucellosis is a neglected zoonotic disease that affects humans and animals and can lead to severe illness in humans and financial losses for households that rear livestock. In human cases, the disease presents with fever, fatigue or malaise, flu-like symptoms, weight loss, headaches and back pains. Most human brucellosis occurs in rural regions where individuals live in close-proximity to their livestock and ingest contaminated raw milk and milk products. In Zambia, there is a paucity of information on the seroprevalence of human brucellosis and its risk factors in the human population. The study aimed to investigate the seroprevalence of human *Brucella* antibodies and associated risk factors among patients seeking medical attention at community hospitals.

A cross-sectional seroepidemiological study was conducted from 21st April 2023 to 12th January 2024 among patients seeking medical attention at health facilities in selected districts of Western province in Zambia. 225 blood samples were collected from consenting participants. Sera were separated and analysed for anti-*Brucella* antibodies using the Rose Bengal Test (RBT) and Competitive enzyme-linked immunosorbent assay (c-ELISA) in serial interpretation. A questionnaire was administered to obtain epidemiological data related to exposure to the *Brucella* pathogen. The data obtained were coded and entered in the Micro-Soft Excel 2013® and analysed using STATA version 15® (Stata Corp., College Station, TX, USA). The odds ratio, 95% confidence interval, and Fisher's exact tests were computed to see the degree of association of the risk factors with *Brucella* seropositivity. Using the cut-off of P.I.< 30% and P.I. ≥ 30% for c-ELISA respectively negative and positive.

Only 197 sera samples were found acceptable for testing and analysis for this study, out of these, the seroprevalence of *Brucella* antibodies was 18.3% (n=36, 95% CI=12.8-23.6) on RBT and 4.57% (n=9, 95% CI=3.25-14.8) on c-ELISA (p-value=0.412) respectively. Among the risk factors considered only the number of animals was statistically significant to *Brucella* seropositivity (OR 6.49, 95% CI=1.10-38.13, p-value = 0.039). *Brucella* antibodies are prevalent among patients attending health facilities in the Western province of Zambia. To reduce the risk of exposure, the general public needs to be educated about the brucellosis disease. Additionally, farmers should be encouraged to vaccinate their animals.

Keywords: ***Brucella* antibodies, Human brucellosis, Risk factors, seroprevalence, Western Province, Zambia**

DEDICATION

This work is dedicated to my loving wife, Charline Ngalya Mambote; my daughter Darline Malaika Mambote; my mother, Alphonsine Dibazola; my sisters, Syntyche Mambote and Espe Mambote; my brother Flavien Bumbangi and his wife Rachel Velu, my brother Maty Ngoma and my sister Lysa, my step family, my family members and friends for their support and encouragement during my studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

BCV	*Brucella-containing vacuoles*
c ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CF	Complement Fixation
CI	Confidence Interval
D.C	*Dendritic cells*
eBCV	Endosomal Brucella-containing vacuole
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
FPA	Fluorescent Polarisation Assay
FRET	Fluorescent resonance energy transfer
i ELISA	Indirect Enzyme-Linked Immunosorbent Assay
OR	Odds Ratio
RBT	Rose Bengal Test
SAT	Standard Agglutination Test
WHO	World Health Organisation

CHAPTER ONE: INTRODUCTION

1.1. BACKGROUND

Brucellosis is an infectious, zoonotic illness caused by intracellular Gram-negative coccobacilli bacteria of the genus *Brucella* (Dong et al., 2022). In humans, Brucellosis is commonly known as "undulant fever", "Mediterranean fever", "gastric remittent fever", or "Malta fever" (*C*orbel et al., 2006; Ducrotoya et al., 2017; Bennett et al., 2022). The disease is widespread, affecting humans, a wide range of wild animals, and economically viable domestic livestock such as cattle, goats, sheep, donkeys, camels, swine, and dogs (WHO, 2016). Currently, 12 species of *Brucella* have been identified; however, only *B. melitensis, B. abortus, B. suis,* and, on rare occasions, *B. canis* are classified as human infections (Corbel 2020). Human brucellosis continues to have a significant global impact (Rossetti et al., 2017; Bosilkovski et al., 2021).

Brucellosis is among the seven neglected zoonotic diseases (WHO, 2016; Mableton et al., 2014). Despite this, more than 170 nations have recorded occurrences and the WHO reports that more than 500,000 new cases of brucellosis are reported in humans on a global scale annually (WHO, 2016). Brucellosis is a significant public health issue identified as a common occupational disease (Saeed et al., 2022). The disease profoundly impacts developing countries with weak public and animal health programs (Mirnejad et al. 2017). In most African countries, *brucellosis* is a neglected zoonosis due to the category of the affected people, particularly the poorly educated, as well as the limited efforts directed towards it is control (Ducrotoy et al., 2015).

Brucella has four primary species: *Brucella abortus* in calves, *Brucella melitensis* in goats and sheep, *Brucella suis* in pigs, and *Brucella canis* in dogs (Rossetti et al., 2017; Bosilkovski et al., 2021). *Brucellosis* can manifest as either an acute or chronic condition, broad or localised (Aysegul et al., 2013). *Brucella melitensis* and Brucella *abortus* pose a risk to humans and livestock (Foster et al., 2018; Dadar et al., 2019). *Brucella pinnipedialis*, *Brucella ceti*, and *Brucella microti* are three novel species isolated from seals, dolphins, and wolves respectively (Foster et al., 2018). Humans can be infected by consuming unpasteurized dairy products and/or direct contact with diseased animals' secretory secretions (Golshani et al., 2017). High-risk occupational groups are primarily affected by *Brucellosis*, including veterinarians, laboratory employees, abattoir workers, slaughterhouse people, livestock caretakers, and

farmers (Mukthar et al., 2022). In humans, brucellosis may present with fever, headaches, physical weakness, sweats, and back pains (Jiang et al., 2019). Humans are incapacitated by the condition, which causes significant debility and a loss of active workdays (Ayoola et al., 2017). *Brucella* is highly likely to infect genital organs (Zolzaya et al. 2014). The main clinical signs in cattle include abortion, reproductive failures (in both genders), and decreased milk production (Zhou et al., 2020). According to Esmaeli et al. (2014), about 20% of milk production losses, 2–3-fold abortions and 10% of the infertility in animals are attributed to *brucellosis*. Abortion-related brucellosis is estimated to be between 30% and 80% in dairy herds under traditional management (Kiros et al., 2016). The economic losses caused by these problems are enormous for farmers and at the national level in countries where the disease is endemic, and as a result, authorities must pay close attention (Zeng et al., 2019; Shelby et al., 2021).

Studies on human *brucellosis* have reported varying seroprevalences in Egypt 31.3% (El-Moselhy et al., 2018); Nigeria 24.1% (Aworh et al., 2013); Cameroon 5.6% (Awah-pendulum et al., 2018); Kenya 5.7% and 31.8% (Ogola et al., 2014); Uganda 17% (Tumwine et al., 2015) and Tanzania 1.41% (Sagamiko et al., 2020). Due to clinical presentation diversity, only 50.0% or 60% of cases are detected and recorded (Dean et al., 2012; Bennett., 2021).

In Zambia, there is scarce information on brucellosis in humans as most studies have focused more on animals (Muma et al., 2007; Muma et al., 2011; Mfune et al., 2021) than humans. Therefore, this study hopes to fill this existing knowledge by determining the seroprevalence and risk factors of human brucellosis among patients seeking medical attention at community hospitals in selected districts of the western province of Zambia.

1.2.Problem statement

Brucellosis has been reportedly endemic among traditional cattle in Southern and Western provinces of Zambia (Mfune et al., 2021). The disease affects both humans and animals, leading to substantial morbidity, mortality, and economic loss. Despite its impact, brucellosis often remains underdiagnosed and inadequately addressed in healthcare settings. Most people in Zambia's cattle-producing areas consume raw milk, which exposes them to *Brucella* infections (Mfune et al., 2021). There is a scarcity of data on human *Brucella* infections in

Zambia. However, seroprevalence is estimated to be at about 5.0 % (Muma et al., 2016) and recently reported at 20.3% in Southern province by Mubanga et al. (2021) mainly on occupationally exposed individuals (farmers, abattoir workers). However, there is a scarcity of studies on clinical cases in hospitals where all patients from different areas come for health care.

Furthermore, human *brucellosis* is not routinely tested for in hospitals, so there is a need to expand disease screening to other provinces. Febrile cases are increasing across the country and are frequently misdiagnosed as malaria, even though the majority of malaria tests are negative. Misdiagnosis of this disease causes disability, economic losses, and mandatory slaughter of animals worldwide owing to *Brucella* infection. The economic losses caused by these difficulties are enormous for farmers and at the national level in endemic countries. Hence, it needs the careful attention of authorities. Brucellosis poses a major public health challenge in Zambia, where rural and peri-urban populations are at high risk due to close contact with livestock. The disease can lead to chronic health issues, including fever, joint pain, and systemic complications, impacting individuals' quality of life.

The disease is often underreported due to nonspecific symptoms and limited diagnostic facilities. This study will help understand the actual burden of the disease and the extent of its spread in the community. Brucellosis can cause a range of symptoms, including fever, joint pain, fatigue, and night sweats. Chronic brucellosis can lead to serious complications such as arthritis, endocarditis, and neurological disorders. In Zambia, brucellosis has been reported to contribute significantly to febrile illness, with studies showing that up to 20% of febrile patients in rural areas may test positive for brucellosis when tested.

While brucellosis is rarely fatal, severe cases can lead to life-threatening conditions if not promptly treated. The disease’s chronic nature can result in prolonged illness and decreased quality of life.

In livestock, brucellosis causes reproductive issues, including abortion, stillbirths, and infertility. It has been estimated that brucellosis reduces milk production and livestock productivity by up to 30% in affected herds.

The economic burden of brucellosis in animals includes the costs of veterinary care, loss of productivity, and trade restrictions. It has been reported that brucellosis-related losses can reach millions of dollars annually in affected regions, affecting the livelihoods of farmers and livestock owners. The disease imposes a significant burden on healthcare systems and rural communities. Patients often experience long-term health issues that impact their ability to work and provide for their families. The economic strain includes both direct costs (medical expenses) and indirect costs (loss of productivity and income).

1.3. Study Justification

Western province is Zambia's second-largest cattle farming area, with documented *Brucella* seropositivity in animals (Mfune et al., 2021). In addition, humans have a tradition of drinking raw milk (Khalid et al., 2021). The province also has several slaughterhouses. Therefore, slaughterhouse workers and ranchers are at risk of contracting disease. Brucellosis is a significant zoonotic disease caused by *Brucella* species, which affects both humans and animals. In Zambia, brucellosis is endemic, particularly in rural areas where livestock farming is prevalent. Despite its impact, the disease often goes underdiagnosed due to a lack of awareness and inadequate diagnostic facilities. In Zambia, this disease is prevalent in rural regions where livestock farming is common. Understanding the seroprevalence and identifying risk factors associated with brucellosis is crucial for effective control and prevention strategies.

Most studies conducted in Zambia about *Brucellosis* were targeted at livestock farms, but this study will help us to have a view of Brucellosis in patients attending hospitals in the community of the western province. This information will be useful in designing intervention measures for human brucellosis in the Western province.

1.4. Research Questions

1. What is the seroprevalence of *Brucella* antibodies among patients seeking medical attention in the Western province of Zambia?
2. What risk factors are associated with Brucellosis among the febrile patients seeking medical attention in the Western Province of Zambia?

1.5. Objectives

1.5.1. General objective

To assess the epidemiological situation of Brucellosis among febrile patients seeking medical attention in the Western province of Zambia.

