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RISK OF HUMAN EXPOSURE TO BRUCELLA PATHOGENS THROUGH CONSUMPTION OF CULTURED MILK IN THE SOUTHERN PROVINCE OF ZAMBIA

BY

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A dissertation Submitted to the University of Zambia in Partial Fulfilment of the Requirements for the Award of the Master of Science in Food Safety and Risk Analysis

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DECLARATION

I, Thendji Muila Lysa, hereby declare that the contents of this dissertation being submitted herein are my original work and have not been previously submitted to any university for the award of a degree or other qualification.

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CERTIFICATE OF APPROVAL

This dissertation submitted by Thendji Muila Lysa is approved as fulfilling the requirements for the award of Master of Science in Food Safety and Risk Analysis of the University of Zambia.

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DEDICATION

This dissertation is dedicated to my loving father, Mvutu Lukunga Jackson and my mother, Kunumana Pwa Marie Claver and my sisters Makeya Mvutu Hadassa Esther, Mvutu Gupalumuga Esperance, Therese Gunumana and my brother Mvutu Mabelo Eliezer, for encouraging me to strive for greater heights and to be a better person.

To Drs Flavien and Rachel Bumbangi for their unwavering commitment to seeing me complete my education and achieve greater success.

ACKNOWLEDGMENT

I am grateful to the African Centre for Infectious Diseases in Humans and Animals (ACEIDHA), School of Veterinary Medicine, University of Zambia, for offering me a scholarship and providing financial support that allowed me to complete my research. I am grateful to my principal supervisors, Professor John Bwalya Muma and my co-supervisor Dr Ethel Mkandwire, for their patience and assistance. I would also like to thank them for the numerous corrections they made to the dissertation, from the concept note to the writing of this dissertation, to make it acceptable.

Professor Eystein Skjerve deeply moved me during the FORTECASE student exchange programme at the Norwegian University of Life Sciences (NMBU) for his critical and analytical approach, advice, and corrections during the dissertation writing process.

Further gratitude is also extended to Ms Rebecca Sikasunge, who assisted me in translating into Tonga, and Mr Michelo Milimo, our driver, who enabled me to visit various farms, milk collection centres and households.

Finally, I would like to express my heartfelt gratitude to the Ministry of Livestock and Fisheries staff and officials in the three districts who assisted in the data collection process. Everyone who has helped me in some way to complete my studies will be remembered for the rest of my life.

ABSTRACT

Brucella is bacteria that causes Brucellosis, one of the most common zoonotic diseases in the world. The disease affects mainly animals but, in some cases, humans who are considered incidental hosts. Brucellosis is transmitted to humans through inhalation of aerosols or contact with skin sores, by the intake of infected animal products such as undercooked meat as well as consumption of raw milk or unpasteurised milk and dairy products. This study aimed to estimate the risk of human exposure to Brucella pathogens through the consumption of cultured milk in the Southern Province of Zambia. The specific objectives were to determine the consumption patterns of cultured milk by the population in the three districts of the Southern province and to estimate the likelihood of being exposed to *Brucella* spp. through cultured milk.

A survey was conducted using a structured questionnaire to interview traditional farmers at the milk collection centres and in selected households. Survey data were analysed in the STATA Statistical package to obtain frequencies on consumption patterns of cultured milk. A risk model was developed to estimate the risk of being exposed to *Brucella* spp. by consuming contaminated cultured milk using the Monte Carlo simulation in ModelRisk®.

The study had 236 males and 87 females, representing 73.1% and 26.9%, respectively. The proportion of farmers that fermented milk using raw milk was 65.3%. Boiled milk was used by 13.3%, and 21.7% purchased milk already fermented, not knowing if it was. The most common milk fermentation period was one day (40.9%), followed by those who fermented for two days (29.1%). Most respondents (63.8%) fermented milk at room temperature, and the majority (52.9%) consumed half to one litre of sour milk per person per day, while 47.1% consumed less than half a litre. The frequency of consumption was two-three times per day (60.7%), followed by one-two times per day (35.6%) and more than once per week (3.7%).

The surveys showed that there were four probabilities to be exposed to Brucella and the numbers of people likely to be exposed with the results are as follows: first probability (cultured milk prepared from raw milk obtained from an infected): 0.019 (90% CI: 0.059-0.022), number to be exposed was 17 (90% CI: 4-24). The second probability (cultured milk prepared from boiled milk obtained from an infected cow): 0.0184 (90%C I: 0.01265-0.037), the numbers to be exposed: 21 (90% CI: 11-39, the third probability (cultured milk prepared from raw milk obtained from a seronegative cow): 0.166(90% CI: 0.00135-0.0154), the numbers to be exposed was 15 (90% CI: 1-16). The fourth

probability (cultured milk prepared from boiled milk obtained from a seronegative cow): was 0.023 (90% CI: 0.0029-0.02609), and the number to be exposed was 24 (90% CI: 2-29).

Most people consumed about 0.5 to 1.0 litres of cultured milk per day, made from raw milk fermented for one day. The consumption of cultured milk in the three districts was high and frequent. Moreover, consuming cultured milk from an infected animal exposed the population to Brucellosis and posed a risk to public health. To reduce the risk of humans being exposed to *Brucella* spp., there is a need to raise awareness among the farmers, the sellers, the consumers and the government about Brucellosis and associated risk factors. Furthermore, studies are required to assess the risk with a larger population.

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LIST OF ACRONYMS AND ABBREVIATIONS

2-ME	2-MercaptoEthanol
CAC	Codex Alimentarius Commission
cELISA	Competitive ELISA
CSH	Clinically Suspected Human
FAO	Food and Agriculture Organization
FPA	Fluorescence Polarization Assay
LFA	Lateral Flow Assay
LMICs	Low-Middle-Income Countries
LPS	Lipopolysaccharide
OEP	Occupationally Exposed People
OEH	Occupationally Exposed Human
RBT	Rose Bengal Test
SAT	Slow Agglutination Test
STAT	Standard Tube Agglutination Test
TFMPS	Traditionally fermented milk products
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

4.1 Background

Milk is a staple food in many people's diets worldwide because of its nutritional content (Górska-Warsewicz, H. *et al*., 2019). It is an essential source of protein and micronutrients in many people's diets, especially for children in many poor and middle-income countries (Prakashbabu et al., 2020). Before consumption, milk often undergoes processing such as pasteurisation, boiling or fermentation (Leone, C. *et al*., 2022; Burke, N *et al*., 2018).

Milk fermented with lactic acid bacteria such as *lactococcus*, *lactobacillus*, and *leuconostoc* is known as cultured milk or fermented milk. Milk has a significant nutritional value not just for new-born mammals and humans but also for microorganisms (Ballard, O., *et al*., 2013).

Because of its nutritive value, milk can be a source of various milk-borne hazards, including bacteria, viruses, protozoa, and chemical hazards (Dhanashekar et al., 2012). Therefore, milk can serve as a source of disease transmission to humans. Milk-borne pathogens represent a significant health burden in high- and low-middle-income countries (LMICs), making the LMICs populations vulnerable (Havelaar, A. H. *et al*., 2015).

One such milk-borne disease is Brucellosis, caused by Gram-negative bacteria of the genus Brucella. Internationally, there are twelve (12) recognised species of Brucella, of which four are pathogenic to humans, which are *B. melitensis*, *B. abortus*, *B. canis* and *B. suis* (Stuart D. Perkins *et al.*, 2010). *Brucella spp*. is transmitted from various animal species (mostly domestic mammals) to humans, who are incidental hosts. Human exposure is often via the mucocutaneous route (contact with an infected animal or contaminated tissues), especially in endemic areas (Bankole, A. A. et al., 2010). Exposure can also occur in the digestive route through the consumption of infected animal products, such as undercooked meat or raw milk, as well as the usage of other animal products that have not been adequately aged or pasteurised *(Dean et al., 2012; Megersa et al*., 2011) and the aerosol route. However, the most common route of Brucella transmission in humans is consuming raw or unpasteurised milk and dairy products from infected animals (Guler, S. *et al*., 2014).

The acidity/alkalinity (pH) and water activity are considered significant factors influencing the survival of Brucella in dairy products (Jansen et al., 2019). Therefore, due to the strictly lactic

fermentation, short duration, and desiccation in fresh cheeses, Brucella survival can be much longer (Brisabois et al., 1997). It was demonstrated that *B. melitensis* could not survive at a pH less than 4, whereas *B. abortus* appeared more acid-tolerant and could even survive for ten days in fermented milk at a pH less than 4.0. (Dadar et al., 2019). Another study by Jansen et al. (2019) reported that Brucella could survive with optimal pH conditions ranging from 6.6 to 7.4 at 37°C and a survival capacity between pH 4.1 and 8.4.

Livestock farming is a substantial socioeconomic activity in Zambia, contributing considerably to the national Gross Domestic Product (GDP) (Muuka et al., 2012). Zambia's cattle industry is essentially split into two primary subsectors: commercial and traditional, and it has a skewed geographical distribution throughout the nation with livestock concentrations in the Western, Southern and Eastern regions (Muma et al., 2011). In the commercial sector, milk is mainly produced by Friesian and Holstein cows, with an average daily yield of 25 litres (Kaluba 1993). Milk is produced in the traditional sector by native cattle, mostly Sanga and Zebu crossbred with Tonga, Barotse, and Angoni breeds (Muma et al., 2011). Milk produced in this sector is traditionally consumed at home as part of a regular diet without defined milk processing.

Zambia has recently seen increased milk production (Mumba C *et al*., 2013), which has increased the number of persons handling and drinking milk, increasing the risk of exposure to milk-borne illnesses. In the absence of adequate disease prevention and milk hygiene, this has the negative consequence of increasing the risk of exposure to milk-borne diseases. Therefore, there is a need to assess the risk of milk-borne diseases continually. According to Dadar et al. (2019), fermented non-pasteurised milk may contain Brucella and cause human contamination.

4.1 Statement of the Problem

Milk is a key source of protein and micronutrients in the diets of a considerable proportion of adults and children across the globe. However, if not well processed, milk can also be a source of several milk-borne hazards ranging from bacteria, viruses, protozoa, and chemical hazards. One such bacterial hazard is Brucella species that causes Brucellosis due to consuming unpasteurised milk. Brucellosis is a significant zoonotic infection causing substantial public health effects, animal industry and economic losses, especially in developing countries (Franc, K. A. *et al*., 2018). As a result of endemic Brucella infections in animals, millions of people are at risk of the disease because of unsafe food preparation methods, lifestyles that bring them in direct contact with

infected animals, consumption of contaminated foods, and occupational contact. It is estimated that approximately 500,000 incident cases of human Brucellosis are reported annually across the globe (Hull, N. C. *et al*., 2018).

Many rural communities in Zambia are active in cattle ranching and generate large quantities of raw milk, making raw milk readily available to households. Among traditional farmers, estimates show that 68.7 % and 87.3% consume raw and cultured milk, respectively (Muma et al., 2008). Furthermore, human seroprevalence studies indicate a 5.0% serological positivity to Brucella species in rural areas (Muma et al., 2008).

In Zambia, Brucellosis has been reported in traditional cattle herds in Southern Province at 22.7 % (Muma et al., 2013). With humans as incidental hosts, there is a need to assess the risk of exposure of humans to Brucella infections by consuming animal products such as milk and meat. Furthermore, in the Southern province, the population has a tradition of drinking raw milk which puts them at risk of exposure to milk-borne zoonoses as it could carry several harmful germs, including *Brucella*.

Another study on Brucella seroprevalence and associated risk factors in occupationally exposed humans conducted in three districts of Zambia's Southern province found that the overall Brucella seroprevalence was 20.3%, with comparable seropositive results among districts showing Namwala with 26.9%, Monze with 19.0%, and Choma with 11.36% seropositivity (Mubanga et al., 2021). There is, therefore, a need to assess the risk and raise the awareness of these communities towards consuming unprocessed and cultured milk.

4.2 Study Justification

Even though Brucellosis's burden is highest in Africa, little has been done to monitor and control this milk-borne zoonosis. Information is needed for the effective implementation of any intervention. This study will estimate the risk of exposure to Brucellosis, which is important information in implementing any interventions. Further, the information will form the baseline information for another study.

4.3 General Objective

To estimate the risk of human exposure to Brucella pathogens through the consumption of cultured milk in the Namwala, Monze, and Choma districts of the Southern Province of Zambia.

4.4 Specific Objectives

1. To determine the consumption patterns of cultured milk in the human population in the three districts.
2. To estimate the likelihood of being exposed to *Brucella* spp. through the consumption of cultured milk.