1.5.2. Specific objectives

1. To determine the seroprevalence of *Brucella* antibodies among febrile patients seeking medical attention in the Western province of Zambia.
2. To elucidate factors associated with Brucellosis among febrile patients seeking medical attention in the Western province of Zambia

CHAPTER TWO : LITERATURE REVIEW

2.0. Introduction

Brucellosis is an infectious bacterial disease that affects various domestic and wild animal species and humans (Moreno et al., 2022). This disease is also known as Malta fever, Bang's disease, undulant fever, or Mediterranean fever in humans, and it is a zoonosis with a wide range of clinical manifestations caused by tiny, aerobic, Gram-negative rods of the genus Brucella (Hayoun et al., 2020). The organism enters the body and multiplies in the lymphatic system before spreading to other organs (James, 2017). The disease can be transmitted from animals to humans by consuming unpasteurised milk and dairy products, consumption of undercooked meat, or skin penetration by persons in touch with cattle (Hayoun et al., 2020).

2.1. Historical Overview of Brucellosis

The story of *Brucellosis* started many years before, but the first one to isolate the *Brucella* organism was Sir David Bruce in 1886, who led the Malta Fever Commission that identified *Brucella melitensis* as the bacteria responsible for the disease (Głowacka et al., 2018). Before Sir David Bruce, the disease was discovered in Malta (Berhanu et al., 2020; Sayer., 2016). David Bruce first named the causative agent of brucellosis as *Micrococcus melitensis*, which he isolated from the spleen of a man who died of Malta Fever (Bruce., 1887). Dr Themistocles Zammit highlighted the disease's zoonotic character, and he was able to isolate the pathogenic bacterium from the milk, urine, and blood of afflicted native goats (Moreno et al., 2014).

2.2. Aetiology

The genus *Brucella* belongs to the family Brucellaceae, order Rhizobiales, class Alphaproteobacteria, and phylum Proteobacteria, all of which are Gram-negative bacteria having an outer membrane primarily made of lipopolysaccharides (Głowacka et al., 2018). Proteobacteria are a prominent phylum of bacteria with several diseases, such as Escherichia, Salmonella, Vibrio, and Helicobacter. Brucellosis is a bacterial disease caused by the genus *Brucella*, which spreads from animal to human (James 2017). Many species have been identified (El-Sayed et al., 2018) with currently 12 recognised species of *Brucella*, all of

which infect specific animal hosts but can also infect other creatures (Richard M., 2021). They are

The *Brucella* species include :

Brucella melitensis, which primarily infects goats and sheep, *Brucella abortus,* associated with cattle, *Brucella suis* found in pigs, *Brucella ovis* affecting sheep, *Brucella canis* affecting dogs, *Brucella neotomae* found in woodrats, *Brucella ceti* affecting cetaceans, *Brucella pinnipedialis* present in pinnipeds, *Brucella microti* seen in common voles, *Brucella inopinata* which can infect humans, *Brucella papionis* associated with baboons, *Brucella vulpis* found in foxes (Rajendhran et al., 2021).

Human Brucellosis is caused largely by *B. melitensis,* followed by *B. abortus*, *B. suis*, and *B. canis,* all of which have zoonotic potential (Fabrizio et al., 2019; Rajendhran., 2021, Whatmore et al., 2016). The ability of *Brucella* to reproduce and stay in host cells is directly related to its ability to induce chronic illness and evade innate and adaptive defenses (Mariana., 2016). The *Brucella* organisms have diverse host ranges, causing disease in various animal species and humans (Mekonnen Addis., 2015).

Four types of *Brucella* species cause most human infections (Molavi et al., 2013). *Brucella melitensis* (sheep and goats) is one of the most pathogenic species that infect humans (Kolo et al., 2022); *Brucella suis* causes disease both in humans and animals (Richard M., 2021). There are five different biovars of *Brucella suis.* However, *B. suis* biovars 1, 2, or 3 cause pig infections, most frequently in farming areas. (Elmonir et al.m 2022). Infections brought on by biovars 1 and 3 are more geographically and host-specific than those brought on by biovar 2 (Fabrizio et al., 2019). Regarding public health, biovar 2 is rarely pathogenic to humans, whereas biovars 1 and 3 are highly pathogenic to humans, producing severe sickness (Young et al., 2022). *B. suis* infection has been established in wild or feral pigs in some regions, and diagnostic procedures for wild and feral pigs are the same as for domestic pigs (Godfroid et al., 2013; Gong et al., 2021). Brucellosis due to *B. Melitensis, abortus,* and *suis* are epidemiologically most meaningful (Rajendhran et al., 2021). There are also novel species with zoonotic potentials, such as an amphibian-type *Brucella* isolate, that have recently been associated with human Brucellosis (Rouzic et al., 2021).

2.3. EPIDEMIOLOGY

2.3.1. Global Distribution of human brucellosis

Brucellosis is a worldwide disease that affects many countries (Noah et al., 2018; Laine et al., 2022). The prevalence of Brucellosis is becoming significant with time (Cardenas et al., 2019). The geographical distribution changes with Brucellosis re-emerging in some areas (Tsokana et al., 2020). However, this geographical distribution varies according to the species of Brucella (Suárez-Esquivel et al., 2020).

B. suis: In the United States, wild pigs are the primary source of *Brucella* infections. The Southeast and California both have high rates of this Brucellosis strain (Miller et al., 2021). Additionally, it can be found in Southeast Asia, Latin America, and Europe (Bennett et al., 2021)

B. canis: The infection spreads from dogs, and it is most often found in North, Central, and South America, Japan, and Central Europe (Miller et al., 2021).

B. abortus: The infection is caused by cattle and global distribution concerns (Miller et al., 2021). Several European countries have been eradicated, including Japan, Israel, Canada, Australia, and New Zealand (Miller et al., 2021).

Brucella melitensis: is the organism most frequently involved and is endemic in many Mediterranean countries, Africa, and Central America *(*Khan., et al. (2011).

2.3.2. Distribution of human brucellosis in Africa

Africa is one of the continents with the highest prevalence of human brucellosis (Krpasha et al., 2021). Several studies have found many brucellosis cases, yet many people are unaware of the disease (Mengele et al., 2023). A recent Ethiopian study found a 4% seroprevalence (Abebe et al., 2022), while a similar study in Nigeria reported a high prevalence of 33.5% of human brucellosis (Philip et al., 2020). Another study conducted in Morocco reported a seroprevalence of 33.2% (Kaoutar Faddane et al., 2022). In South Africa, *the gold standard of Brucellosis seroprevalence and culture prevalence* among the slaughtered cattle at the Gauteng abattoirs was estimated at 5.5% (Francis. et al., 2019). In Tanzania, a study reported an overall human seroprevalence of 10.9% (Robert Makala., 2020). In Rwanda, a study reported a seroprevalence of 6.1% among patients attending a District hospital (Gafrita et al., 2017), while another study

in Nigeria on seroprevalence and risk factors of human *Brucellosis* among patients attending healthcare facilities in Bauchi found a seroprevalence of 14.9% (Halilu et al., 2023).

Species of Brucellosis in Africa

In Africa, Brucellosis is caused by several *Brucella* species, impacting various animal hosts and potentially affecting human health. Brucella species observed in Africa include *B. abortus, B, melitensis ; B. suis and B. canis.*

Brucella abortu affects cattle and is a major cause of brucellosis in African livestock and has been isolated from cattle in Zambia (Muma, et al. (2006). *Brucella melitensis* primarily affects goats and sheep. It is one of the most common species in Africa and is also a significant zoonotic risk (Godfroid., et al. (2013). *Brucella suis is* typically associated with pigs, but can also infect other species. It has been reported in some parts of Africa, though it is less prevalent than *B. abortus* and *B. melitensis* (Scholz et al. (2008). *Brucella canis* affects dogs and has been reported in several African countries, although its prevalence is less well-documented compared to other species (Mantip, et al. (2016). *Brucella ovis* affects sheep and is present in some African regions, though less common compared to *B. melitensis (*Khan., et al. (2011).

Species of Brucellosis in Zambia

In Zambia, Brucellosis is primarily caused by several *Brucella* species, each affecting different animal hosts and potentially posing risks to human health. *Brucella abortus**:*** Affects cattle and is a major concern in Zambia's livestock sector (Chitambo, et al. (2021). This study explored the prevalence of *B. abortus* in cattle in Zambia and its implications for livestock health (Chitambo, et al. (2021). *Brucella melitensis* primarily affects goats and sheep and is also a significant zoonotic risk **(**Muma, et al. (2019).There are no reports of brucella melitensis in Zambia, through the risk exist considering that Zambia imports goats from Nambia where the diseases is present. *Brucella suis **is*** typically associated with pigs, but its presence in Zambia is less common **(**Peters et al. (2020). *Brucella canis* affects dogs and may be found in Zambia, though it is less documented (Kampamba et al. (2022).

2.3.3. Human brucellosis in Zambia

In Zambia, 5.03% *Brucellosis* seroprevalence was reported in humans by Muma et al. (2008). However, a recent study in the Southern province reported an overall seroprevalence of 20.3% in

humans (Mubanga et al., 2021), while another study by Mfune et al. (2021) in Southern and Western reported the overall seroprevalence in animal and herd levels of 7.53% and 21.14% respectively. These studies acknowledge the prevalence and importance of brucellosis as a zoonotic disease problem that requires concerted One Health approaches in Zambia. However, these studies were mostly limited to Southern Province, hence the need to expand the investigations to other provinces. These previous studies were limited to a particular group of individuals. However, our study focused more on patients with brucellosis-related clinical symptoms.

Implementing One Health in Zambia

Several measures are required to effective control brucellosis to protect the public and also prevent animal infections. These include the following:

(a) Developing Comprehensive Surveillance Systems

Implementing integrated surveillance systems that monitor both animal and human health is critical to control of brucellosis. This system should track brucellosis cases and outbreaks, using data to inform control measures (Fèvre, et al. (2019). Regular testing of livestock helps in identifying and managing outbreaks. This includes serological tests and sometimes culture methods (Huang, et al. (2023).

(b) Enhancing Veterinary and Medical Training

Providing training programs for veterinarians and healthcare workers on brucellosis management, ensuring they are equipped with the latest knowledge and techniques is essential in brucellosis infection prevention and control (Munyeme., et al. (2020). Monitoring programs help track the prevalence and effectiveness of control measures (Kumar, et al. (2023).

(c) Strengthening Collaboration

It is equally important to foster collaboration between government agencies, NGOs, and local communities to create a coordinated approach to brucellosis control. This includes sharing information, resources, and best practices (Morris, et al. (2021).

(d) Promoting Public Awareness

Equally important in brucellosis control is launching campaigns to raise awareness about brucellosis prevention, focusing on practices like avoiding consumption of unpasteurized dairy

products and adhering to proper animal handling protocols (Gao, et al (2023). Raising awareness among people who handle animals or consume unpasteurized dairy products (Singh, et al (2021) is useful in brucellosis control.

(e) Improving Infrastructure

The government needs to invest in infrastructure to support disease control efforts, including veterinary clinics, diagnostic laboratories, and public health facilities (Liao, et al (2019).

(f) Biosecurity Measures

Biosecurity measures need to be implemented to protect both humans and animals against brucellosis. This includes:

- **Hygiene Practices:** Proper disposal of aborted fetuses and placenta, and maintaining clean environments reduce transmission (Fariña, et al. (2019); Singh,. et al (2021).
- **Quarantine:** Isolating new or infected animals helps prevent the spread (Feng, L., et al. (2021).

By adopting a One Health approach, Zambia can address brucellosis more effectively, reducing its impact on both animal and human populations and enhancing overall health outcomes (Murray, et al (2022)

2.4. Modes of Infection and Transmission

Brucellosis is usually transmitted to humans through direct or indirect contact with infected animals or by consuming contaminated products from infected animals (Molavi et al., 2013). Therefore, eradicating animal bacteria is essential for preventing human infections (Bonnett et al., 2021).