CHAPTER TWO: LITERATURE REVIEW

2.1 General Overview of Milk

Raw milk is the source of all milk products. However, before the milk is placed on the consumer's table, it goes through various steps. These procedures are used to ensure the quality and safety of the milk. The type of treatment applied impacts the milk's final quality (Leyou and Bouguetaib, 2014). Codex defines milk as follows: ‘Milk is the normal mammary secretion of milking animals obtained from one or more milking without either addition to it or extraction from it, intended for consumption as liquid milk or further processing’ (Codex., 1999).

2.2 Composition of Milk

Due to its unique composition and properties, milk is a significant source of bacterial infection and a substrate for bacterial growth. Milk is a complex food rich in nutrients. It has over a hundred components, which are in solution, emulsion, or suspension in water, accounting for over 90% of milk's composition (Wattiaux, 2001). According to (Grimaud et al., 2007), milk's composition varies greatly and is unstable. First and foremost, the composition changes based on the animal species. However, there are also noticeable differences between breeds and between specific animals within a breed (Grimaud et al., 2007). Depending on feeding and climate, the composition could shift throughout the day.

In addition to genetics, udder health (clinical and subclinical mastitis), milk production level, lactation stage, season, cow age, environmental temperature, and light/dark ratio can impact milk composition (Lujerdean et al., 2007). Average figures for milk composition from cows, sheep and goats are given in Table 1. Several elements, including calcium, potassium, vitamins, and protein, are abundant in milk, making it a nutrient-dense diet.

Table 1. Milk composition of domestic ruminant

	Cow milk	Goat milk	Sheep milk
Water	87.2%	85.8%	81.6%
Total solids	12.8%	14.2%	18.4%
Fat	4.0%	4.9%	6.5%
Protein	3.4%	4.3%	6.7%
Lactose	4.5%	4.1%	4.3%
Ash (Minerals)	0.9%	0.9%	0.9%

Adapted from Pandey & Voskuil (2011)

2.3 Physico-Chemical Properties of Milk

The main physicochemical properties used in the dairy industry are described including the freezing point, the density of milk, the boiling point and milk acidity (Table 2).

Table 2. Physico-chemical properties of milk

Milk parameters	Value
The density of milk at 15°C	1.028 to 1.035
Freezing point	-0.530°C to -0.575°C
Boiling point	100.5°C
Milk acidity	0.13 and 0.17
pH of milk	6.6 to 6.8

Adapted from Amiot *et al*. (2002)

2.4 Milk As a Source of Pathogens

Milk is an excellent substrate for microbial growth because of its physical-chemical properties. Because of this, it is merely a sporadic vector for other widespread or dangerous microbes (Brahimi and Mohammadi,2019). Milk microorganisms are categorised into two primary types based on their significance: the indigenous or original flora and the contaminating flora (Yobouet, 2016). In addition to helping with digestion and offering defence against other infections, some of the bacteria found in milk (such as *Lactobacillus spp*. or *Bifidobacterium spp*.) are also found in the healthy human gastrointestinal tract. However, other bacteria can harm human health (Dhanashekar et al., 2012).

2.4.1 Original or indigenous flora in milk

Milk obtained under aseptic circumstances from a healthy animal should generally contain less than 5000 cfu/ml (Berhe, G., et al., 2020). The original flora of dairy products is defined as the collection of microorganisms found at the udder's exit. These bacteria (such as *Lactobacillus spp*. or *Bifidobacterium spp*.) are found in milk and the healthy human gastrointestinal tract. These prevalent microorganisms interact with the feed, exist close to the feed, and have no noticeable impact on the quality or production of milk (Sepulveda, D. R et al., 2005).

2.4.2 Infected flora by milk

This flora is a group of bacteria contaminating milk at every stage, from manufacturing to consumption. It may contain pathogenic flora that is hazardous to human health and alter flora, resulting in sensory abnormalities or reducing the product's shelf life (Sepulveda, D. R et al., 2005).

a) Spoilage flora

Spoilage flora takes advantage of sensory flaws (taste, aroma) or shortens the shelf life of dairy goods. However, some spoilage microbes might also be dangerous. The rotting (spoiling) flora comprises three identical taxa: coliforms, yeasts, and moulds (Ledenbach, L. H et al., 2009).

b) Pathogen flora

Pathogen flora is a component of the milk-contaminating flora. Bacteria that are dangerous to humans may be present in raw milk and dairy products made from it. The bacteria which can be responsible for milk-borne diseases., include; *Brucella spp, Campylobacter jejuni*, *Bacillus cereus*, Shiga toxin-producing *E. coli* (*E. coli* O157:H7), *Coxiella burnetii*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avium* subspecies *paratuberculosis*, *Salmonella spp*, *Yersinia enterocolitica* and certain strains of *Staphylococcus aureus* which are capable of producing highly heat-stable toxins (Havelaar et al., 2015; Jans et al., 2017; Kirk et al., 2015).

2.5 Cultured Milk

The term “cultured milk” also known as “fermented milk” or sour milk, refers to inoculating milk with various microorganisms that convert lactose to lactic acid. The metabolic processes of these bacteria produce carbon dioxide, acetic acid, diacetyl, acetaldehyde, and various other substances,

giving the end products their distinct flavours, textures, and aromas. Fermented milk originates in the Near East and quickly spreads throughout Eastern and Central Europe. The earliest fermented milk was most likely created by nomads who fermented milk from goat or sheep milk (Bintsis, T., & Papademas, P., 2022). Since then, the production of fermented milk products has evolved in various European areas and nations, resulting in cheeses such as Parmesan cheese from Parma, Switzerland, Gouda from the Netherlands, Brie from France, and Cheddar from the United Kingdom (Moonga, 2019). Sub-Saharan Africa is not well-known for cheese production, and there are no well-known indigenous fermented milk products such as yoghurt or kefir (Moonga, 2019). Most countries, however, have traditional popular fermented milk products that are still made at home using traditional methods and for which manufacturing processes have not been recorded or are simply scarce (Moonga, 2019).

Fermentation is one of the methods of milk preservation that has been used for centuries. Fermented milk products serve several purposes in rural developing countries (Gonfa, A. et al., 2001). They are consumed as food and drinks, and their market value and shelf life outperform raw milk (Motarjemi and Nout, 1995). According to popular wisdom and specific study findings, they have been hailed as more nutritious and health-promoting than fresh milk. According to Platt (1964), Fermented milk is high in B vitamins, particularly vitamin B12. There is more evidence that fermentation improves milk protein digestion (Marshal, 1986).

Lactic acid bacteria are the most common beneficial microorganisms in food fermentation. Homo- and hetero-fermentation are two types of lactic acid fermentations that can be distinguished by the results of glucose hydrolysis (Adams and Moss, 2008). Milk fermentation is a very complex process because it usually involves the product interacting with a group of microorganisms. This means that any changes occurring during fermentation will be determined by the available nutrients and nutrient precursors in the raw milk, the metabolic activities of the microorganisms responsible for fermentation, and any potential interactions between these elements (McFeeters, 1988).

2.6 Traditionally Fermented Milk Products

Traditionally fermented milk products (TFMPs) are made from raw or pasteurised milk through natural or spontaneous fermentation. Most commercial fermented milk products use traditional milk fermentation recipes like cheese and yoghurt. Fermented milk products have traditionally

played an important role in rural diets, nutritional security, and an increasing number of urban African households (Moonga, 2019). TFMP production is primarily associated with pastoral ethnic groups who own cattle in many African countries (Moonga, 2019). Population growth and rapid urbanisation are expected to drive up demand for TFMPs. Attempts to commercialise such products are underway in some countries. However, because many of the African population are lactose intolerant, TFMPs are a better option for their dairy intake because they contain less lactose (Moonga, 2019).

In Africa, various traditionally prepared fermented milk products are produced at the household level in rural areas. An example, in Zambia, according to Schoustra et al. (2013), a traditional fermented milk product called *Mabisi* is made by spontaneous fermentation of raw milk at room temperature for 48 hours in a calabash (gourd), then stirred/sieved before eating (Schoustra et al., 2013), This results in a sour milk product with a pH range of 4.0 to 4.5 and a thick, smooth or lumpy consistency. The calabash is not usually washed to provide starting culture for the subsequent fermentation batch. The completed product has a mildly acidic flavour (sour taste) and a firm texture. This spontaneous fermentation involves microbes from the raw milk, the containers, and the immediate environment (Moonga, 2019). The resulting milk fermentation products are called *'Nunu*' from Ghana, *'kivuguto*' from Rwanda, *'Mursik*' from Kenya, *'Ergo*' from Ethiopia, *'Iben'* from North Africa, *'Omashikwa*' and *'Mabisi*' from Namibia, and *'Amasi*' from Zimbabwe and South Africa are some of the TFMPs produced on the African continent. Most are made from unpasteurised raw milk, except for *'Mursik*', which has a pasteurisation step (Moonga, 2019).

Food-borne pathogens are often isolated from traditionally fermented milk (Beukes et al., 2001) (Savandago et al., 2004; Akabanda et al., 2010; Schutte, 2013). This is of concern because these foods are also used as weaning foods, and according to Nout et al. (1989), mortality and morbidity rates due to diarrheal diseases are highest in infants during the weaning period. Therefore, fermented milk products' safety becomes a significant public health concern. Consumption of sour milk as half-done also contributes to developing Brucellosis (Omore et al., 2002). An acidic condition of sour milk inhibits many pathogenic bacteria (Lund et al., 2000) but does not eliminate all the pathogenic bacteria.

2.7 Brucella Pathogen

2.7.1 Microbiological characteristics

Brucella species are Gram-negative coccobacilli bacteria (short rods) measuring about 0.6 to 1.5 μm by 0.5-0.7 μm; they are slow-growing aerobic organisms (Galinska & Zagórski, 2013), non-sporing and non-encapsulated or flagella and, therefore, are non-motile. Currently, the genus Brucella contains twelve (12) species: *B. abortus*, *B. suis*, *B. ovis*, *B. melitensis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti*, *B. microti* and *B. inopinata* (Galinska & Zagórski, 2013), *B. vulpis* (Holger, 2016), *B. papionis* (Adrian, 2014). Brucella species are classified as smooth or rough depending on colony morphology during culture. Smooth Brucella species, such as *B. melitensis*, *B. abortus*, and *B. suis*, are commonly considered pathogenic, whereas rough forms, such as *B. canis* and *B. ovis*, are less so pathogenic (Corbel, 2006; Godfroid, 2017). *B. abortus* (from cattle), *B. melitensis* (from goats and sheep), *B. suis* (from pigs), and *B. canis* (from dogs) are the most pathogenic and invasive species in humans (Godfroid et al., 2013; Pappas et al., 2005). *B. melitensis*, the most virulent and invasive species, causes more severe symptoms in humans than the others (Godfroid, 2017).

The isolation of a distinct Brucella strain, tentatively named *B. maris*, from marine animals in the United Kingdom, Australia, and the United States broadens the genus' ecological range and its potential as a zoonosis (Cloeckaert et al., 2001; Ross et al., 1994). *B. maris* has two subspecies: *B. cetaceae* from otter/seal and *B. pinnipediae* from whale/porpoise (Cloeckaert et al., 2001).

The pathogen Brucella causes Brucellosis, which affects mainly animals. Human illness is spread primarily through consuming raw milk or milk products and contact with animals or animal food items such as undercooked meat (Rust, 2006). Laboratory personnel, veterinarians, agricultural workers, and slaughterhouse employees are generally in danger (Wafa et al., 2006).

2.7.2 Culture of Brucella species

Gram-negative bacteria are difficult to grow in ordinary media but resist external media such as soil or slurry. The brucella metabolism is primarily oxidative, and its culture in a selective medium demonstrates action on carbohydrate components. They are aerobic, but certain species, such as *B. abortus*, require a CO_2-enriched atmosphere (5 to 10%) (Pérez-Etayo et al., 2018). Growth factors found in blood or serum promote cell multiplication. At 37^0C, brucella culture is always

auxotrophic (requires thiamine, niacinamide, and biotin) and requires non-inhibitory peptones (some peptones release inhibitory sulphur from the cysteine) for adequate growth. The medium brucella culture should be selective by adding antibiotics such as cycloheximide, bacitracin, and polymyxin B (possibly vancomycin, nalidixic acid and nystatin) (De Miguel et al.,2011).