In animals, the source of infection and transmission mode is vertical and horizontal (Mahendra et al., 2020). Horizontal transmission occurs through consumption of contaminated feed, skin penetration, via conjunctiva, inhalation, and udder contamination during milking, or by sucking the discharge of an animal, new-born calf, or retained foetal membrane (Mahendra et al., 2020). Congenital infection occurs after parturition and is typically eradicated, with just a few animals remaining sick as adults (More et al., 2017). Venereal infections can also occur and are most commonly found with *B. suis* infections (More et al., 2017). Vertical transmission occurs

transplacental and during delivery (Mahendra et al., 2020). This pathway also disseminates *Brucella suis* and *B. canis* (Mahendra et al., 2020). Although *Brucella abortus* and *B. melitensis* can be identified in sperm, venereal transfer of these organisms is rare (Elmonir et al., 2022). Some species of *Brucella* may be found in organic secretions such as urine, faeces, hygroma fluids, saliva, and nasal and ocular secretions (Ibarra et al., 2023). These sources appear to be rather unimportant in transmission in most situations; however, some may assist in accounting for the direct non-venereal transmission of B. *ovis* between rams (Salih., 2016). Transmission of brucellosis to humans is possible through the ingestion of unpasteurized dairy products from small herds, commonly sold door-to-door at low costs (Fargol et al., 2020 ; Almashhadany et al., 2020). Human infection is also transmitted by skin tears caused by direct touch with tissues, blood, urine, vaginal secretions, aborted fetuses, or placentas; occupational aerosol infection in laboratories and abattoirs has also been documented (Salih., 2016). Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human illnesses (Ulu et al., 2015).

2.5. Pathogenesis of Brucellosis

Brucella has a high tissue tropism for the lymphoreticular and reproductive systems as well as its intracellular lifestyle, which protects it from antibiotic actions and promotes clinical illness symptoms and pathology while limiting exposure to innate and adaptive immune responses (Figueiredo et al., 2016; Wareth et al., 2020). *Brucella* was initially reported as a facultative intracellular parasite bacterium capable of replicating in both professional phagocytes like macrophages, dendritic cells (D.C.), granulocytes and nonprofessional phagocytes including epithelial, fibroblastic, and trophoblastic cells (Luizet., 2019). *Brucella* engages with macrophage cell membranes through lipid rafts, allowing it to enter host cells and form Brucella-containing vacuoles (BCVs) that are encased in phagocytic vesicles (Hanwei et al., 2021). After cellular entry, from 8 to 12 hours, BCV acquires some host marker molecules via interactions with lysosomes and endosomes, develops endosomes in membrane-bound vacuoles, and generates acidified endosomes, and BCV is now known as endosomal Brucella-containing vacuole (eBCV) (Jioa et al., 2021). The Type IV secretory system (T4SS) mediates the interface between the effector protein and the endoplasmic reticulum (E.R.) exit site as BCV develops and matures, obtaining E.R. and Golgi apparatus-derived membranes (Jiaoa et al., 2021). Following the loss of the early host marker molecules, the eBCV acquired Lys marker molecules (such as Rab7, LAMP-1, etc.) (Sonia., 2022). Escaped Lys degradation,

BCV will enter the E.R. and merge with the E.R. in a Sar1 and Rab2-dependent way (Sonia., 2022). The BCV is now known as repetitive *Brucella*-containing vacuole (RBC). rBCV will be changed into autophagic *Brucella*-containing vacuole (aBCV) later in the infection (Sonia., 2022). *Brucella* can infect the host through eating, inhalation, conjunctiva, or skin abrasions (Sonia., 2022). After infecting the host, the pathogen is sequestered within cells of the reticuloendothelial system and the smooth lipopolysaccharides that cover the bacterium, as well as proteins involved in signalling, gene regulation, and transmembrane transportation (Mekonnen Addis., 2015). Then, during the early stages of infection, smooth, non-endotoxic lipopolysaccharides inhibit the development of innate and specific immunity (Mekonnen Addis., 2015). Furthermore, smooth lipopolysaccharide in *Brucella* may be involved in the inhibition of apoptosis of infected cells because resistance to apoptosis of infected cells has been observed in patients with acute and chronic disease, causing arthritis, orchitis, hepatitis, encephalomyelitis and endocarditis (Hanwei et al., 2021).

2.6. Clinical manifestation

Brucellosis is a systemic disease with several clinical manifestations (Yang et al., 2015), which may be difficult to specify because they are confused with other diseases like malaria and salmonellae (Ulu et al., 2015; Mehari et al., 2021). After exposure to the disease, these clinical manifestations can appear after 2 to 4 weeks (Ulu et al., 2015). Some appear about five to 30 days after contact with the bacteria and go on for months or years (Bosilkovisk., 2014). The clinical presentation of *Brucellosis* varies from an acute, nonspecific febrile illness to chronic and might begin with a complicated form such as osteoarticular and neuropsychiatric abnormalities or other symptoms (Amar et al., 2017). These symptoms depend on the type of *Brucella* that has infected a person (Amonov et al., 2020):

- *B. melitensis* may cause sudden and severe symptoms, leading to disability;
- *B. suis* may cause areas of infection (called abscesses) in different organs;
- *B. abortus* usually causes mild or moderate symptoms, but they are more likely to become chronic (long-lasting);
- *B. canis* symptoms may come and go. They are similar to *B. abortus* infection, although people with *B. canis* often have vomiting and diarrhoea.

However, in humans, *Brucellosis* is distinguished by symptoms such as persistent, intermittent, or irregular fever of variable duration, headaches, weakness, profuse sweating, chills,

depression, and weight loss, as well as the acute or insidious onset of fever and one or more of the following: Night sweats, arthralgia, headache, weariness, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or localised organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly) are all symptoms of meningitis (Mahendra et al., 2020). *Brucellosis* sometimes presents clinical signs similar to those of other febrile diseases, making it difficult to distinguish between these pathologies (Amar et al., 2017; Matthew., 2013).

2.7. Diagnosis

To control and eradicate brucellosis is extremely important to diagnose it promptly and accurately. Brucellosis diagnostic tests fall into two categories: those that demonstrate the presence of organisms and those that detect an immune response to their antigens.The clinical indications of *Brucellosis* are diverse and nonspecific, and adequate treatment requires laboratory testing to confirm the presence or absence of the disease (Pablo et al., 2017). Culture, serological testing, and nucleic acid amplification assays can be used to diagnose the condition (Giovanni et al., 2021). It should be noted that the diagnosis of Brucellosis is based on clinical manifestations and laboratory tests, with serological diagnostics favoured for disease detention (Giovanni et al., 2021). Human *Brucellosis* can be diagnosed using a variety of laboratory procedures such as blood culture, serological assays, and skin tests (Battikh et al., 2021). However, serological diagnostic tests are becoming more common (Battikh et al., 2021). *Brucella* antigen (Ag) is detected in serum 1-2 weeks after infection using a serological assay (Battikh et al., 2021). A study by Muma et al. (2007) assessed the sensitivity (Se) and specificity (Sp) of the Rose Bengal test (RBT), Fluorescent Polarisation Assay (FPA), and Enzyme-Linked Immuno-Sorbent Assay (ELISA) in traditional Zambian cattle and discovered that RBT and FPA had better test performance indices.

2.7.1. Bacteriological culture detection of *Brucella*

The bacteriological culture of *Brucella* organisms is the gold standard method, although the sluggish growth of species of the genus, laboratory safety concerns, and lower sensitivity in chronic disease and focused infections make culture identification of *Brucella* germs difficult (Pablo et al., 2017).

Types of blood culture

Culture can confirm or detect the existence of the disease in blood in its early stages when serological test results are still negative or show low or borderline antibody titers, and culture has the benefit of providing a diagnosis in individuals who do not exhibit clinical indications (Giovanni et al., 2021; Ostrominski et al. 2023).

2.7.2. Serological test

Indirect methods, or immunological methods, are used to detect an immune response to Brucella antigens. They are often used for simplicity of performance and interpretation and are based on antibody detection. The detection of these Brucella-specific antibodies in milk or serum samples may be performed through numerous immunological diagnostic tests assays, including milk ring test (MRT), buffered Brucella agglutination tests (i.e., Rose Bengal test (RBT); Card Test (CT), and buffered plate agglutination test (BPAT), complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA) and fluorescence polarisation assay (FPA). Most of the indirect tests do not have high test index (sensitivity and specificity) and it recommended to use several techniques to increase the level of detection (GarinBastuji et al., 2006). Indirect tests are used globally as screening tests. In animals, these test contribute to eradication policies and to study herd/flock prevalence of infection and surveillance (OIE, 2016). The World Organisation for Animal Health (OIE) emphasizes that no single serological test is appropriate in all epidemiological situations, since all have limitation.

For human brucellosis diagnosis, as the Brucella organism grows very slowly in vitro, serological tests are used as screening tests for preliminary diagnosis of brucellosis (Khan et al., 2017). The serological test, which is based on a method of testing the patient's immune system in search for antibodies that attest to previous exposure to the disease, is still one of the most significant tests for detecting or confirming Brucellosis, particularly in endemic areas (Pablo., 2020).

Brucella Antigens for serodiagnosis: LPS and Cystol Proteins

Wright and Smith invented this test in 1897 with simple agglutination for the diagnosis of human Brucellosis, and it was later adapted for veterinary medicine as well (Pablo et al., 2017; Pablo., 2020).

Serological Tests that Target Brucellar S-LPS

Rose Bengal Test (RBT)

The RBT detects nonagglutination and agglutination antibodies (William et al., 2017; Sevliya et al., 2020). RBT is a rapid and simple screening test used primarily for cattle (William et al., 2017). It detects agglutinating antibodies in serum and it remains a valuable screening tool, particularly in field conditions (William et al., 2017). However, its specificity can be affected by cross-reactivity with other diseases (Dadar et al. (2021). Despite its advantages, RBT can produce false positives due to cross-reactivity (Khan et al. (2020). Khan et al explored methods to improve the specificity of RBPT by using purified antigens and reducing non-specific reactions (Khan et al. (2020).

The Standard Agglutination Test (SAT)

The Standard Agglutination Test (SAT), invented by Bruce, is the most often used serodiagnostic assay for diagnosing *B. abortus*, *B. melitensis*, and *B. suis* infections (Pablo., 2020). Although the SAT identifies antibodies to brucellae S-LPS, it is ineffective in identifying diseases caused by rough B. canis species (Pablo., 2020; Xu et al., 2023; Eko et al., 2022).

Brucella Coombs Gel Test

The Brucella Coombs gel test (Odak test) is a novel, easy-to-perform agglutination experiment in microcolumns comprising a gel matrix and Coombs antibodies (Baris et al., 2016).

The Complement Fixation Test (CFT)

This test involves inactivating the patient's complement by heating the serum at 56°C for 30 to 60 minutes, then serially diluting the serum and adding entire dead Brucella cells and pre-titrated rabbit complement to the test tubes (Pablo., 2020; Mahboubeh et al., 2020).

Immunocapture agglutination test

Traditional agglutination tests are disadvantageous because they are labour-intensive and time-consuming (Pablo., 2020).

IgG avidity ELISA

The IgG zeal, a modification of the standard ELISA, is based on the idea that low-affinity IgG antibodies are gradually replaced by high-affinity antibodies over time (*Engelmaie et al., 2022*).