Brucella colonies appear in two or three days on a suitable solid medium. Their culture reveals two strains: S (smooth) and R (rough). The S colonies are small, round, and convex, but dissociation with loss of the (lipopolysaccharide) (LPS's) O chains frequently occur, resulting in rough variants. Because *B. canis* and *B. ovis* LPS lack O-chains, the latter is natural. This phase of dissociation is significant in vaccination. It is especially important in selecting vaccine strains, some of which are non-agglutinogen and prepared from R strains. They are abortus and melitensis antigens, respectively (nominally only because of some biotypes and conversely pure melitensis). In serology, Brucella S colonies almost entirely react with each other but not with R colonies. A monospecific serum reacting only with the A or M antigen has been prepared, and antibodies specific for A and M have now been identified, indicating that each type of chain has at least one unique epitope (Forsyth 2005).

2.7.3 Growth and survival of Brucella

2.7.3.1 Survival of Brucella in the Environment

In contrast to most bacteria, which do not produce spores, Brucella is extremely resistant outside their hosts (Freycon, 2015). In addition to the animal reservoir, the organism's potential survival in the environment, which may play a role in disease epidemiology, must be considered.

Brucella survival is influenced by several factors, including product type, water content, temperature, pH changes, the biological action of other bacteria present, and the duration and storage conditions of the product (Brisabois et al., 1997; FAO.2005). Brucella is resistant to drying and can survive in biological material for extended periods, particularly at low temperatures. Brucella can survive for several months in water at 4-8°C, 2.5 years at 0°C, and several years in frozen tissue or media. Survival in moist soil is possible for more than 60 days and more than 144 days at 20°C and 40% relative humidity (Bervas et al., 2006). They are sensitive to a wide range of disinfectants, including formaldehyde, hypochlorite, iodophors, and phenols, as long as there is no excess organic matter (Aggad & Boukraa, 2006). The environment is not considered an important source of infection for humans; despite its relatively high survival capacity and over

extended periods when conditions are favourable to the bacteria, it may play an important role in disease spread.

2.7.3.2 Survival of Brucella in food products

Many factors, including the product's type and age, the medium's humidity, the temperature, pH variations, the humidity of the product, the biological activity of the other bacteria present, and storage conditions, influence the survival of Brucella in milk and milk products. Dairy products' pH and water activity are considered significant factors influencing Brucella survival (Jansen et al., 2019). In raw milk, Brucella survives 24 hours at 25-37^{0}C, 48 hours at 8^{0}C, and 2.5 years at -40^{0}C. At low concentrations in a liquid medium, Brucella is quite heat sensitive. Thus, bacterial suspensions diluted in milk can be easily inactivated by pasteurisation or 10 minutes of prolonged boiling.

Previous research has shown that the pH of dairy products plays an important role in the survival and growth of Brucella spp., and it has also been argued that the role of dairy products as a vehicle for transmitting several pathogens can be predicted by determining the pH values of the products (Estrada et al., 2005). Davies et al. (1973) demonstrated the effect of pH change on the survival of *B. abortus* in milk and milk products, implying a direct relationship between the microorganism's survival and the pH. Nonetheless, Estrada et al. (2005) found that even at pH 4.0, the starter culture did not inhibit the survival of *Brucella abortus* in milk fermented with a yoghurt starter culture. Some studies have indicated that fat milk content may affect *Brucella*'s survival (Estrada et al., 2005).

Although some studies have indicated that survival rates could be higher in milk with higher fat contents, this has not been established (Falenski et al., 2011). It was discovered that *B. melitensis* could not survive at pH levels below 4.0, whereas *B. abortus* strains appeared to be more acid tolerant and could even survive for ten days at pH levels below 4.0 in fermented milk (Dadar et al., 2019). According to Jansen et al. (2019), Brucella can survive with optimal pH conditions ranging from 6.6 to 7.4 at 37 °C and a survival capacity ranging from pH: 4.1 to 8.4. *Brucella* can survive for much more extended periods; strictly lactic fermentation, short duration, and desiccation are all factors that flavour their survival (Brisabois et al., 1997).

2.8 Brucella Epidemiology

Because transmission occurs from animals to humans, human data are correlated with animal data.

2.8.1 Animal epidemiology

Brucella primarily infects ruminants (cattle, goats, and sheep) and pigs, responsible for nearly all human infections (Chakroun & Bouzouaia, 2007). This acceptance of animals has spread to aquatic mammals (dolphins, seals and certain river fish) (Maurin, 2005). On a global scale, bovine Brucellosis is the pinnacle of livestock disease. However, the prevalence of infection varies by country. The prevalence of bovine Brucellosis in some of the endemic countries in Africa is presented in Table 3. *Brucella melitensis* is less common worldwide than *Brucella abortus* (Cisse Aissa, 2015). Animal Brucellosis is frequently chronic and well tolerated, but it is responsible for repeated abortions in females. The economic impact of chronic Brucellosis in cattle is significant due to the reduced fertility and health risk associated with the disease (Chakroun & Bouzouaia, 2007).

2.8.2 Brucellosis in human

Brucellosis in humans almost always originates from an animal reservoir (Godfroid et al., 2013). The prevalence of the human disease is difficult to estimate due to clinical polymorphism and underreporting. Although the disease's incidence is declining in developed countries, it can reach alarming levels in developing countries. The range of reported incidence of human brucellosis cases per 100,000 people per year is 0.28-268.81 in North Africa and the Middle East, 34.86 in Sub-Saharan Africa, 0.03-32.49 in Western Europe, 88.0 in Central Asia, 12.84-25.69 in Central and Southern Latin America and 0.02-0.09 in North America (Dean et al., 2012).

Table 4. shows the prevalence of human Brucellosis in some endemic countries. Human cases can be used to predict the presence of disease in animal populations. It is also necessary to determine whether the infection was acquired locally or elsewhere and if food products are involved, whether they were produced locally or imported (FAO, 2006).

Table 3. Reported prevalence of bovine Brucellosis in some endemic African countries

Country	Sample size(herd/animal)	Study level	Test used	Herd prevalence (95% CI)	Cattle prevalence (95% CI)	Reference
Algeria	95/1032	National	RBT	26.3% (17.8-35.4)	8.2% (6.6-10.1)	Aggad and Boukraa (2006)
Cameroon	146/1377	National	cELISA	20.3% (4.2-77.6)	3.1% (1.8-4.4)	Scolamacchia *et al.*, 2010
Egypt	1966	Sub-national	RBT	-	4.9% (4.1-6.0)	Samaha *et al*., 2008
Ethiopia	903/7196	Sub-national	RBT, CFT	20.4% (17.8-23.2)	4.3% (3.6-4.5)	Ibrahim et al., 2010; Mekonnen et al., 2010; Megersa et al. (2011); Adugna et al., 2013
Kenya	398	Sub-national	c-ELISA	-	16.8 (13.2–20.4)	Okumu et al. (2019)
Libya	42	Sub-national		-	42.1% (20.3-66.5)	Ahmed *et al*., 2010
Namibia	49718	-	RBT, CFT	9.3% (7.5-11.4)	0.49% (0.43-0.56)	Madzingira et al., 2020)
Niger		Sub-national	iELISA	14.9% (12.4-17.8)	3.2% (2.7-3.9)	Boukary et al., 2013
Nigeria	271/4745	Sub-national	cELISA	77.5% (68.6-84.5)	26.3% (22.1-31.0)	Mai *et al*., 2012
Tanzania	2048	-	cELISA	-	2.4% (0.02-0.31)	Mengel et al. 2013
Turkey	626	Sub-national	RBT	-	35.3% (31.6-39.2)	Sahin et al., 2008
Zambia	179/2537	Sub-national	RBT, cELISA	56.4% (48.8-63.8)	16.3% (14.9-17.8)	Muma et al., 2006; Chimana et al., 2010; Muma et al., 2013
Zimbabwe	156	-	RBT	30.1% (18.4-61.7)		Vhoko et al. l 2018

Table 4. Reported prevalence/incidence of human Brucellosis in some endemic countries in the World.

Country	Sample size	Type of sample	Test used	Prevalence/incidence	Reference
Egypt	4490			64-70 cases per 100000 individuals	Jennings et al.,2007
Ethiopia	541	PUO patients, OEP	RBT, LFA, 2-ME	9.9% (7.6-12.8)	Kassahun et al.,2006; Regassa et.,2009
Tanzania	199	OEP	RBT	5.5% (2.8-9.7)	Swai and schoonman.,2009
Togo	683	OEP and general people	RBT, iELISA	1.0% (0.4-2.1)	Dean et al. (2012)
Iran	39359			0-37.3% cases per 100000 individuals	Mollalo et al.,2014
Iran	1681	Referred patients, OEP	iELISA	4.1% (2.8-5.8)	Esmaeili et al.,2014; Nikokar et al.,2011
Kenya	1022			35.8 (32.8–38.8)	Kairu-wanyoike et al. (2019)
Namibia	971		CSH	17.9% (15.6-20.5)	(Oscar et al., 2021)
Turkey	2038	Farmers, Veterinarians and general people	RBT, STAT	8.8% (7.6-10.1)	Cetinkaya et al.,2005; Otlu et al.,2008; Kutlu et al.,2014
Zambia	153	OEH	iELISA, cELISA	20.3% (14.6-27.5)	(Mubanga et al., 2021)

2.9 Pathogenesis

2.9.1 In human

Brucellosis is transmitted to humans through consuming contaminated unpasteurised milk or other animal food products such as undercooked meat (Rust, 2006). Bacteria enter the bloodstream and circulatory system from the gastrointestinal tract via the mucosa. Transmission can, however, occur through cuts, abrasions, inhalation, and direct contact with the mucous membranes of infected animals (Rust, 2006). Pathophysiology is divided into several stages. During the incubation period, which lasts about 15 days on average, the bacteria migrate to the first lymph node and multiply. In its acute phase, Brucellosis is distinguished by lymphatic septicaemia in which bacteria colonise organs rich in reticulohistiocytic cells (lymph nodes, liver, spleen, bone tissue, genitalia) and form intra-cellular bacterial foci surrounded by a histiomonocytic and lymphocytic inflammatory reaction (Maurin, 2005). An autophagosome is where intracellular multiplication occurs. Acute clinical manifestations of the disease occur during this stage, and blood cultures are positive. From the second week on, the appearance of specific serum antibodies (Ig G, Ig M, Ig A) will partially counteract the development of the infection, which will clinically subside even in the absence of treatment (Chakroun & Bouzouaia, 2007). The disease may then progress into a subacute phase, with the appearance of one or, in rare cases, several secondary localisations. These include osteoarticular, neurological, testicular, hepatosplenic, and other conditions (Maurin, 2005) (Janbon,2000; AFSSA, 2006). Tissue infection causes a cellular reaction that results in the appearance of granulomas that are limited by a lymphoplasmacytic cellular reaction arranged in a crown. In humans, suppurative and necrotic lesions are unusual. Malignant poly visceral involvement may be determined by the strain's exceptional virulence and deficient terrain. Chronic Brucellosis is defined as a course that lasts more than a year, with or without discovering a secondary location (Janbon, 2000). Brucella is facultative intracellular bacteria that secrete a factor that prevents apoptosis in infected macrophages, which accounts for their long-term survival in the body. Reticular endothelial cells, particularly macrophages, are also preferred in animals and are humans' chief site of infection (McDermott & Arimi, 2002; McGill & Oyoo, 2002).

2.9.2 In animal

Chronic Brucella infections in animals have been linked to survival mechanisms, specifically initial survival and organism dissemination (Riley & Robertson, 1984). The virulent factor is primarily lipopolysaccharide (LPS), which protects the organism from complement-mediated lysis and increases intracellular survival (Rege et al., 2006). The dissemination is based on inhibiting polymorph neutrophil primary degranulation and oxidative bursts, which prevents phagolysosomal fusion (Harmon et al., 1988; Riley & Robertson, 1984) (Frenchick et al., 1985). This is more likely to happen when the initial antibiotic treatment for Brucellosis is insufficient. Although the nature of humoral immune mechanisms' participation in acute infection control is unknown (Cloeckaert et al., 2001; Muñoz et al., 2005) (Rust, 2006). Because of the intracellular response achieved by Brucella organisms in the liver and bone marrow, the capacity of humoral immune mechanisms to influence the course of the infectious reaction is likely to be limited (Seema et al., 2001). Chronic Brucella infections in animals have been documented.