Serological Tests That Target Cytosolic Proteins

Indirect Enzyme-Linked Immunosorbent Assays (i-ELISA) or Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) is becoming more widely used for same-day brucellosis diagnosis (Pablo., 2020). The test is carried out in precoated 96-well microtiter plates with Brucella antigen (whole cells, sonicate protein extracts, or another antigen). Although the ELISA method can detect S-LPS, plates are typically coated with cytosolic protein antigens. The serum from the patient is serially diluted and placed into the wells before the plates are incubated (Pablo., 2020). Following a washing stage, an enzyme-conjugated anti-human IgG, IgM, or IgA (typically alkaline phosphatase or horseradish peroxidase) is applied (Pablo., 2020). Following the addition of the enzyme-specific substrate, the optical density of the wells at the relevant wavelength is measured (Pablo., 2020). The sensitivity of in-house ELISAs is often good, but the specificity is lower than that of agglutination tests (Pablo., 2022). Commercial ELISA kits typically perform poorly when used in endemic areas and should be examined with the epidemiological context in mind (Pablo., 2022). For difficult, focused, and chronic situations, some authors recommend ELISA. The ELISA is suggested when other serodiagnostic tests are negative, but patients have a clinical picture that suggests brucella infection (Pablo., 2020). When conducted on CSF specimens, the test is also indicated for the serodiagnosis of neurobrucellosis (Pablo., 2020). Because it allows for the examination of several samples at the same time, ELISA is also commonly employed in epidemiological surveys. It is also the most commonly used method for detecting specific IgG isotypes. False-negative results for anti-Brucella IgM antibodies, on the other hand, may occur from an excess of IgG. Hence, both IgG and IgM isotypes should be tested concurrently (Pablo., 2022). False-positive results due to the presence of rheumatoid factor are possible, and they should be routinely eliminated by absorption of the serum before testing for Brucella IgM antibodies (Pablo., 2020). The competitive ELISA is a competitive binding process executed by the original antigen (sample antigen) and add-in antigen. The procedures of competitive ELISA are different in some respects compared with Indirect ELISA, Sandwich ELISA and Direct ELISA (Sana., et al. 2022): A simple procedure list is as follows:

1. Primary antibody (unlabeled) is incubated with sample antigen.
2. Antibody-antigen complexes are then added to 96-well plates which are pre-coated with the same antigen.
3. Unbound antibody is removed by washing the plate. (The more antigen in the sample, the

less antibody will be able to bind to the antigen in the well, hence "competition.")

4. The secondary antibody that is specific to the primary antibody and conjugated with an enzyme is added.

5. A substrate is added, and the remaining enzymes elicit a chromogenic or fluorescent signal.

6. For competitive ELISA, the higher the sample antigen concentration, the weaker the eventual signal.

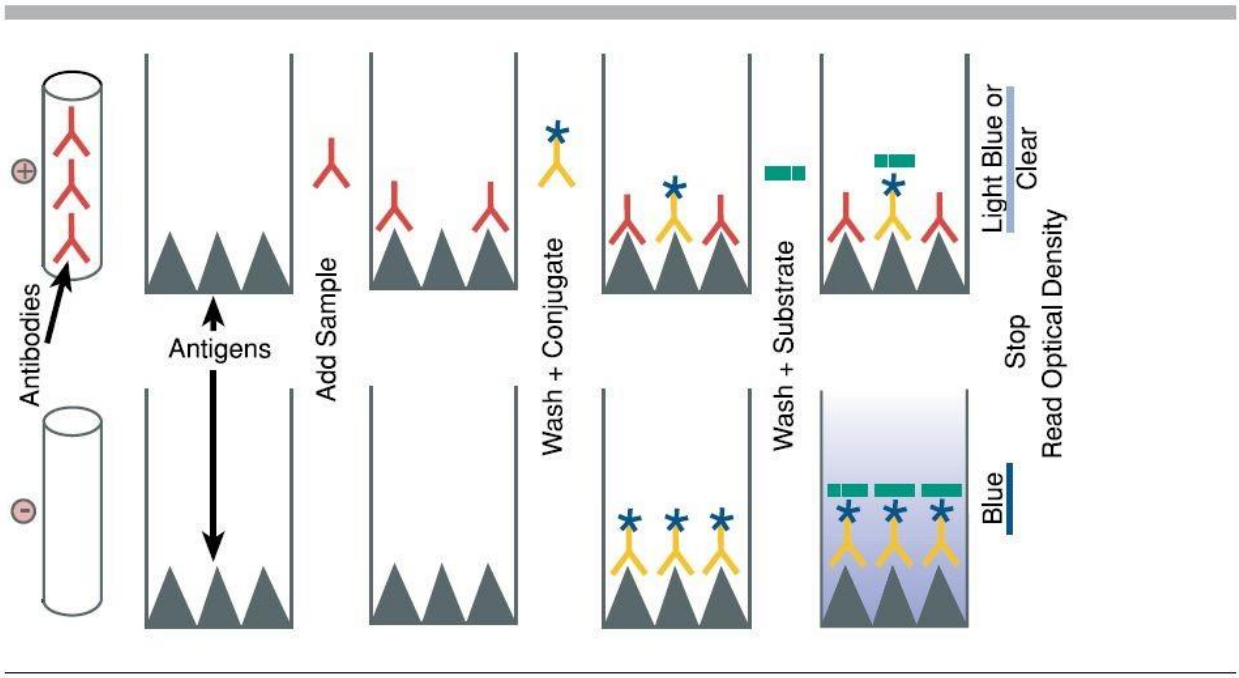

Figure 1: Pictoral Presentation of the Procedure for Competitive c-Elisa

The major advantage of a competitive ELISA is the ability to use crude or impure samples and still selectively bind any antigen that may be present. (Note that some competitive ELISA kits include enzyme-linked antigens rather than enzyme-linked antibodies. The labelled antigen competes for primary antibody binding sites with your sample antigen (unlabeled). The more antigen in the sample the less labeled antigen is retained in the well and the weaker the signal). Commonly, the antigen is not first positioned in the well.

Laboratory Analysis: Competitive ELISA for Brucellosis

Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) is a widely used method for the diagnosis of brucellosis, particularly in livestock. It is valued for its sensitivity and

specificity. Here, we discuss the principle behind C-ELISA, its diagnostic performance characteristics, and recent insights from the literature (Pablo., 2020):

Principle of Competitive ELISA

Competitive ELISA is based on the principle of competition between an antigen in the sample and a labelled antigen for a limited number of antibody binding sites on a solid phase. Here's a step-by-step explanation of the process:

1. **Coating**: The wells of a microtiter plate are coated with a specific antibody that recognizes the antigen of interest.
2. **Blocking**: The wells are then blocked to prevent non-specific binding of proteins.
3. **Competition**:
    - The sample (containing the target antigen) is added to the wells, along with a fixed amount of enzyme-labelled antigen (which is a competitive marker).
    - Both the sample antigen and the labelled antigen compete for the same antibody binding sites on the plate.
4. **Binding**: The antigen in the sample binds to the antibody, reducing the amount of enzyme-labeled antigen that can bind.
5. **Detection**:
    - After washing away unbound antigens, a substrate for the enzyme is added.
    - The enzyme-substrate reaction produces a colorimetric signal proportional to the amount of enzyme-labelled antigen that was not bound (i.e., inversely proportional to the amount of antigen in the sample).
6. **Quantification**: The intensity of the color is measured using a spectrophotometer. A higher color intensity indicates lower levels of the antigen in the sample, while a lower color intensity indicates higher levels of the antigen.

Sensitivity and Specificity of C-ELISA

The performance characteristics of C-ELISA, particularly sensitivity and specificity, are crucial for reliable diagnosis:

Novel Serodiagnostic Tests

TR-FRET assay

Fluorescent resonance energy transfer (FRET) occurs when donor and acceptor fluorophores with appropriate spectrum characteristics are situated close together and correctly orientated exchanged energy (Pablo., 2020).

Fluorescence Polarisation Immunoassay

The fluorescence polarisation immunoassay (FPA) works by measuring the rotational velocity difference between a tiny antigen molecule in solution labelled with a fluorochrome and the same antigen molecule coupled with its antibody (Giovanni et al., 2021; Hendrickson et al., 2020).

Rapid Point-of-Care Tests

Shepherds of goats, sheep, or camels living in remote rural areas who migrate annually in search of fresh pastures and ship cooled or frozen blood specimens from the field to distant centralised testing laboratories are common in endemic brucellosis populations (Pablo., 2020).

Dipstick assay

The dipstick assay is a quick test for detecting Brucella-specific IgM antibodies. It comprises a nitrocellulose strip with S-LPS generated from *B. abortus* as the antigen applied in a separate line (Pablo., 2020; Verónica., 2019).

LATERAL FLOW ASSAY The lateral flow assay (LFA) is a streamlined variant of an ELISA housed in a suitable plastic device (Pablo., 2020).

RBT is a rapid diagnostic test

In recent years, the rose bengal test (RBT) has gained popularity in hospital emergency rooms for the quick diagnosis or exclusion of brucella infections in patients presenting with a feverish illness (Pablo., 2020; Ying-Hock et al., 2017; McGiven., 2015; Gamal et al., 2020).

PCR based methods

Nucleic acid amplification assays can also diagnose *Brucellosis* (Pablo., 2020).

Nucleic acid amplification assays are crucial for detecting and quantifying nucleic acids (DNA or RNA) in various biological samples.

The principles of these assays involve several key steps (Pablo., 2020):

1. **Target Nucleic Acid Detection**: The assay begins with the identification of a specific nucleic acid sequence that is of interest. This involves using primers or probes that are complementary to the target sequence.

2. **Amplification**: The nucleic acid is amplified to produce multiple copies of the target sequence. This is achieved through various methods such as Polymerase Chain Reaction (PCR), Reverse Transcription PCR (RT-PCR), or Loop-Mediated Isothermal Amplification (LAMP).

3. **Signal Generation**: During amplification, a signal is generated that can be detected and measured. This may involve fluorescent dyes, enzymes, or other reporter molecules that indicate the presence and amount of the target nucleic acid.

4. **Quantification and Analysis**: The amplified nucleic acids are quantified and analyzed to determine the presence and concentration of the target sequence. This may involve comparing the signal to a standard curve or using specific thresholds for detection.

2.8. Treatment

Although various studies have been conducted, early detection and treatment are critical for preventing morbidity and death, and treatment is anti-biotherapy, which can be monotherapy, biotherapy, or therapy, depending on clinical indications (Bosilkovisk., 2023). The commonly used drugs for *brucellosis* treatment are doxycycline and rifampin in combination, which are given for a long time, either 6 or 8 weeks (Zambia Standard Treatment Guideline 2020).

Animal Brucellosis treatment, prevention and control

Antibiotic therapy of infected animals, or those who may have been exposed to *Brucellae* agents, has not been widely used and should be avoided as a treatment option for Brucellosis (WHO., 2017; Pérez-Sanchp et al., 2015). A few studies have demonstrated that treating the flock herd reduces the incidence of Brucellosis. However, this approach is regarded to be limited in practice. It is almost always more cost-effective and practicable to avoid diseases than to try to control or eliminate them. Preventive methods for *Brucellosis* include selecting

replacement animals with care (Moreno et al., 2022). Vaccination of animals typically leads to the elimination of clinical disease and a reduction in the number of organisms expelled by sick animals. Vaccination is the only feasible and cost-effective method of controlling animal brucellosis in many endemic countries. While there is no perfect vaccination, attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats, as well as *B. abortus* strain 19, have proven to be superior to all others (Moreno et al., 2022, FAD PReP/NAHEMS Guidelines., 2015).

Human Brucellosis Treatment Prevention and Control

The administration of efficient antibiotics for an acceptable period is critical in treating human *Brucellosis* (Sara et al., 2019). Furthermore, this antibiotic treatment should be started as soon as possible, even in individuals who appear to improve independently (Lokamar et al., 2020). In those patients with complications, additional treatment, including, in some cases, surgical intervention, will be necessary (Arzu et al., 2020). Because direct or indirect contact with infected animals or their products is the ultimate source of human *Brucellosis*, prevention must focus on avoiding such contact. In many cases, there is little choice but to try to lessen the disease's impact and risk of infection through personal cleanliness, safe working practices, environmental protection, and food hygiene (Luelseged et al., 2018). Because no safe, effective, widely available vaccinations are approved for human use, prophylaxis currently plays a minimal role in disease prevention (Luelseged et al., 2018). Food safety is one of the primary pillars on which human health protection is based; humans normally get infected by Brucella mostly through improperly prepared and/or maintained animal-derived food (WHO., 2017). Laws, regulations, and veterinary policy initiatives will not achieve the desired objectives unless the entire community participates in health education in schools, the workplace and the general public (WHO., 2017). For starters, the higher the level of self-sufficiency and social awareness, the more individuals and families will assume responsibility for protecting their animals and themselves from disease threats conveyed directly through the food of animal origin or by fomites (Ning Zhang., 2020).

2.9. Public Health Importance and Risk factors

2.9.1. Public Health Importance

Brucella is a significant problem in many resource-limited countries (Moriyon et al., 2023). Since this disease is one of 7 neglected diseases (Franc et al., 2018). Governments do not invest

much in resources such as medicines, diagnostic tests, vaccines against Brucellosis in animals and other materials that can help the vulnerable population eradicate the disease (Moriyon et al., 2023). This causes a serious problem that requires special attention. Brucellosis is believed to be one of the world's major causes of illness in animals and people (Franc et al., 2018). It is a public health issue in most nations, with health consequences for animals and humans and economic consequences for people and communities (Franc et al., 2018). It is an occupational disease, with those most vulnerable being laboratory personnel, veterinarians, abattoir workers, farmers, and animal keepers who work with animals or handle aborted foetuses and animal products infected with *Brucella* agents (Mfune et al., 2021). Millions of people are in danger worldwide, particularly in areas where infection in animals or humans has not been controlled, heat treatment of milk processes such as pasteurisation is not frequently used, and hygiene standards are low (Mukthar et al., 2020). Brucellosis has a huge impact on living creatures, affecting their health and social and economic lives, and this is especially true in rural areas where livestock husbandry is the primary source of income (Lokamar et al., 2020). The species of Brucellosis determines the risk of the disease to a patient (*Sabra et al., 2021*). *Brucella melitensis* is the most commonly reported and isolated variety as a cause of human disease. It is the most dangerous and is linked to severe acute illness (Lauren W et al., 2021). Contrary to popular belief, *B. melitensis* remains fully pathogenic to humans after infecting cattle (Sandip et al., 2020). Bovine infection is very difficult because of the massive volume of infected milk that a single cow may produce and the extensive environmental pollution that even single miscarriages or sick births can cause (Sandip et al., 2020).

2.9.2. **Risk Factors for Brucellosis Occurrence**

Occupational and Environmental Exposure: Individuals who work with livestock, including farmers, veterinarians, and slaughterhouse workers, are at higher risk due to direct contact with infected animals or their secretions. Studies have shown that occupational exposure significantly increases the risk of brucellosis in humans (Pappas., et al. (2019). Poor animal husbandry practices, such as inadequate sanitation, improper disposal of animal waste, and lack of regular veterinary care, can increase the risk of brucellosis transmission and highlight that farms with suboptimal biosecurity measures are more likely to experience brucellosis outbreaks (Maboni et al. (2020).

Consumption of Unpasteurized Dairy Products: Consumption of unpasteurized milk and dairy products from infected animals is a well-documented risk factor for brucellosis and it was

found that individuals consuming raw dairy products in endemic regions had a higher incidence of brucellosis (Villar et al, (2021)**.**

Geographic and Environmental Factors: Brucellosis is more common in regions with a high prevalence of the disease in animals. In endemic areas, such as parts of Africa, the Middle East, and Latin America, the risk is higher due to the widespread occurrence of the infection in livestock (Hussein, et al. (2022).

Factors such as climate, altitude, and environmental conditions can influence the prevalence of brucellosis and it was noted that wetter climates and high humidity can facilitate the persistence of *Brucella* in the environment (Ishii et al. (2019).

Animal Factors: Certain animal species and breeds may be more susceptible to brucellosis. For example, *Brucella abortus* is prevalent in cattle, while *Brucella melitensis* often affects goats and sheep and Genetic susceptibility of different breeds can also play a role in disease prevalence (Galińska, et al. (2021). Infected animals, particularly those that are pregnant or have recently aborted, can shed *Brucella* bacteria in their secretions, increasing the risk of transmission to other animals and humans (Feng, al. (2019).

Socioeconomic and Cultural Factors: In lower-income regions, a lack of resources for proper veterinary care and sanitary conditions increases the risk of brucellosis (Rashid et al. (2021). Certain cultural practices, such as traditional methods of animal slaughter or unpasteurized milk consumption, can increase the risk of brucellosis (Almeida et al. (2022)

Vaccination and Control Measures: In regions where vaccination programs for livestock are not implemented or are poorly executed, the risk of brucellosis is higher and the gaps in vaccination coverage contribute to ongoing brucellosis outbreaks (Ortega et al. (2020).

Lack of Disease Aware: In humans, lack of awareness about brucellosis and its transmission can lead to higher risk. Public health education and training for those at risk are essential for prevention and highlighted the importance of educational programs in reducing brucellosis incidence (Hernandez et al. (2023).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area

The study was carried out in the Western province of Zambia, an area known to be endemic for brucellosis in livestock. The study was conducted in three districts: Mongu, Limulunga and Senanga. Western Province covers an area of about 126 386 km2. The prominent physical features are the Barotse floodplain of the Zambezi River and the sandy soils. The province lies between the latitudes 14º S and 17º S and longitudes 22º E and 25º E (Sinkala, 2015). The core area of this research covers the central Barotse plain fed by the Zambezi River. This area is characterised by the annual floods, to which three-quarters of Barotse cattle are subjected in the Limulunga, Mongu and Senanga areas. The main economic activity for the people of the Western province is livestock production, especially cattle rearing, followed by fishing and crop production. The choice of province and district was based on the fact that the Western province is one of the major livestock-producing provinces, and brucellosis has been reported (Muma et al., 2012). A study conducted in the Western Province by the District Veterinary Office in 2015 showed that the overall seroprevalence of brucellosis was 16%.

In 2022, Western Province had a total human population of 1 363, 520 and the area covered is 126,386 Km2 with a density of 10.79/ km2 (CSO). Mongu district had a population of 197,816 with 33.19/ km2, Limulunga district with a population of 61,102, and Senanga district with a population of 112,040 with 10.60/ km2 (CSO, 2022). Senanga is located 543 kilometers from Lusaka, 601.2km from Lusaka and Mongu is 607.47 km from Lusaka. These individuals were grouped into categories depending on their level of daily activities that could lead to direct contact with suspected *Brucella*-infected animals or the use of infected animal products. Three health facilities were selected in Senanga District namely Ngundi, Sikumbi and Lui Wanya Health Centres, while two were included from Mongu (Lealui Mini Hospital and Sefula Rural Health Centre) and Limulunga Districts (Limulumga Mini Hospital and Ikwichi Rural Health Centre) respectively.

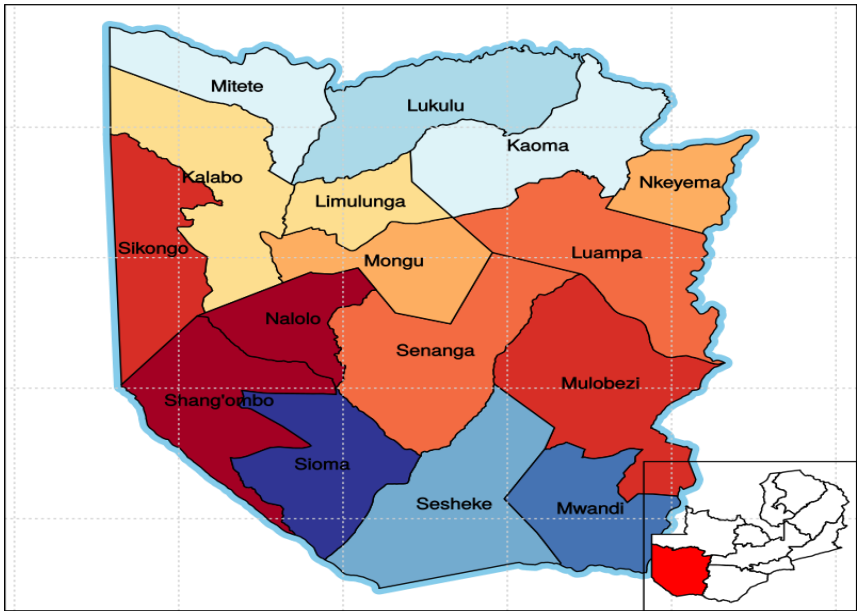

Figure 2: Map Western Provinces of Zambia (Operational Data Portal, July 2020 Zambia)

3.2. Study design

A cross-sectional study was conducted among consenting febrile patients seeking medical attention at selected health facilities in Senanga, Limulunga and Mongu districts of Western Province in Zambia the three districts were selected because they are home to the central plain where farmers take cattle for grazing. Blood samples were collected from symptomatic patients who visited the health facilities following their consent. A questionnaire was administered to the participants to collect epidemiological information.

3.3. Sample size

The sample size was calculated and estimated using the Ausvetepitool software (http://epitools.ausvet.com.au/) based on the following assumptions :

a) Expected prevalence of 20% (Mubanga et al., 2021), the results showed that 20.3% (95% Cl: 3.68 to 6.38) of people had serological evidence of exposure to Brucella spp. Antigen.
b) Desired absolute precision (+/-) =5%.
c) Confidence level =95%

The required sample size was calculated using the formula based on these assumptions.

$$n = \frac{Z^2 * p * (1 - p)}{d^2}$$

Where z = 1.96, p = the expected seroprevalence of 20%, d = the desired absolute precision of 9% and confidence level of 95%.

$$n = \frac{1.96^2 * 0.05 * (1-0.05)}{(0.09)^2}$$

$$n = 225$$

We targeted a sample of persons from each district. The sample size was distributed in the three districts according to the weight index (human population) for each district (Table 1).

Table 1: Sample size of humans weighted by district population

District	**Weighting index (Human Population**	**Number of persons to be sampled**
Mongu	197816	120
Senanga	12040	68
Mongu	61102	37
All areas	**370958**	**225**

3.4. Inclusion criteria

The inclusion criteria were all consent patients at hospitals between 1st April and 31st January 2024 who presented any of the following signs and symptoms: intermittent or persistent fever, headache, weakness, profuse sweating, chills, arthralgia, weight loss and joint pain fever, with a negative result for malaria were included in the study.

3.5. Exclusion criteria

Patients with another confirmed diagnosis, such as smear-positive tuberculosis, malaria, salmonella, febrile disease and those who did not consent were excluded from the study. Patients who presented with different signs and symptoms and/or were unwilling to participate were excluded from the study

3.6. Sampling strategy

The study included a purposive sampling of human populations in three districts of the Western province who were occupationally exposed to brucellosis. The individuals were grouped into categories depending on their level of daily activities that could lead to direct contact with suspected *Brucella*-infected animals or the use of infected animal products.

3.7. Sampling and Laboratory analysis

3.7.1. Blood sampling

Two hundred and twenty-five individuals were purposively sampled from the Senanga, Mongu and Limulunga districts in the Western Province of Zambia. A list of all health facilities in each district was obtained and used to select individuals to screen randomly. A clinical officer collected four (4) ml of blood via the median cubital vein and stored it in sterile plain tubes at +4 ºC.

3.7.2. Laboratory analysis

Blood samples were screened using c-ELISA. The figure below displays the reagents used during the analysis.