Nonetheless, immunoglobulin M (IgM) antibodies begin to rise at the end of the first week after infection and typically peak at around one month, when immunoglobulin G (IgG) antibodies appear. IgG antibody titres decrease over time, whereas IgM antibody titres remain elevated for years. Immunoglobulin A (IgA) antibodies are produced late and can last long periods (Muñoz et al., 2005) (Rust, 2006). The two scenarios of antibodies and transaminase enzymes suggest that the liver and bone marrow are involved in the acute phase (Chimana, 2012). Without specific activation, macrophages mediate infection control during this phase. However, after the first two weeks of infection, sensitised T lymphocytes specifically activate the macrophage response, significantly reducing the survival rate of Brucella organisms in most infected individuals' liver and spleen (McCullough and Paulson, 1998; Rust, 2006). The Rose Bengal test shows classic positivity at this stage due to the high levels of antibodies. There is melitococcemia, which is the presence of Brucella in the blood, which, if left untreated, causes the localisation of infection and progresses to a chronic stage (Rienzo, 1948; Villafane et al., 1948). These focalisations usually occur in bones and joints, leading to lumbar spondylodiscitis with sacroiliitis, a disease symptom. In animals, the incubation period lasts between 30 and 60 days. When infection occurs in pregnant animals, the initial lesion is in the uterine wall and later spreads to other parts of the organ. Brucella organisms' proliferation rate is related to erythritol production (Nicoletti, 1980; Radostits et al.,

1994). This causes severe ulcerative endometritis of the inter-cotyledonary spaces, affecting the allantoic chorion, foetal fluids, placental cotyledons, and villi destruction (Radostits et al., 1994). Following bacteraemia, there is localisation in the cow's gravid uterus, resulting in placentitis, which increases prostaglandin production, curtailing the corpus luteum, and then abortion occurs (Roushan et al., 2006) (Woods and Jan 2005). In cases where the animal is not pregnant, there is localisation in the udder, resulting in interstitial mastitis and involvement of the mammary glands, which may cause the organisms to be excreted in milk for months or even years, causing the animal to become a carrier (ÇokÇa et al., 1999; Mdegela et al., 2005) (Akay et al., 2007).

2.10 Symptoms and Complications

2.10.1 In animal

In animals, the disease is characterised, for example:

a) In Bovine brucellosis

Abortion is most common in females around the sixth or seventh month of pregnancy. Brucella metritis endometrial lesions heal in a few weeks, resulting in temporary infertility (Tassadit., 2014). Brucella mastitis (mammary inflammation) reduces milk production by 10% (Cisse, 2015). Symptoms are uncommon in males. However, orchitis, which may be associated with epididymitis, can be observed. Extra-genital symptoms and lesions such as arthritis and frequent knee hygroma may also occur (Cisse, 2015).

b) In sheep and goats

They are similar to bovine Brucellosis regarding genital involvement, abortion (usually beginning in the third month of pregnancy), and placental retention (Garin-Bastuji, B et al., 2006). In males, infection is generally undetectable (though cases of orchitis, epididymitis or reduced fertility have been reported) (Garin-Bastuji, B et al., 2006). One-third of the infected animals usually abort at six months or later, and there is sterility or infertility of either the male or female (Chimana, 2012).

2.10.2 In humans

Brucellosis is distinguished by polymorphism, which results in non-specific clinical manifestations, particularly at the beginning. The most common form is acute Brucellosis, also known as sweat fever, which is now rare. The onset is typically progressive and insidious, if not

brutal, after a 15-day silent incubation period (8-21 days). It is frequently characterised by an influenza-like illness with fever, asthenia, diffuse pain, and general malaise, prompting the patient to seek medical attention.

The symptomatology during the state phase combines three significant symptoms: fever, sweat, and pain. Rippling fever is the most common, but it is becoming increasingly rare (Chakroun & Bouzouaia, 2007) (Chakroun & Bouzouaia, 2007). It often appears as a plateau, remittent, or pseudo-malaria. It is characterised by excessive sweating, especially at night, a distinctive "wet straw" odour, and diffuse algias such as headache, myalgias, mobile and fleeting arthralgias, and arthromyalgia (Chakroun & Bouzouaia, 2007). The general condition is maintained for an extended period, and weight loss occurs later.

Acute brucellosis symptoms among others include rash, testicular discomfort, among others; abdominal discomfort, diarrhoea, nausea, vomiting, constipation, and anorexia are common gastrointestinal symptoms some people encounter (Galinska and Zagorski, 2013; Wojno et al., 2016). Physical examination may reveal moderate splenomegaly, hepatomegaly, cervical and axillary adenopathy, and bronchial rales (Chakroun & Bouzouaia, 2007). At this stage, two visceral locations are suggestive of Brucellosis. These are orchiepididymitis and sacroiliitis.

In addition to this form, most brucellosis cases are asymptomatic or only mildly symptomatic. The disease frequently goes unnoticed, and the diagnosis can only be made based on a serology test after a proven exposure. Typhoid-like forms have symptoms similar to typhoid fever (Chakroun & Bouzouaia, 2007). Polyvisceral malignant forms, with a poor prognosis, have been observed in immunocompromised subjects and have become exceptional. In pregnant women, Brucellosis can cause abortion, premature delivery, death, and death in utero (Chakroun & Bouzouaia, 2007). The disease does not manifest specific clinical features in people infected with the human immunodeficiency virus. Subacute brucellosis cases have symptoms comparable to those described above but are milder (Galinska and Zagorski, 2013). Chronic Brucellosis can take several forms and might even be asymptomatic.

2.11 Complications

In cases of Brucellosis, complications mainly affect the musculoskeletal system, but they can also affect the central neurological and cardiovascular systems (Galinska and Zagorski, 2013, Daff,

2016; Mangalgi et al., 2016; Wojno et al., 2016). Complications can also occur in other systems, such as gastrointestinal, hepatobiliary, respiratory tract, genitourinary, pregnancy and breastfeeding, cutaneous, and ophthalmic (Corbel, 2006). It is now recognised that Brucellosis can affect people of all ages, particularly in areas where *B. melitensis* is the dominant species. Regardless of age, the course of infection and the incidence of complications appear to be similar.

2.12 Paraclinical Diagnostic

2.12.1 In animal

Animal disease diagnosis and control must be made on a herd level. Some infected animals may have a very long incubation period, and individuals may be serologically negative for a long time after infection. The presence of one or more infected animals is sufficient evidence that infection exists in the herd, and those other serologically negative animals may be incubating the disease and present at the time of infection (Corbel, 2006). Diagnostic tests are classified into two types: those demonstrating the presence of the organisms and those detecting an immune response to its antigens. Isolation of Brucella is conclusive proof that the animal is infected, but not all infected animals produce a positive culture, and the methods and facilities required are not always readily available. Antibodies or hypersensitivity reactions provide only a preliminary diagnosis, but they are the most practical and cost-effective method of diagnosis in practice (Corbel, 2006).

a) Bacteriological procedures

Isolation and identification of Brucella may be useful for epidemiological purposes and monitoring the progress of a vaccination programme because it provides a definitive diagnosis of Brucellosis from genital secretions (swabs), milk, runt (stomach, spleen, lung), foetal membranes, semen, or joint fluid (Corbel, 2006). The lymph nodes of the cephalic region, the genital and mammary regions, the uterus, the udder, and the testes are the most used samples from deceased animals (Colatrella, 2000).

b) Serological techniques

Detecting specific antibodies in serum or milk remains the most practical method of diagnosis of Brucellosis. Typically, the most efficient and cost-effective method is to screen all samples with a low-cost, quick test that detects a high proportion of infected animals. Each test batch must have adequate quality control, and tests must be repeated if the quality control criteria are not met

(Corbel, 2006). Diagnostic tests are used in livestock for screening or prevalence studies, confirmatory diagnosis, trade certification, and post-disease surveillance (Higgins, 2015). As with any laboratory-based diagnosis, it is critical to correctly identify the "audit trail" of individual animal identity, sample number, and test result so that the linkage between animal and result is completely certain.

2.12.2 In human

Because of the wide range of clinical manifestations of human brucellosis, bacteriological and Serological tests are necessary. All physicians who treat a febrile patient who lives in an endemic area or has recently visited a country where Brucellosis is endemic ("travel-associated disease") must be aware of the risk of Brucella infection (Corbel, 2006). As a result, a thorough clinical history is essential for orienting the diagnosis, and the importance of some fundamental questions (occupation, diet, contact with animals, and travel to endemic areas) must be emphasised. In addition, a rapid screening test is required. Despite being a sensitive and quick screening test, bacteriological and other serological tests should confirm the Rose Bengal plate results. Additional tests should be performed to confirm the result if the screening test is negative in the face of a history and clinical presentation. Adherence to these practices will aid in the prevention of delayed diagnosis (Corbel, 2006).

2.13 Risk Analysis

Risk is the probability of an adverse effect occurring and the magnitude of that effect consequential to a hazard in food. Risk Analysis is a systematic way of carrying out science-based analysis and controlling food safety hazards to help protect consumers' health and ensure fair practices in the food trade. It is a decision-making tool for hazard management and is used to estimate the risks to human health and safety, identify and implement appropriate measures to control the risks and communicate with stakeholders about the risks and measures applied. According to the Codex Alimentations Commission (CAC) Framework (CAC, 1999), risk analysis has three (3) components, distinct but closely related and complementary:

i. **Risk assessment**: described as the characterisation of the potential hazards and the associated risks to life and health resulting from exposure of humans to hazards present in

food over a specified period. The risk assessment will answer the following types of questions:

a. What is the source of the hazard?
b. How much is hazardous?
c. What is the important route of transmission?
d. What are the effects on the consumer?
e. How serious are the effects in case of exposure?
f. How frequent is the hazard contamination?
g. Are there some more vulnerable groups?
h. How many cases or outbreaks have been reported?
i. What factors impact the growth and survival of the microorganism?

A scientifically based process, it is typically carried out in accordance with the four steps of the Risk Assessment Process (Figure 1), which are based on the Codex Committee on Food Hygiene Principles and Guidelines for the Conduct of Microbiological Risk Assessment (CAC, 1999) and include the following steps: (1) Hazard identification, (2) Hazard characterisation, (3) Exposure assessment, and (4) Risk characterisation:

a) Hazard identification: The first step in conducting microbiological risk assessment focuses on gathering and collating existing information on the characteristics of the hazard. Hazard identification looks at three types of hazards: biological, physical and chemical. This risk assessment will primarily focus on Brucella, a biological hazard in milk and milk products.

b) Hazard characterisation: Once the potential hazards have been identified, the nature of the hazard must be characterised, and the probability of adverse human effects as a function of viable pathogen numbers ingested should be known. Hazard characterisation describes the relationship between levels of a pathogen consumed (dose) and the probability of subsequent development and severity of illness or another adverse health outcome (response). Data types that can be used to establish dose-response relationships include animal toxicity studies, clinical human exposure studies and epidemiological data from illness investigations. There is a level of exposure known as a threshold dose below which

adverse health effects do not occur. Above a threshold, the effects will be more severe when the exposure increases.

c) **Exposure assessment**: It estimates how probable a person, or a community will be exposed to a microbiological danger and how many organisms will likely be consumed. (Lammerding and Fazil, 2000). According to Codex, exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of a microbial hazard via food as well as exposure from other sources if relevant or with the potential to cause an adverse health effect (FAO/WHO, 2008). Risks from microbial hazards are typically evaluated in terms of single exposures to contaminated food. Exposure assessment is based on the factors such as consumption: the amount of food consumed (g/kg bw/day), the frequency (consumption pattern) and based on an estimated food contamination level at the very moment of consumption. Exposure assessment can be qualitative, semi-qualitative or quantitative assessment. Quantitative assessment is subdivided into a deterministic approach (which uses a random estimate for each model variable, for example, an average) to determine the model's results or a probabilistic approach (model variables are handled as distributions in the probabilistic method).

d) **Risk characterisation**: The final stage of the risk assessment is an "opinion," which combines data from hazard characterisation and exposure assessment to give scientific guidance for risk managers (Renwick et al., 2003). It entails risk calculation and risk description, and it is based on data and expert opinions. Risk characterisation, according to the CAC (2004), is "the qualitative and/or quantitative estimation, including associated uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation, and exposure assessment" (CAC, 2004).

Risk Assessment in Milk

Milk is an important source of vital nutrients for humans, but its composition encourages the growth of microorganisms, including pathogenic ones like Brucella (Berhe et al., 2020). In a study conducted in Sudan to evaluate the associated risk factors of Brucellosis as well as to estimate the qualitative risk of Brucellosis to public health, the overall risk estimation for Brucellosis was found to be high, which means the risky event is likely to occur among farms workers and consumers from consuming raw milk contaminated with Brucella (Mohammed & Salman, 2020). In another

study in Nigeria, 62 milk samples from cattle herds were collected, and 40 milk samples from milk vendors sold fermented milk; 17.7% of milk samples from herds and 12.5% of samples from milk vendors were positive for Brucella antibodies (Daniel & Cornelius, 2015). Further, the risk assessment in milk was conducted in Tanzania by Ndaki et al. (2022) where the model showed that the consumption of cultured milk, defined as half-cooked/half-done milk, has a risk of getting Brucellosis which was described as the probability of getting an infection to be 0.4 (95% CI 0.32-0.48).

ii. **Risk management:** the process of weighing policy alternatives considering the results of the risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures. Risk management of microbiological food-borne-food-borne risks to consumers is best performed by adhering to the general risk management framework (RMF), which consists of four key stages and several activities: Preliminary microbiological risk management (MRM) activities, identification and selection of MRM alternatives, Implementation of MRM options, monitoring, and review.

iii. **Risk communication:** The CAC defines risk communication as "the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions. Two basic methods are often utilised to support the continued engagement of all key stakeholders:

- Model 1: A risk management team member oversees the overall coordination of all risk communication responsibilities, often performed by others.
- Model 2: As part of the risk analysis team, one or more risk communication specialists oversee the planning, creating, and implementing of the risk communication process.

Whatever model is adopted should identify and outline the roles for risk communication from the start of the risk analysis process.

2.14 Knowledge gaps

It has been reported in a previous study conducted by Muma et al. (2008) among traditional farmers in Zambia that 68.7% consume raw milk, while 87.3 consume cultured milk, but there is no

available information on consumption patterns and serving portions of milk in the Southern province, which can lead to an infection. Moreover, there is a lack of knowledge on human exposure to Brucella through the consumption of sour milk in Zambia.

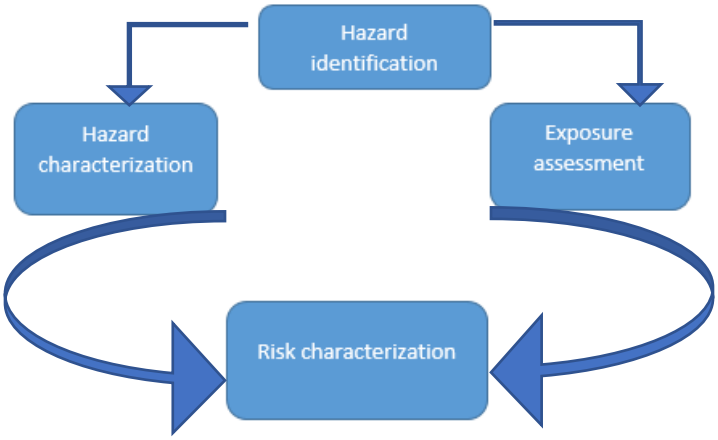

Figure 2.5: *Protocol for microbial risk assessment based on the CAC*

CHAPTER THREE: MATERIAL AND METHODS

3.1 Research Design

This was a two-tier study consisting of a cross-sectional questionnaire survey and designing a risk model for exposure to Brucellosis. The primary data came from a questionnaire survey to collect information on cultured milk intake. Secondary data was gathered from prior research, a study of scientific peer-reviewed articles, and grey literature. Both primary and secondary data were utilised in designing the risk model.

3.2 Study Area

The research was conducted in selected districts of Zambia's Southern Province that are known to be endemic to Brucellosis in cattle and are the primary producers of livestock in the country, particularly in the districts of Monze, Choma, and Namwala (Mfune et al., 2021; Muma et al., 2006; Muma et al., 2013) (Figure 2). Another reason we chose Southern Province was that cultured milk, known as Mabisi in Zambia, was mostly produced in the Southern Province (Moonga, 2019). Southern province, located between latitudes 16 0S and 30 0S and longitudes 270E and 00 0E, with a total land surface area of 85,283 Km^2 and an estimated human population of 1,907,784 (CSO, 2018), is a particularly fertile cattle-producing area. According to the 2017-2018 report, Zambia had a cattle population of 3,714,667 in 2018, with the Southern Province having the most significant number at 1,315,238, accounting for 35.4% of the national total (Zambia Reports, 2019).

3.3 Study Population and Sampling Frame

The research comprised a sample size guided by demographic statistics to generate a representative estimate of portion sizes and consumption patterns in the Southern Province's three districts of Monze, Choma, and Namwala. This sample size was calculated using Epitools Epidemiological Calculators (https://epitools.ausvet.com.au/) with a large population, a desired level of precision, desired confidence level and the expected true proportion.

Where:

- Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

- P is the expected true proportion (=100)
- e is desired precision (=0.05)

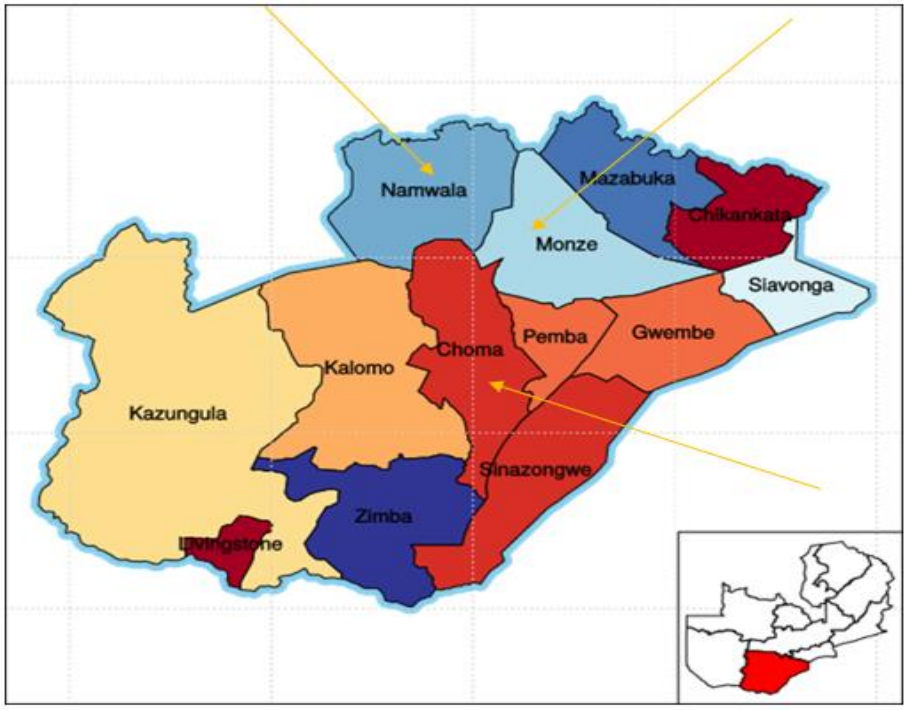

Figure 3.1*: Districts in the Southern province showing the study area. (Source: http://www.sou.gov.zm/)*

Based on the assumed data inputs, the estimated sample size (n) was 323. As a result, a sample of 323 respondents conveniently selected from homes that created their cultured milk and those that made *Mabisi* for sale at milk collection centres were interviewed as part of the survey.

3.4 Instruments for Data Collection

To collect primary data, a structured questionnaire was used (Appendix 5). Secondary data from published literature were collected in accordance with a quantitative risk assessment and the Codex Alimentarius Commission food safety risk assessment framework based on key search phrases such as quantitative risk assessment, Brucellosis, and the intake of cultured, soured, or fermented milk.

3.5 Risk Assessment Process

A Quantitative risk assessment of the health impacts of consumption of traditional fermented milk was performed, following the two steps of the Codex Alimentarius nomenclature: (1) Hazard

identification, (2) Exposure assessment. The risk assessment was incomplete due to a lack of information on the dose effect. Because parameters describing Brucellosis's dose-response relationship were unavailable, no hazard characterisation was performed. In a risk assessment, the risk is typically calculated using exposure to the hazard and the dose-response relationship. However, the risk characteristics could not be calculated due to this limitation. The inputs from primary and secondary data were used to calculate and estimate the probability of being exposed to Brucella and the presence of Brucella in the entire food chain following these steps.

Based on the habits of people living in the three districts:

- In the first scenario, people did not screen the animal: milk from infected animals is milked and using raw milk for fermentation. It is the first probability for exposure assessment (P1)
- In the second scenario, people did not screen the animal: milk from infected animals is milked and boiled before fermenting. It is the second probability for exposure assessment (P2)

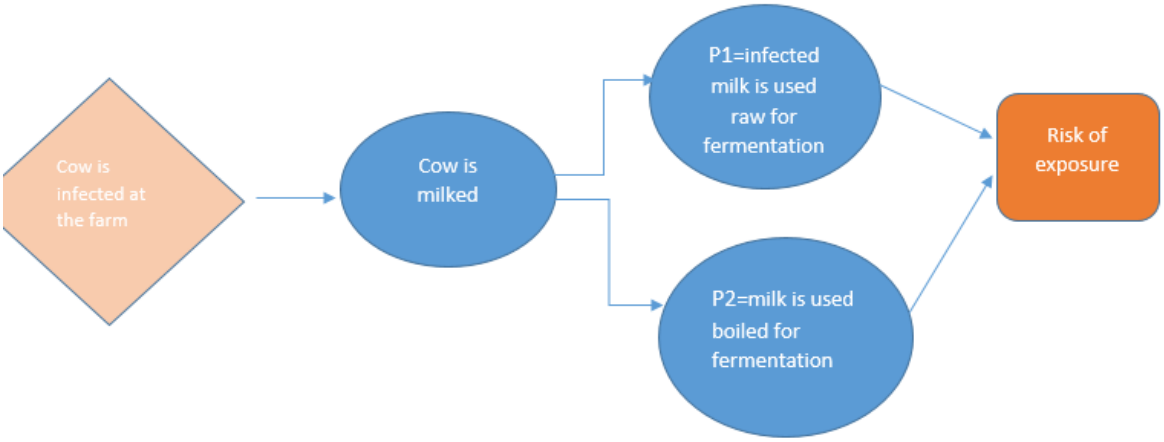

Figure 3.2: *Flow chart illustrating possible exposure to Brucella through consumption of cultured milk prepared from raw milk (Scenario 1) and boiled milk (Scenario 2) obtained from an infected cow.*

We assumed the cow was screened but not detected with Brucellosis: 2 scenarios.

- The first scenario: includes people who performed the screening test, but the animal was seronegative: the animal is milked. The milk was positive, and the milk was fermented using raw milk for fermentation. It is the third Probability (P3).

- The second scenario includes people who performed the screening test, but the animal was seronegative: the animal is milked, and the milk is positive and boiled before fermenting. It is the fourth Probability (P4).

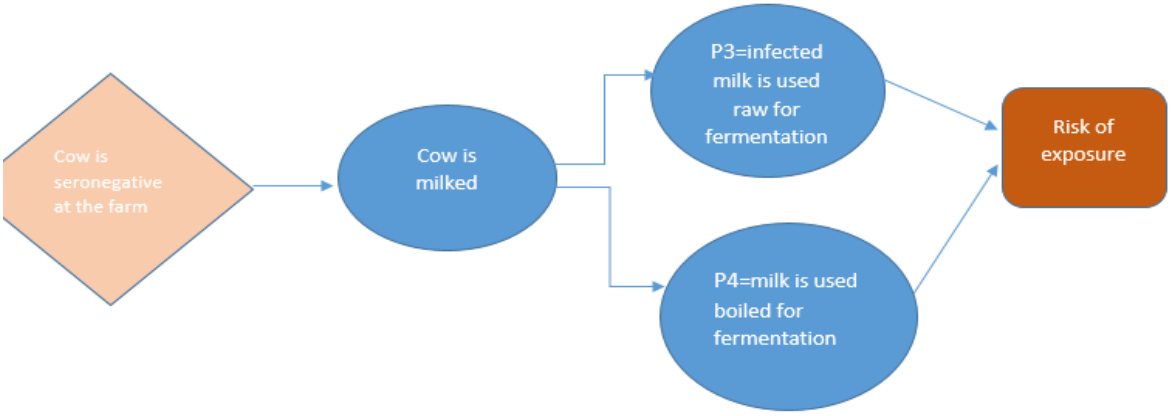

***Figure 3.3**: Flow chart illustrating possible exposure to Brucella through consumption of cultured milk prepared from raw milk (Scenario 3) and boiled milk (Scenario 4) obtained from seronegative cows.*