Figure 3 *: ELISA reagents displayed on the work station*

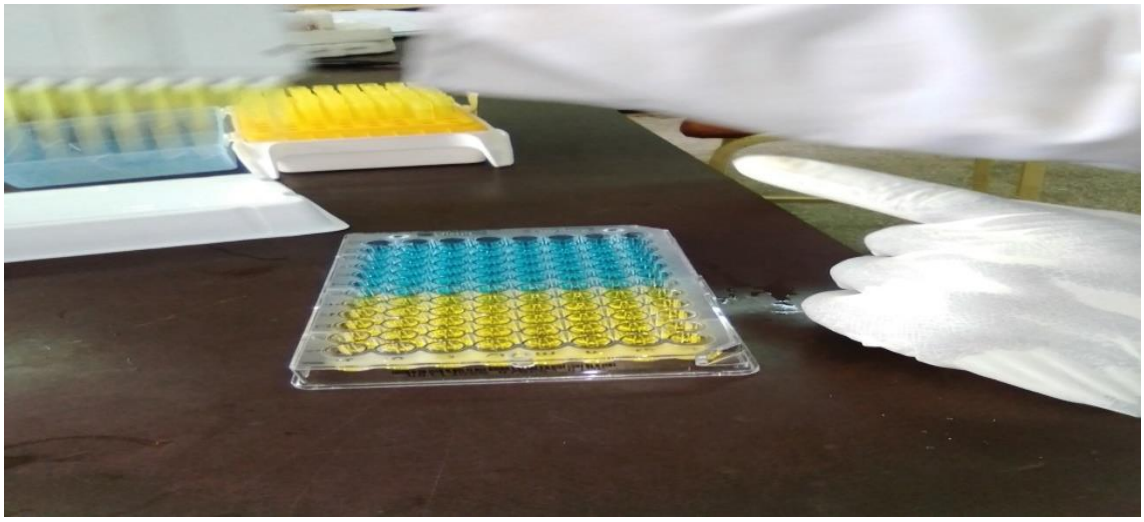

Figure 4*: c-ELISA plate impregnated with antigen-antibody read for reading*

c-ELISA analysis was done according to the manufacturer's guidelines and reagent kit manual (SVANOVIR^{R} *Brucella* –Ab c-ELISA, Boehringer Ingelheim Svanova, Sweden).

Briefly, 50uL of Mab-solution was added into all wells used for controls and samples (time difference between controls (A positive, weak positive, and negative control were included in a 96-well plate.)/samples and Mab-solution addition did not exceed 10 minutes). The plate was sealed, and the reagents were thoroughly rinsed for 5 minutes using a plate shaker. The plate was incubated at room temperature (25^{0} C) for 30 minutes. The plate was rinsed four times with PBS-Tween buffer, and the wells were filled up at each rinse. The plate was then emptied, and the plate was tapped hard to remove all remaining fluid. Then, 100uL of the conjugate solution was added to each well, and the plate was sealed and then incubated at room temperature for 30 minutes. Rinsing was repeated, as explained above. Then, 100uLsubstrate solution was added to each well and incubated for 10 minutes at room temperature (timing began after the first well was filled). The reaction was stopped by mixing 50uL of stop solution in the same order as the substrate solution. Then, the optic density (OD) of controls and samples was measured at 450nm in a microplate photometer (the air was used as blank). The OD was measured within 15 minutes after the addition of the stop solution to prevent fluctuation in OD values. The OD of the positive control was the one with which the OD of each test serum was compared to establish the final result (negative or positive). Determination of the positive and negative tests using the cut-off was provided in the cELISA kit guide. Negative results were determined by a percent inhibition (PI) of < 30%, while positive results were ≥ 30%.

3.8. Epidemiological data collection (Questionnaire Survey)

Epidemiological information was collected from the participants using a structured questionnaire which was adopted from a similar study by Mubanga et al 2021. The questionnaire consisted of four parts: (I) sociodemographic characteristics of the study participants; (ii) types of slaughtering activities; (iii) work hygiene-related factors (i.e., wearing personal protective equipment, contact with blood or faeces, and presence of skin wound); (iv) other potential risk factors (cattle breeding, and consumption of raw beef, by products and milk), (v), knowledge about brucellosis and (vi) clinical and symptoms. The questionnaire was pretested in three similar districts before the commencement of the study and minor corrections were made accordingly.

3.9. Data analysis

The data obtained was coded and entered in Microsoft Excel 2013®, exported, cleaned and analysed using STATA version 18® (Stata Corp., College Station, TX, USA). Categorical data were expressed in percentage, and seroprevalence was calculated by dividing the number of positive sera samples by the total samples examined. The odds ratio, 95% confidence interval, and Fisher's exact tests were computed to see the degree of association of the risk factors with Brucella seropositivity. Using the cut-off of P.I. < 30%, and P.I. ≥ 30% for c-Elisa, respectively, the independent effects of categorical risk factors on anti-Brucella spp. The associations between the risk factors and the outcomes were assessed using Fisher's exact test. Variables with a p-value ≤ 0.25 from the univariable analysis were used as candidate variables in the logistic model.

3.10. Ethical Consideration

The Ethical approval for the study protocol was obtained from Excellence in Research Ethics and Science (ERES) (Ref No. 2018-Dec-004). The authority to conduct the research was granted by the National Health Research Authority (NHRA), while permission to conduct the study at the health facilities was obtained from the Provincial Health Director in Western Province before the commencement of the study. Written informed consent was obtained from all the participants enrolled in the study. For participants below 18 years, written informed consent was obtained from their parents or legal guardians, followed by assent, to ensure they understood the study before agreeing to participate voluntarily. The participant consent

outlined the purpose of the study, procedures, risk of minimal pain and discomfort at the injection site during blood sample collection, benefits, and the right to withdraw at any time. The site of needle puncture during blood collection and their right to withdraw at any given time during the study. All data for the study were restricted to the investigators and treated in confidence, no participant identifiers were used.

The aim of the study was explained to the research assistants and individuals included in the study. Upon arrival at the households, the study objectives were explained to the household heads, and permission was sought to conduct the study.

CHAPTER FOUR : RESULTS

4.1 Socio-demographic characteristics of study participants

The study had more female 110 (55.8%) than male 87 (44.2%) participants. The mean age of the participants was 36 years, ranging from 10 to 81 years. More than half, 107 (54.31%) of the participants were married. Most participants, 109 (55.33%) had achieved a primary level of education, and 107 (54.31%) were married. Most participants, 134 (68.02%), were unemployed. Senanga District had more participants, 93 (48.23), than Mongu and Limulunga districts (Table 2).

Table 2: Socio-demographic variables of study participants

Variables	**Categories**	**N = 197**	**Per cent**	**95% CI**	**P-value**
Gender	Male	110	55.84	55.76-55.92	0.582
	Female	87	44.16	44.08-44.24	
Age	10-20	49	24.97	24.90-25.04	0.811
	21-35	52	26.40	26.33-26.47	
	36-60	79	40.10	40.03-40.17	
	>60	17	8.63	8.59-8.67	
Level of education	None	15	7.61	7.57-7.65	0.92
	Primary	109	55.33	55.24-55.42	
	Secondary	62	33.50	33.46-33.54	
	Tertiary	7	3.55	3.53-3.57	
Marital status	Single	73	37.06	37.02-37.10	0.627
	Married	107	54.31	54.22-54.40	
	Divorced	9	4.57	4.55-4.59	
	Widowed	8	4.06	4.04-4.08	
Occupation	Abattoir worker	1	0.51	0.50-0.52	0.225
	Health worker	3	1.52	1.51-1.53	
	Livestock farmer	25	12.69	12.64-12.74	
	Student	26	13.19	13.16-13.22	
	Other	8	4.06	4.04-4.08	
	Unemployed	134	68.02	67.94-68.10	
District	Mongu	66	33.50	33.46-33.54	0.975
	Limulunga	36	18.27	18.24-18.30	
	Senanga	93	48.23	48.14-48.32	

4.2. Seroprevalence of human *Brucella* antibodies

From the 197 sera samples that were acceptable for testing and analysis, the estimated seroprevalence of *Brucella* antibodies among the patients attending the health facilities in Western Province was 18.3% (n=36, 95% CI=25.4-46.7) on RBT and 4.57% (n=9, 95% CI=3.25-14.8) on c-ELISA (p-value=0.412) respectively. The seroprevalence was higher in Senanga and Mongu (2.54%) than Limulunga district (Table 3).

Table 3: Distribution of *Brucella* antibody seropositivity per study district

DISTRICT	Number tested	RBT		c-ELISA	
		Seropositive Participants (%)	95%CI	Seropositive participants (%)	95%CI
Mongu	66	10 (5.08)	6.49-23.81	4 (2.03)	1.00-9.90
Senanga	93	19 (9.64)	12.23-28.63	4 (2.03)	1.00-9.90
Limulunga	38	7 (3.55)	6.07-30.77	1 (0.51)	0.00-7.71
Total	**197**	**36 (18.27%)**	12.8-23.6	**9 (4.57%)**	3.25-14.8

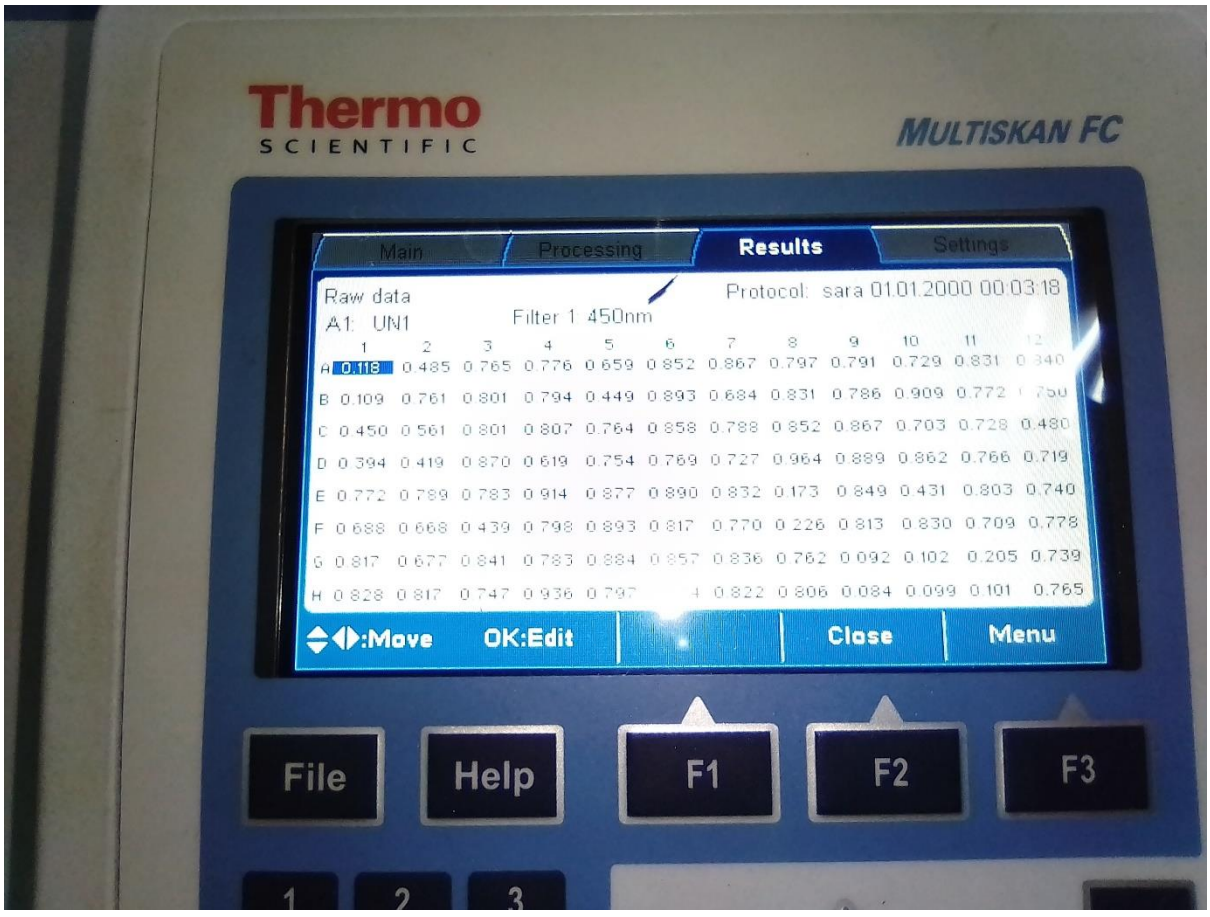

Figure 5 : ***ELISA reading from the spectrometer***

4.3. Knowledge and attitudes of participants regarding Brucellosis

Most of the participants 114 (57.87%) had obtained their information on brucellosis from veterinary officers, while only a few, 13 (6.60%), were aware that brucellosis can affect humans. Most participants, 132 (67.01%), were ignorant about the mode of transmission to humans while only 16 (8.12%) stated the symptoms of Brucellosis as shown in Table 4.