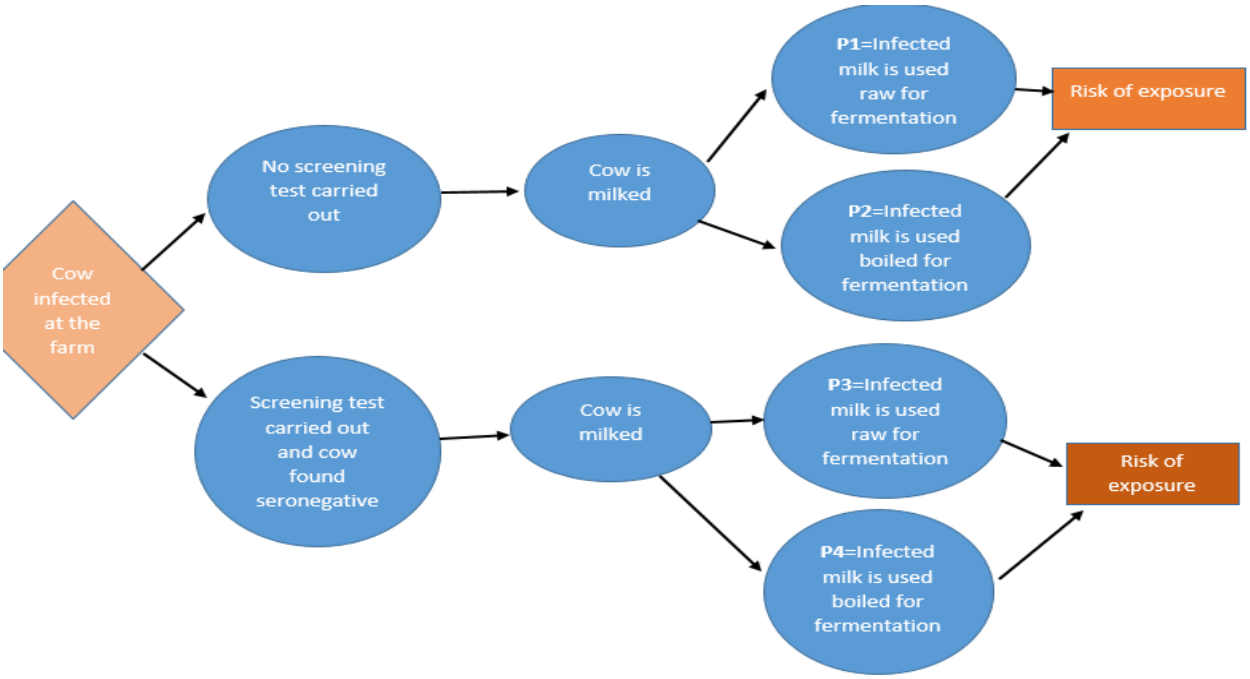

***Figure 3.4:** Flow chart illustrating all the possible probabilities of exposure through the consumption of cultured milk*

3.5.1 Input parameters for risk assessment

The population in the Southern province used in this model was estimated to be 1907784 (CSO, 2018). The consumption period considered in this study was one year (365 days), as shown in Table 5.

Table 5. Input parameters for risk assessment

Variable	Parameter	Data from literature	Source of information
N1	Population size	1907784	CSO, 2018
N2	Quantity of milk consumed/pers/day/l	0.5 - 1	Survey
N3	Period of consumption	365	
P1	The probability that cows are infected at the farm	0.07 (-0.0897 - 0.11)	(Mubanga et al., 2021)
P2	The probability that raw fermented milk is infected	N=40 P= 5	(Ior & Chukwu, 2015)
P3	The probability that pasteurised/boiled, fermented milk is infected	N=28 P=7	(Moslemi et al., 2018)
P4	The probability that the infected cow was not detected and milked	N=65 P= 2	(Sabrina et al., 2018)

3.5.2 Description and distribution of variables and models for risk assessment

The distributions used for each parameter are described in Table 6

Table 6. Description and distribution of variables and models for risk assessment

Variable	parameter	Distribution/model
N4	Quantity of milk consumed/pop/day l	N2 *N1
N5	Quantity of milk consumed/pop/year l	N4*N3
N2	Quantity of milk consumed/pers/day	Uniform (0.5,1)
P1	The probability that cows are infected at the farm	Triangle (0.07,0.0897,0.11)
P2	The probability that raw fermented milk is infected	Beta (5+1,40-5+1)
P3	The probability that pasteurised/boiled, fermented milk is infected	Beta (7+1,28-7+1)
P4	The probability that the infected cow was not detected and milked	Beta (2+1,65-2+1)
P5	Probability of exposure from farm to consumption scenario 1	p2*p1
P6	Probability of exposure from farm to consumption scenario 2	p3*p1
P7	Probability of exposure from farm to consumption scenario 3	p2*p4
P8	Probability of exposure from farm to consumption scenario 4	p3 *p4
N6	Number of people likely to be exposed for 1000 cases scenario 1	P5*1000
N7	Number of people likely to be exposed for 1000 cases scenario 2	P6*1000
N8	Number of people likely to be exposed to 1000 cases	P7*1000
N9	Number of people likely to be exposed to 1000 cases	P8*1000

3.6 Primary Data

A pre-tested comprehensive questionnaire Appendix (1) was used to fill the information gaps on consumption patterns of cultured milk in Southern Province. The questionnaire involved collecting primary data related to milk consumption, the methodology of fermented milk products, and food

safety Practices. The interviews were conducted by convenience. Interviews were conducted with traditional farmers at the milk collection centre and in selected households.

3.7 Secondary Data

We online searched for secondary data through the literature review containing terms relevant to our study, such as Brucella, fermented milk, raw milk consumption, and quantitative risk assessment to fill in the model's input parameters.

3.8 Ethical Approval

Ethical clearance was obtained from Excellence in Research Ethics (ERES) Converge, Ref No.2022-Aug-003. The authority to conduct the study was granted by the Ministry of Livestock and Fisheries of the Choma district. Informed written consent was sought from the respondents willing to participate after fully explaining the whole research process, benefits, and their rights in participation. Participants could leave the survey at any time without feeling obligated to continue. Anonymity and confidentiality were maintained since we did not include the respondent's name, which means we could not link any participant to their data. Before beginning the study, we informed the participants that their responses would be kept confidential, and we also urged the participants to do the same for the respondents to be aware.

3.9 Data Management and Analysis

Data from a questionnaire were collected using Epicollect5 (a digital questionnaire), imported into Excel®, coded, and analysed using Stata Statistical Software to produce a descriptive statistic by calculating the proportion. Furthermore, using primary and secondary data, the risk model was simulated using the Monte Carlo simulation in ModelRisk® Software platform embedded in Excel® We described the numbers of people likely to be exposed in a population of 1000 for each scenario and the probability of exposure from farm to consumption for each scenario.

3.10 Assumptions and Limitations

Assumptions:

- We assumed that the secondary data gathered from different African countries would also be applied to Zambia.

- We assumed that the milk met the boiling point required, similar to pasteurisation.
- We also assumed that pasteurised cheese is similar to pasteurised fermented milk because fermented milk is used to make cheese.

Limitations:

- Time constraints to isolate Brucella in milk.
- Lack of funds.
- Hazard characterisation and risk characterisation has not been calculated.

CHAPTER FOUR: RESULTS

4.1 Demographic Information of the Respondent

The primary demographic information about respondents is given in Table 7. The study had 236 males and 87 females, representing 73.1% and 26.9%, respectively. Most respondents had primary (30.9%) and secondary from grade 10-12 (30.0%) level of education, but they could understand and respond in English or their mother tongue.

Table 7. Demographic information of the respondent (n=323)

Variable	Variable	Proportion/mean	95% CI
Sex	Male=236	73.10%	67.9-77.6
	Female=87	26.90%	22.3-32.1
Level of education	Primary school (grade1-7) =100	30.90%	26.1-36.2
	Junior secondary (grade 8-9) =90	27.90%	23.2-33.0
	Senior secondary (grade 10-12) =97	30.00%	25.3-35.3
	Tertiary=8	2.50%	1.2-4.9
	University=8	2.50%	1.2-4.9
	No education=20	6.20%	4.0-9.4

4.2 Consumption Pattern of Sour Milk in the Southern Province of Zambian

4.2.1 Method of preparing fermented milk

A summary of the method for preparing fermented milk is shown in Table 8. The proportion of farmers that fermented milk using raw milk was 65.3%. Boiled milk was used by 13.3%, and 21.7% purchased milk already fermented, not knowing if it was prepared from raw milk or boiled milk. The most common milk fermentation period was one day (40.9%), followed by two days (29.1%), 6-12 hours (0.6%), 12-18 hours (5-6%), and less than 6 hours (2.5%). Majority (63.8%) fermented milk at room temperature, whiles 3.7% fermented milk in the coldest part of the house, 5.6% in the shade, and 5.6% in the sun.

Table 8. Method of preparing fermented milk

Variable	Categories	Proportion	95% CI
Treatment of milk before fermentation	Not applicable=69	21.70%	17.5-26.5
	No=211	65.30%	59.6-70.1
	Yes=43	13.30%	10-17.5
Time of fermentation	No application=69	21.30%	17.2-26.2
	1 day=132	40.90%	35.6-46.3
	2 days=94	29.10%	24.4-34.3
	6-12 hours=2	0.60%	0.2-2.5
	12-18 h=18	5.60%	3.5-8.7
	Less than 6 hours=8	2.50%	1.2-4.9
Temperature of fermentation	Not applicable=69	21.40%	17.2-26
	Room temperature= (206)	63.80%	58.3-68.9
	under the shade=18	5.60%	3.5-8.7
	under the sun=18	5.60%	3.5-8.7
	keep to the coldest part of the house=12	3.70%	2.1-6.4
Type of container	Not applicable=69	21.36%	17.2-26.2
	Plastic=211	65.32%	59.9-70.3
	Metal=28	8.66%	6.04-12.3
	Clay pot=10	3.09%	1.7-5.7
	Plant material (wood, calabash) =5	1.54%	0.6-3.6

4.2.2 Consumption of fermented milk

Data on the amount of milk consumed among respondents is shown in Table 9. Most of the respondent (52.9%) consumed 0.5 to 1 litre of sour milk per person per day, while 47.1% consumed less than 0.5 litres. The frequency of consumption was two-three times per day (60.7%), followed by one-two times per day (35.6%) and more than once per week (3.7%).

Table 9. Consumption patterns of fermented milk

Variable	Category	Proportion	95% CI
Quantity of milk/day (Size of milk portion per day)	Less than 0.5 litres =152	47.10%	41.6-52.5
	0.5-1 litters=171	52.90%	47.5-58.4
How often do you consume sour milk(frequency)	2-3 times/day=196	60.70%	55.2-65.9
	1-2times/day=115	35.60%	30.5-41
	More than once time/week=12	3.70%	2.1-6.4

4.3 Food Safety Practices

The essential food hygiene practices when milking and handling milk are shown in Table 4.4. Most of the participants (42.2%) who did the milking process did not clean the udder, and 61.3% always cleaned with soap any surface and equipment used during milk handling, preparation and drinking before reusing on other food.

Table 10. Food hygiene practices when milking and handling milk

Variable	Category	Proportion	95% CI
Milking process (n=254)	Not applicable=64	19.80%	15.8-24.6
	Do not clean the udder=136	42.20%	36.81-47.59
	Clean the udder=123	38.08%	32.92-43.53
Cleaning with soap surfaces and equipment used milk handling, preparation and drinking before reusing on other food. (n=323)	Always	61.30%	55.8- 66.5
	Sometimes	21.70%	17.5-26.5
	Most times	7.12%	04.8-10.5
	Never	4.63%	02.8-07.6
	Not often	5.20%	03.2-08.5

4.4 Risk Assessment

4.4.1 Hazard Identification

A hazard is something that could be harmful to animals, humans, plants, or the environment in general (FAO, 2011). In our study, hazard refers to bacterial contaminants in milk, whereas risk refers to the probability of consuming contaminated cultured milk. Unpasteurised milk has been

linked to various serious diseases, and bacteria can contaminate raw milk and cause food-borne-food-borne illnesses (FAO,1998). *Brucella* spp. was identified as a potential hazard in this study, as cells of *Brucella* spp. can survive in acidic environments in dairy products for weeks to months (El daher et al., 1990; Davies and Casey., 1973). It has also been demonstrated that the fat content of dairy products can protect *Brucella* cells and increase their survival capacity (Bejaoui et al., 2022). The number of viable bacteria in high-fat yoghurt decline more slowly (five days at 3.5% fat) than in low-fat yoghurt (two days at 10% fat) (Falenski et al., 2011).