Table 4: Knowledge and attitude of participants about Brucellosis

Variables	Categories	N =197	Per cent	95% C. I	P-value
Information about Brucellosis	Yes	114	57.87	51.09-64.65	0.8
	No	83	42.13	35.35-48.91	
Source of information	Veterinary officer	53	46.49	36.96-56.02	0.734
	Neighbour	12	10.53	6.20-14.87	
	Health worker	39	34.21	26.77-41.65	
	Media	4	3.50	1.36-5.66	
	Patients with Brucellosis	6	5.26	2.52-7.99	
Can humans be affected ?	Yes	13	6.60	3.79-9.41	0.663
	No	67	34.01	29.06-38.97	
	I do not know	117	59.39	52.92-65.86	
Mode of transmission	I do not know	132	67.01	60.78-73.24	0.167
	Contact with an infected animal	25	12.69	9.48-15.90	
	Eating undercooked meat and drinking raw milk	40	20.3	16.50-24.11	
Symptoms of Brucellosis	Yes	16	8.12	5.56-10.68	0.004
	No	181	91.88	88.88-94.88	

4.4. Hygienic and protection practices of study participants regarding Brucellosis

A high proportion of the participants drank raw milk 27 (13.71%) and consumed undercooked meat 170 (86.29%). Most participants kept animals 114 (57.87%), and among these about 40 (48.19%) had more than ten animals, while 17 (20.48%) had their animals vaccinated against brucellosis (Table 5).

Table 5: Hygienic and vaccination characteristics of study participants about Brucellosis

Variables	Categories	N (197)	Per cent	95% C.I	P-value
Drinking raw milk and eating undercooked meat	No	27	13.71	9.45-17.97	0.647
	Yes	170	86.29	81.29-91.29	
Keeping animal	No	114	57.87	51.65-64.09	0.818
	Yes	83	42.13	35.91-48.35	
Type of animal owned (N=83)	Only goat	14	16.87	6.22-27.51	0.308
	Only sheep	19	22.89	10.02-35.76	
	Only pigs	8	9.64	3.48-15.79	
	Cattle	25	30.12	16.56-43.68	
	Dogs	9	10.84	3.46-18.22	
	Other	8	9.64	3.48-15.79	
Number of animals (N=83)	One animal	5	6.02	3.87-8.17	**0.049**
	1-10 animals	38	45.79	28.41-63.15	
	>10 animals	40	48.19	30.82-65.55	
Vaccinated animal (N=83)	Yes	17	20.48	4.30-36.66	
	No	66	79.52	63.34-95.69	0.421

4.5. Clinical signs and symptoms of the study participants

The main reason for seeking medical attention by most participants was fever 161 (81.72%) with a p-value of 0.004 followed by malaise, 17 (8.63%). Participants who had the onset of symptoms from 2 to 6 days, 161 (81.72%), were the most likely to attend the health facilities (Table 6).

Table 6: Clinical symptoms of study participants in the study area (Mongu, Limulunga, Senanga)

Variables	Categories	N (197)	Per cent	CI value	P-value
Symptoms	Fever	161	81.20	74.70-88.76	**0.004**
	Malaise	17	8.63	3.02-14.25	
	Weakness	5	2.54	0-6.08	
	Weight lost	6	3.04	0-6.55	
	Flu-like symptoms	8	4.06	0-8.01	
Onset of symptoms	One day	28	14.21	6.05-22.36	0.847
	2-6 days	161	81.72	74.70-88.76	
	7-13 days	5	2.54	0-6.08	
	>14 days	3	1.52	0-4.58	

4.6. Potential Risk factors associated with Brucella seropositivity

The univariable analysis first screened the association between the dichotomous outcome variable seroprevalence and potential risk factors (Table 7). All variables with $p \leq 0.25$ were selected for further analysis to build the multivariable logistic regression model.

Table 7*: Univariable analysis of Potential Risk factors associated with Brucellosis*

Variables	Categories	Total	Seroprevalence	Per cent	P-value
Gender	Male	110	7	6.3%	0.592
	Female	87	4	4.6%	
Age	10-20	49	4	8.1%	0.811
	21-35	52	2	3.8%	
	36-60	79	4	5.06%	
	>60	17	1	5.8%	
Level of education	None	15	1	6.6%	0.924
	Primary	109	6	5.50%	
	Secondary	66	4	6.06%	
	Tertiary	7	0	0%	
Marital status	Single	73	3	4.11%	0.627
	Married	107	7	6.5%	
	Divorced	9	0	0%	
	Widowed	8	1	12.5%	
Occupation	Abattoir worker	1	0	0%	0.225
	Health worker	3			

			0	0%	
	Livestock farmer	25	4	16%	
	Unemployed	134	5	3.73%	
	Student	26	2	7.69%	
	Other	8	0	0%	
District	Mongu	66	4	6.06%	0.975
	Limulunga	36	2	5.56%	
	Senanga	93	5	5.38%	
Heard about Brucellosis	Yes	114	6	5.26%	0.8
	No	83	5	6.24%	
Source of information	Vet officer	53	3	5.8%	0.734
	Neighbour	12	1	3.85%	
	Health worker	39	2	5.26%	
	Media	4	0	0%	
	Patients with Brucellosis	6	0	0%	
Can humans become infected?	Yes	13	0	0%	0.663
	No	67	4	5.97%	

	I do not know	117	7	5.98%	
Mode of transmission	I do not know	132	6	4.54%	0.167
	Contact with the infected animal	32	1	3.1%	
	Eating raw meat and drinking raw milk	33	4	12.12%	
Knowledge about Symptoms of Brucellosis	Yes	16	0	0%	0.310
	No	181	11	6.08%	
Drinking raw milk and eating undercooked meat	No	27	1	3.70%	0.647
	Yes	170	10	5.88%	
Keeping animal	No	114	6	5.26%	0.818
	Yes	83	5	6.02%	
Type of animal owned	Goat	25			

(N=83)			1	4%	0.308
	Sheep	3	0	0%	
	Pigs	12	2	16.67%	
	Cattle	15	2	13.33%	
	Dogs	11	0	0%	
	Other	17	0	0%	
Number of animals (N=83)	One animal	17	0	0%	**0.049**
	1-10 animals	50	2	4%	
	>10 animals	16	3	18.75%	
Vaccinated animal (N=83)	Yes	9	0	0%	0.421
	No	74	5	6.76%	
Symptoms	Fever	161	6	3.80%	**0.004**
	Malaise	17	2	11.76%	
	Weakness	5	2	40%	
	Weight lost	6	2	33.33%	
	Flu-like symptoms	8	0	0%	
	Weight lost	6	1	16.67%	

Onset of symptoms	One day	28	1	14.81%	0.847
	2-6 days	161	10	6.21%	
	7-13 days	5	0	0%	
	>14 days	3	0	0%	

4.7. Multivariable logistic regression analysis for brucellosis risk factors in humans

In the multivariable logistic regression, only the number of animals ($p<0.049$) was statistically significantly associated with Brucellosis, and the logistic regression model adequately fit the data (Hosmer and Lemeshow test) with ROC areas around 0.760 (an ROC area of 0.50 indicated no explanatory power) with a ($p>0.785$) (Table 8).

Table 8*: Standard Multivariable logistic regression analysis for Brucellosis risk factors in humans*

Variables	OR	(95%CI)	P-value
Type of animal owned	0.72	0.42-1.23	0.226
Number of animals	6.49	1.10-38.13	**0.039**

The Hosmer and Lemeshow test showed that the model fitted the data, thus increasing its reliability in predicting with ROC areas around 0.760, as confirmed by a ($p>0.785$).

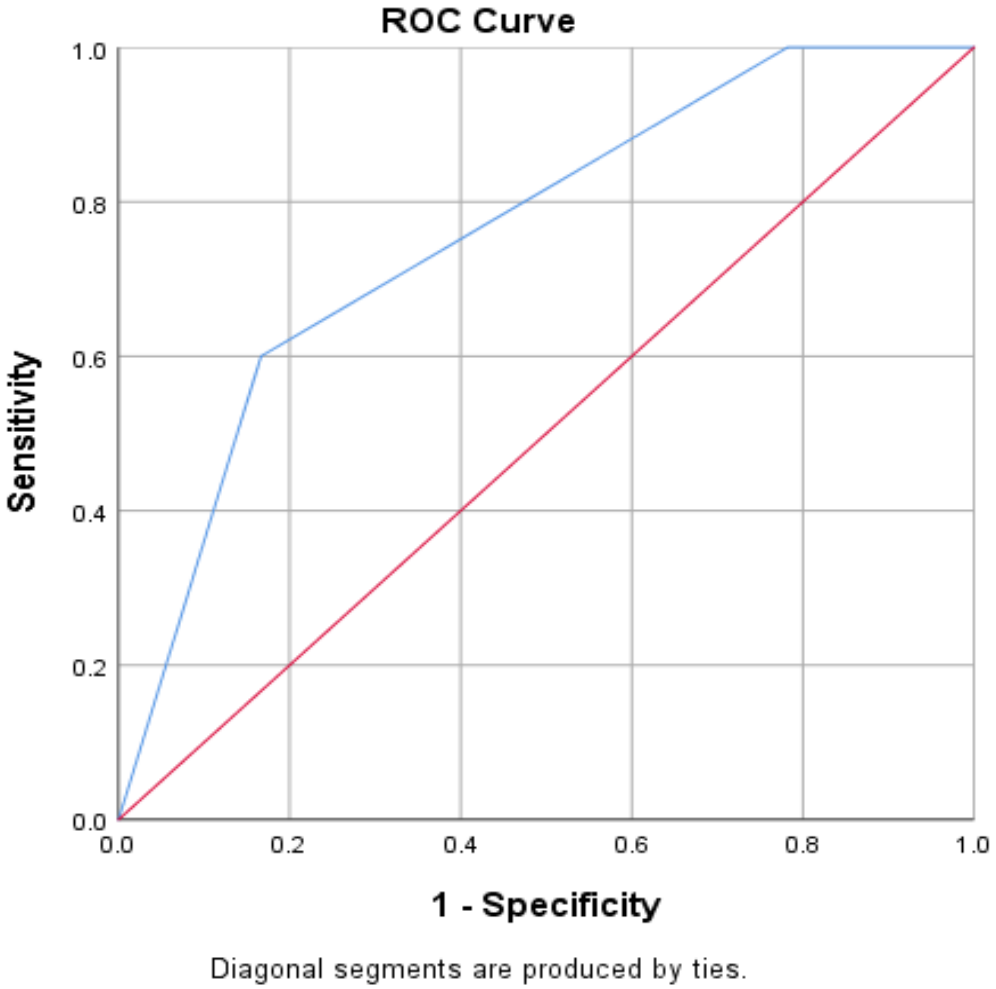

Figure 6*: ROC curve demonstrating the predictability of the model (Area under the curve=0.760)*

CHAPTER FIVE : DISCUSSION

5.1. Introduction

This study aimed to determine the seroprevalence and associated risk factors for human Brucellosis in the Western province of Zambia. This is the first study to document the seroprevalence of *Brucella* antibodies among patients seeking medical attention in community health facilities in Western province of Zambia.

5.2. Seroprevalence of Human Brucellosis

The seroprevalence of human *Brucella* antibodies among patients seeking medical attention was 4.57%. The current study was based on community health facilities and highlights the seropositivity in patients from three districts (Mongu, Senanga and Limulunga) in the Western Province of Zambia. The 4.57% prevalence in our study corroborates the findings in the seroprevalence of 5.03% recorded by Muma et al. (2008) in Southern province under similar settings. It is similar to the 6% reported among febrile patients attending a District hospital in Rwanda (Gafirita et al., 2017).

However, the prevalence in this study is lower than the 20.26% reported in the Southern Province of Zambia (Mubanga et al., 2021). While this present study was focused on screening febrile patients seeking medical attention, that of Mubanga et al. (2021) targeted occupationally exposed humans (herders and abattoir workers) who were at high risk of exposure to brucellosis. This could explain the observed differences in the studies.

The study findings are also lower than the 14.9% prevalence observed among community hospital patients in South Western Uganda (Migisha et al., 2018). This variation can be due to various serodiagnostic methods for the illness, which may be responsible for this variation. Also, South Western Uganda has a high production of milk, with a production of 100,000 litres per day, or 35% of the country's production. Therefore, the population is likely to be exposed within this community due to cultural practices of consumption of raw milk in the Western region of Uganda (Tijjani et al., 2015). A study in Saudi Arabia reported a seroprevalence of 12.8% among patients with fever (Abdullah et al., 2020). The difference could be that in Saudi Arabia, *Brucella melitensis* has been reported to be the most prevalent pathogen causing human brucellosis in the country (Abdullah et al., 2020), while in Zambia, only *Brucella abortus* has

been reported (Muma et al., 2007). *Brucella melitensis* is known to be the most pathogenic species among the brucella species (Dadar et al., 2019).

5.3. Knowledge and attitude of participants about Brucellosis

The majority of respondents, 57.87%, were knowledgeable about the disease. Many studies have demonstrated low knowledge levels of the disease to be a risk factor that increases the risk of Brucella infection in the community, has a detrimental effect on brucellosis control measure compliance, and may contribute to underreporting of disease incidence in the nation (Yamen et al., 2016; Lumumba et al., 2019). In contrast, only 6.60% of participants were aware that Brucella can affect human beings, and most respondents 67.01% were ignorant about the mode of transmission of Brucellosis in humans, which agrees with the study finding reported in Uganda (Kansiime et al., 2014) and Jordan (Musallam et al., 2015). This is also similar to the findings by Munyeme et al. (2010), who reported that the population's low awareness of Brucellosis was caused by a lack of health education programs, inadequate training in handling and rearing animals, a lack of extension services, the absence of health facilities, and remote participant locations. This result is low compared to the study conducted in Iran (Masoomeh et al., 2008). The poor awareness of human Brucellosis in this study may be because few people in the research locations received formal education. Because zoonotic disease education is scarce, it was explained by Bouzoukeev et al. (2014) that farmers who were well-informed about the disease's transmission paths might take preventative measures against Brucellosis. Brucellosis was observed more in the Senanga than in the Mongu and Limulunga districts. This can be because most of the samples collected in the Senanga districts are from rural centres compared to Mongu and Limulunga districts, where most are from mini hospitals. In rural health centres, knowledge levels concerning disease can positively impact preventive measures regarding consuming raw meat and unpasteurised milk, which are major risk factors in the transmission of Brucellosis disease (Mwatondo et al., 2023). According to Ruano and Aguayo (2017), low levels of awareness, like those found in the current study, put the populace at risk for contracting Brucella and have a detrimental effect on compliance with brucellosis control measures. It may cause underreporting of disease occurrence in the nation. It is also noted that some rural people keep animals in their houses (Franc et al., 2018).

5.4 Risk factors associated with Brucella seropositivity

In this study, the risk factors considered statistically associated with *Brucella* seropositivity were the number of animals. The close contact between domestic animals and human beings is still a critical mode of disease transmission. Most humans have animals that can transmit *Brucella* pathogens, so the exposure risk is higher. Among the number of animals, those who had more than ten animals, 23.08% (3/13) were more likely to be exposed (Lokamar et al., 2022). The importance of the number of animals favours migration or mobility, which increases the risk of Brucellosis transmission (Awah-Ndukum et al., 2018). The study conducted in Kenya by (Kairu et al., 2019) found that the number of animals was a risk factor associated with brucellosis. There was a significant correlation with herd size, with larger herds having a significantly higher risk of exposure to the disease. Large herds are often associated with poor sanitation, clustering of animals, and mixing of animals from different herds and species. Several African studies have found a similar correlation between brucellosis seropositivity and several animals. Another study in Mexico by (Solorio et al., 2007) found that the number of animals and/or flocks has been a risk factor.

5.5 Study limitations

The sample size was less than the target but enough to carry out the planned analysis of this study without affecting its validity. However, the same samples collected were hemolysed with a possibility that others would be positive cases among those samples because those samples were not taken into account. It was not practical to analyse all the samples collected because other samples were hemolysed. Despite all these weaknesses highlighted, this study provides information that can help the prevalence of brucellosis and risk factors in health facilities in Zambia.

CHAPTER SIX : CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

Brucella antibodies were present at 4.57% among patients in community health facilities in Western Province. Most participants were unaware that *Brucella* can affect humans and were ignorant about the mode of disease transmission to humans. The number of animals was significantly associated with *Brucella* seropositivity.

6.2. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

a) Western Province is an agricultural district with most of the population living in rural areas. Depending on livestock production, human Brucellosis must be notified properly and promptly so that livestock and human contacts can be screened.

b) More studies are needed on bacteriological and molecular factors to understand the epidemiology of brucellosis further.

   Being a zoonotic disease, addressing Brucellosis in animals can be the most efficient method of controlling human disease. Farmer should be encouraged to vaccinate their animals against Brucellosis.

c) Health authorities should organise public awareness campaigns, especially in rural areas, to educate people about Brucellosis, its risk factors, modes of transmission, and how to prevent it. Consumption of unpasteurised milk and its products should be particularly discouraged.

d) There is a need to conduct regular surveillance of brucellosis in humans and animals.

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APPENDICES

Appendix 1

BRUCELLOSIS RISK FACTORS QUESTIONNAIRE

This oral questionnaire will be administered to the patients attending the hospital in order to collect information on the risk factors associated with exposure and ultimate development of brucellosis.

Section A: Socio-demographic data

DATE ☐ GPS reading _____

_____

District ☐ _____

Ward _____ Village ☐

Name of person being interviewed ☐

Gender of respondent Male ☐ Female ☐

Age (Years) ☐

Level of Education: Tertiary ☐ Seconadry ☐ Primary ☐ Non ☐

Marital status: Married ☐ Divorced ☐ Widowed ☐ Single ☐

Occupation:

Teacher ☐ Unemployed ☐

Abattoir worker ☐ Students ☐

Veterinarian ☐ Others ☐

For how long have you been in this profession? ☐

Section B: Possible risk factors for Brucellosis

Do you have cattle Yes ☐ No ☐

What other animals do you keep?
Goats ☐ Sheep ☐ pigs ☐ Others ☐

For What purpose do you keep your livestock?
For own consumption ☐ To sell meat ☐ To sell milk ☐

To sell livestock ☐ Economic investment ☐ others ☐ Non ☐

What type of production system do you have? Individual ☐ Communal pasture ☐ other ☐

Have your animals been vaccinated against brucellosis? yes ☐ No ☐

Have your animals been diseased in the last year? Yes ☐ No ☐

If yes, what kind of symptoms have they shown? Coughing ☐
Abortion ☐ Nasal discharge ☐ Diarrhea ☐ Fever ☐

other ☐

How many livestock have you bought in the last year? ☐

Where did you buy your livesock from? ☐

Friends/Neighbour ☐ Local market ☐

Farm ☐ others ☐

Where do you sell your livestock? I don't sell my animals ☐

To friends/ neighbour ☐ others ☐

What breeding method is used on your farm?

Artificial Insemination ☐ Both ☐

Natural breeding ☐

If natural breeding is used, is the male used on other farms? Yes ☐ No ☐

What is the average number of cows your bull service in a month? ☐

Knowledge and Awareness of Brucellosis (All respondents)

Have you heard about a disease called Brucellosis? Yes ☐ No ☐

If yes, what was the source of that information? Patient of brucellosis ☐

Friends ☐ Others ☐

Vet office ☐ Health worker ☐

What is the local name for brucellosis in your area? ☐

Which animal species can become infected by brucellosis?

Cattle	☐	Sheep	☐	Goat	☐	Wildlife	☐
Pigs	☐	Horses	☐	Dogs	☐	Don't know	☐

Which animal species cannot be infected by brucellosis?

Cattle	☐	Sheep	☐	Goat	☐	Wildlife	☐
Pigs	☐	Horses	☐	Dogs	☐	Don't know	☐

Can humans become infected? Yes ☐ No ☐ I don't know ☐

Is there any vaccination for animals against brucellosis? Yes ☐ No ☐

Do you have previous history of brucella infection within your herd? Yes ☐ No ☐

Do you have previous history of brucella infection within the household? Yes ☐ No

Do you vaccinate your animals against the disease? Yes ☐ No ☐

Do you drink milk from the animals? Yes ☐ No ☐

If yes , how do you prepare it for drinking? _____

Have you assisted your cow in any difficult calving? Yes ☐ No ☐

If yes to the above did you use any protective clothing? Yes ☐ No ☐

How do you dispose of animal waste?

Use it to grow crops ☐
Sell it ☐
Use it to make biogas ☐
Bury it in the ground ☐
Nothing ☐
Other ☐

Personal Protective Attire

Slaughterhouse scale (cattle per day)

< 50 ☐
50-99 ☐
above 100 ☐

Wearing protective glasses

Always ☐
Sometimes ☐
Rarely ☐
Non ☐

Wearing a protective apron

Always ☐
Sometimes ☐
Rarely ☐
Non ☐

Wearing protective boots

Always ☐
Sometimes ☐
Rarely ☐
Non ☐

Taking a shower after work

Always ☐
Sometimes ☐
Rarely ☐
Non ☐

Contact with blood around the mouth

Yes ☐
No ☐

Contact with blood around the body

Yes (once a week) ☐
No ☐

Contact with feces/urine around the mouth or body

Yes(once a week) ☐
No ☐

Presence of wound on skin

Yes(within a year) ☐
No ☐

Do not separate pregnant uterus from other raw meat

Yes ☐
No ☐

I don’t know ☐

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16th December, 2022

Ref. No. 2018-Dec-004

The Principal Investigator
Dr. Ruth L. Mfune,
The University of Zambia,
School of Veterinary Medicine,
Disease Control Department,
P. O. Box 32379,
LUSAKA.

Dear Dr. Mfune,

RE: RENEWAL AND END OF YEAR REPORT: CHARACTERIZATION OF HUMAN AND BOVINE BRUCELLA SPECIES IN SOUTHERN AND WESTERN PROVINCES OF ZAMBIA.

We acknowledge receipt of your end year progress report Plus your publication, Congratulations.

The study is to proceed; the new expiry date is **12th January, 2024**.

Yours faithfully,
ERES CONVERGE IRB

Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Soc., PhD
CHAIRPERSON


MINISTRY OF HEALTH
NATIONAL HEALTH RESEARCH AUTHORITY
The Health Research Act
(Act No. 2 of 2013)
CERTIFICATE OF REGISTRATION
THIS IS TO CERTIFY THAT
Mambote Mayindu Armand
has been registered as a Health Researcher
Dated this 25th November 2023
Registration number NHRAR-R-1098/24/11/2023
ACTING DIRECTOR/CEO
PROF. VICTOR CHALWE