As a result, the maturation period, acidic pH and the potential production of antagonistic molecules by lactic acid bacteria are major factors influencing *Brucella* survival in dairy products (Jansen et al., 2019). However, the bacteria can survive in alkaline (maximum pH 8.4) and acidic (minimum pH 4.1-4.5) environments. The fact that this hazard can spread quickly and be transmitted to humans adds to its gravity. People infected with *Brucella* typically experience symptoms resembling severe influenza and may present themselves with undulant fever. Although undulant fever does not frequently kill its victims, it is too serious to be taken lightly.

4.4.2 Exposure Assessment

Exposure assessment predicts the probability of an individual is to be exposed to a microbial hazard and the number of people likely to be exposed. To estimate the probability of exposure, and numbers exposed were simulated for four scenarios:

i. Fermentation of milk without boiling milk from an infected cow
ii. Fermentation of boiled milk from an infected cow
iii. Fermentation of milk without boiling from seronegative cow
iv. Fermentation of milk boiled from seronegative cow.

The Monte Carlo simulation was run for 5000 iterations using the software Model Risk (Vose Software, an add-on to Excel). The probability distribution of input and output parameters are shown in Tables 1-4. In the present study, the average milk consumed per person per day was simulated using the uniform input function 0.77 L (90%CI 0.5-1.0 L) of milk consumption.
A graphical image of the simulated volumes for this consumption is found in Figure 4.5.

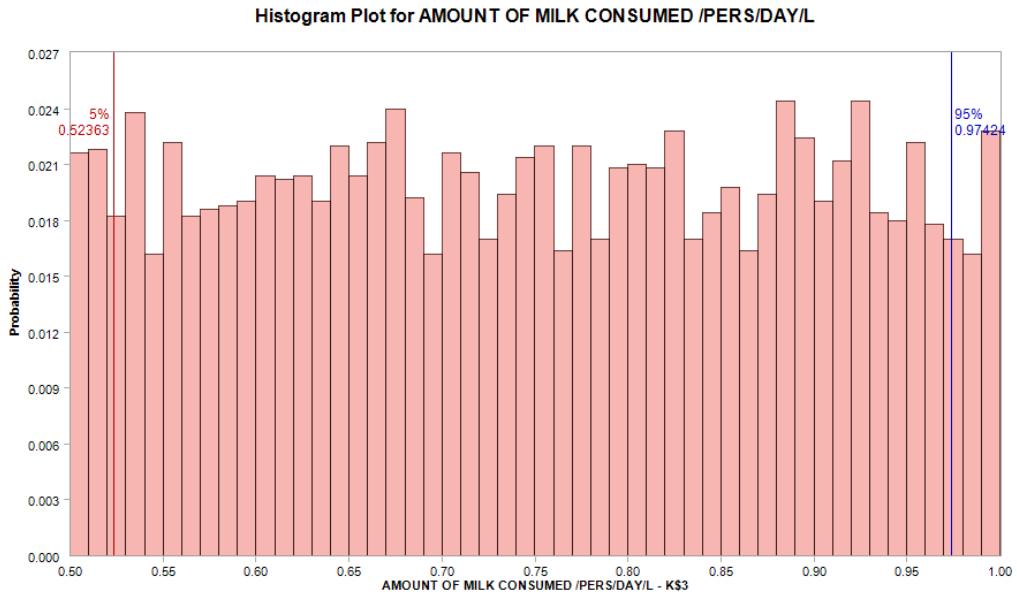

Figure 4.5*: Simulated amount of milk consumed /pers/day/ based on a uniform distribution (MIN 0.5, MAX 1.0).*

- **Simulated probability of exposure**

The probability of being exposed from farm to consumption was calculated according to the four scenarios:

i. The first scenario simulated probability to be exposed from farm to consumption was 0.0191 (90% CI 0.0059-0.022), as shown in **Figure 4.6**.

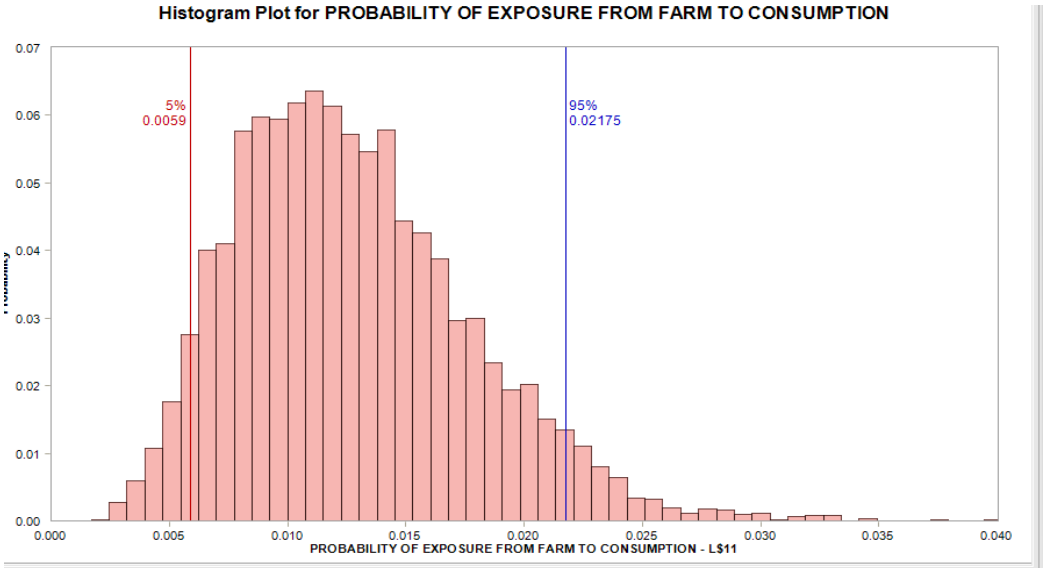

Figure 4.6*: The probability of exposure from farm to consumption under scenario 1*

ii. The simulated probability of exposure from farm to consumption under the second scenario was 0.018 (90% CI 0.013-0.037), as shown in **Figure 4.7.**

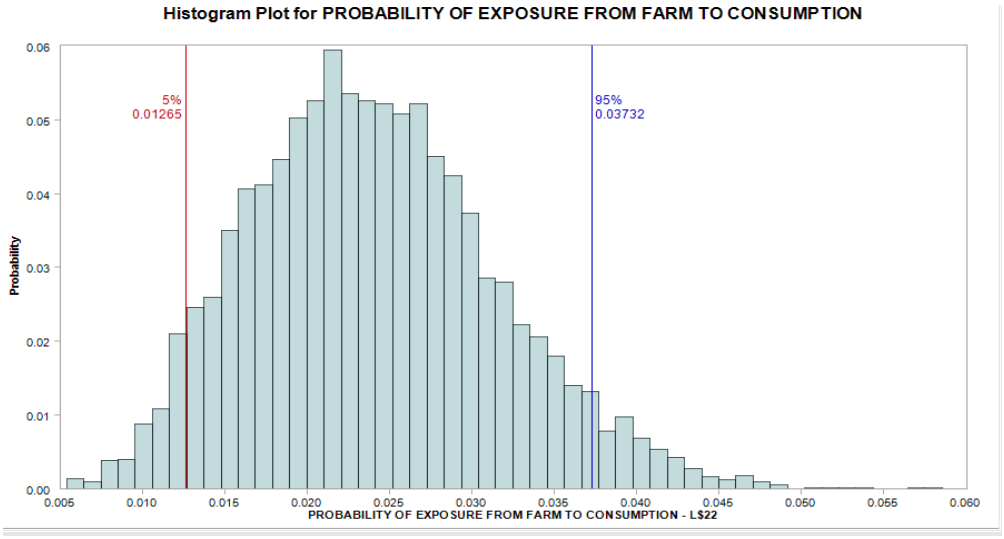

Figure 4.7: *The simulated probability of exposure from farm to consumption under the second scenario*

iii. The probability of exposure from farm to consumption under the third scenario was 0.012 (90% CI 0.0014-0.015), as shown in Figure 8.

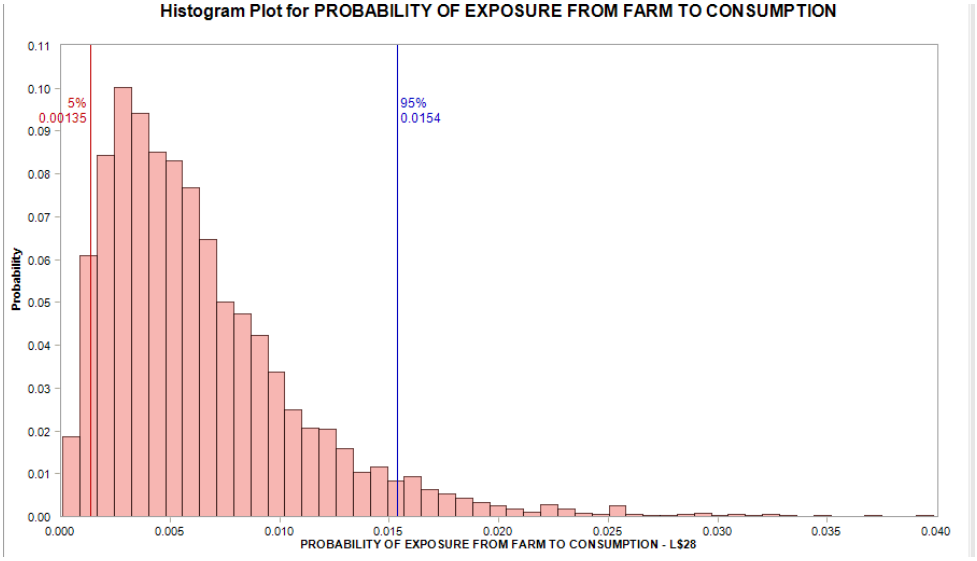

Figure 4.8*: The simulated probability of exposure from far to consumption under the third scenario.*

iv. The simulated probability of exposure from farm to consumption under the fourth scenario was 0.023 (90% CI 0.003-0.027), as shown in Figure 4.9.

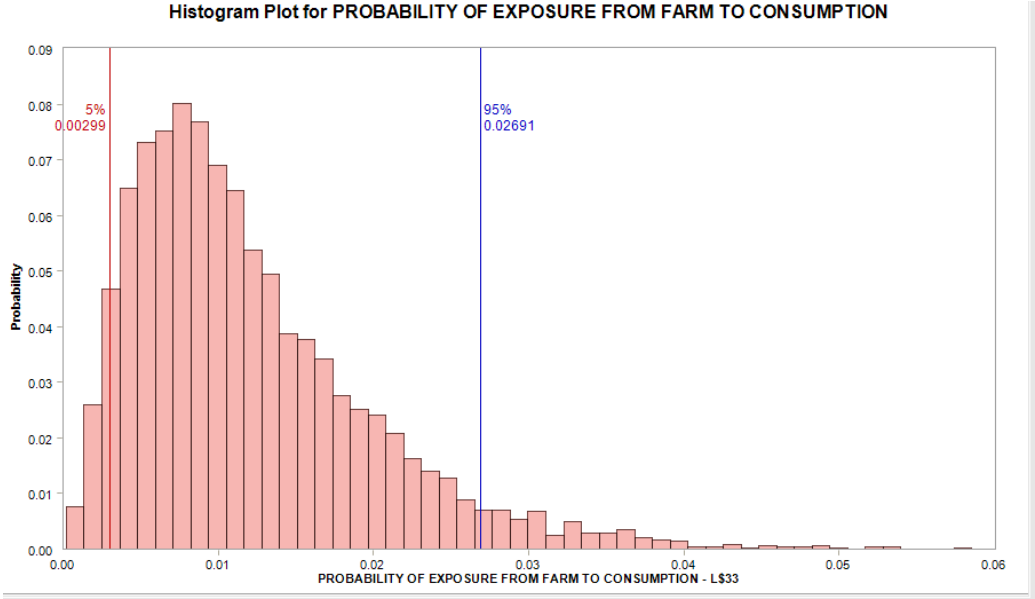

***Figure 4.9**: The probability of exposure from farm to consumption under the fourth scenario*

- **The number of people exposed to *Brucella* from a population of 1000 under the four scenarios.**

The number of people exposed to *Brucella* following the four scenarios was simulated, assuming the number of people under each scenario to be 1000. The simulations were implemented using the binomial function in Model risk with n=1000 and p from the previously reported simulation probabilities. The simulated expected number of people exposed to *Brucella* under scenario one was 17 (90% CI 4-24), as shown in Figure 4.10.

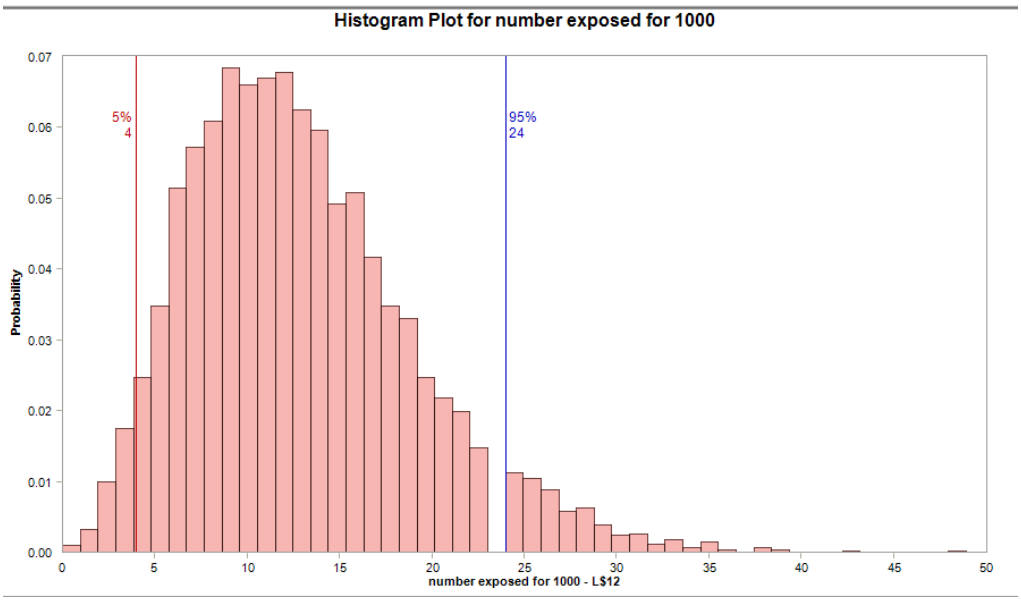

Figure 4.10. *The simulated number of people exposed under scenario one*

The simulated expected number of people exposed to *Brucella* under scenario two was 21 (90% CI: 11-39), as shown in **Figure 4.11**.

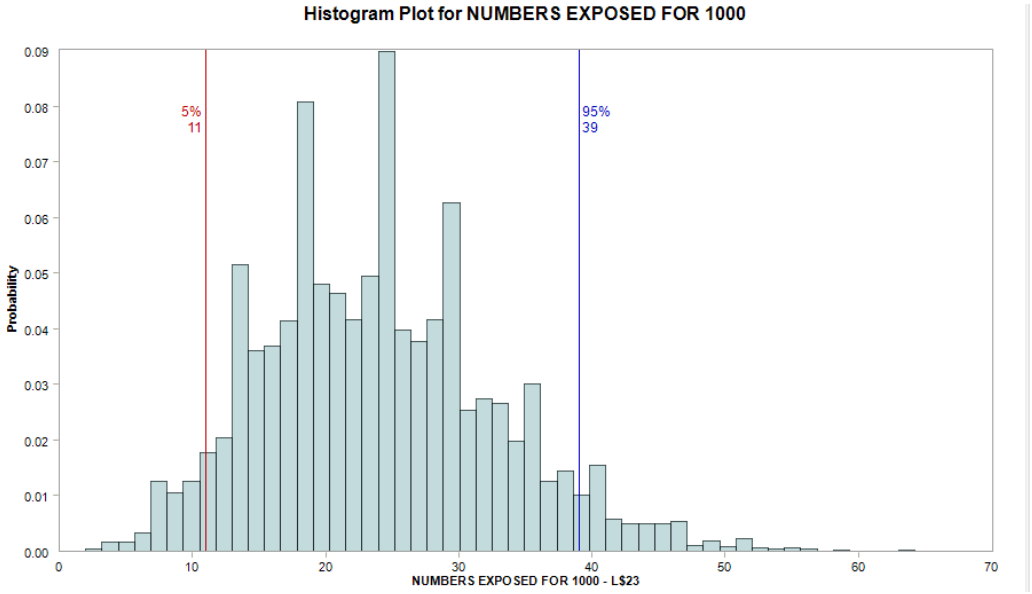

Figure 4.11. *The simulated number of people exposed under scenario two.*

The simulated expected number of people exposed to *Brucella* under scenario three was 15 (90% CI 1-16), as shown in **Figure 4.12.**

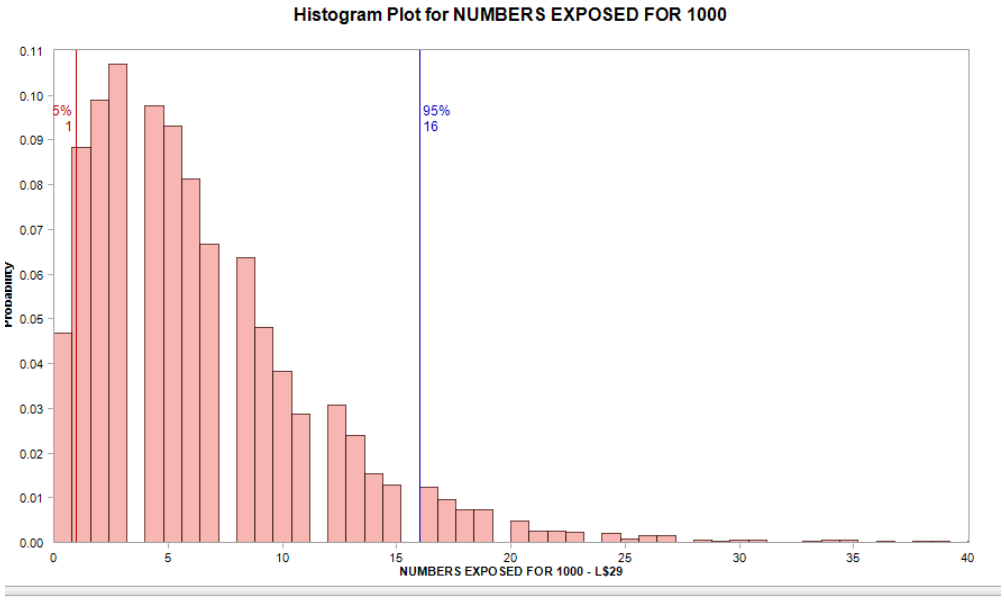

Figure 4.12*. The simulated number of people exposed under scenario three.*

The simulated expected number of people exposed to *Brucella* under scenario four was 24 (90% CI 2-29), as shown in Figure 4.13

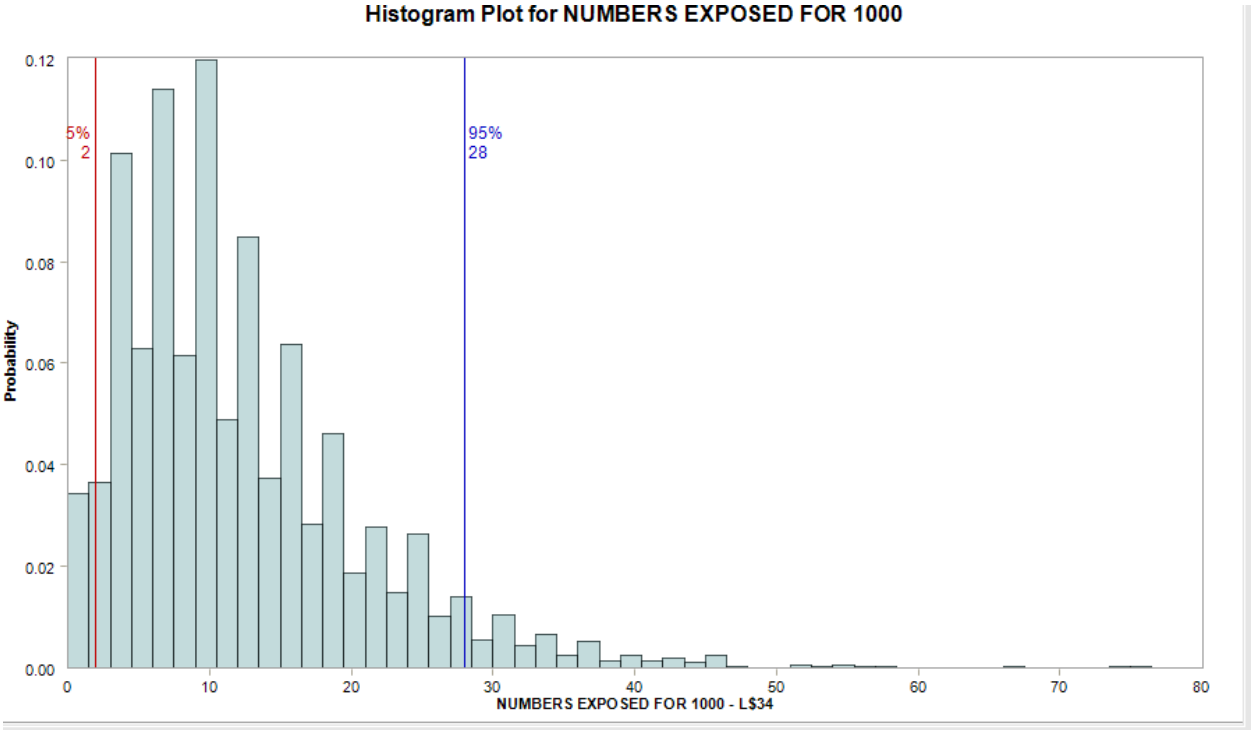

Figure 4.13*: Number of people likely to be estimated (fourth scenario)*

CHAPTER FIVE: DISCUSSION

Brucellosis is endemic in humans in Zambia. This study aimed to investigate the consumption patterns of fermented milk (*Mabisi*) among residents of the three districts of Sothern Province in Zambia and the associated risk of exposure to *Brucella*.

5.1 Demographic Characteristics of the Respondents

According to the findings of this study, 73.1% of respondents were male, and 26.9% were female, which is similar to the observations made in Tanzania, where respondents were 76.2% male and 23.2% female (Ndaki et al., 2022). The similarity could be because, in African culture, in some tribes, women are not expected to speak first in matters of public discussion before men, which may have influenced the number of male respondents observed in this study (Ndaki et al., 2022).

Contrary findings were reported in Nairobi, where the study found that more than half of the respondents (67.3%) were female and 32.7% were male (Muriungi,2016); similar findings as in Nairobi were also reported in southern Ethiopia, where men are involved in the breeding and milking processes while women are responsible for domestic care (Abebe et al., 2013). These findings could be because**,** in Kenya, several households keep dairy animals in their backyards under zero grazing (Nguhiu-Mwangi et al.,2013), often taken care of by women. This is opposed to the practice in our study areas, where animals are taken to communal grazing land (Muma et al., 2006).

5.2 Consumption Pattern of Sour Milk in the Southern Province of Zambian

5.2.1 Method of preparing fermented milk

In this study, 13.3% boiled milk before fermentation, 65.3% did not, and others were unsure because they bought already fermented milk at a local market (21%). The latter assumed the milk was not boiled. Therefore, the total number of people who did not boil milk before fermenting was 86.3%. Several authors reported similar findings indicating that households producing traditional fermented milk did not boil raw milk before fermentation (Muriungi et al., 2016; Mwangi et al., 2013; Wayua et al., 2012; Njarui et al., 2011). The respondents believed that boiled milk lost nutrients during boiling and that the taste and flavour were altered after boiling. A study conducted by Olsen et al. (2017) found that a complete elimination of viable Brucella bacteria (*B abortus, B*

suis, and *B melitensis*) within 30 to 60 minutes required temperatures approaching boiling (at 95^0C, whereas lower temperatures required much longer heating times (hours).

In this study, the most common milk fermentation period was one day (40.9%), followed by two days (29.1%), 6-12 hours (0.6%), 12-18 hours (5-6%), and fewer than 6 hours (2.5%). Our findings are similar to a previous study conducted by Moonga (2019), who reported that the most common fermentation time was one day (30%), followed by two days (26%) and three days (20%). However, the fermentation time varied depending on the type of *Mabisi* production method used and the prevailing ambient temperatures, which are influenced by the seasons. For example, during the cold season (June to July), fermentation took an extra 1-2 days, according to 93% of respondents. The climate in Southern Zambia: Average daytime and night-time temperatures in June are (26 ^{0}C and 8,3 ^{0}C) and in July (27.2 ^{0}C and 8.8 ^{0}C). Pathogen survival is affected by temperature and time during the incubation and storage of fermented dairy products (Gadaga et al., 2004).

According to a study conducted in Nairobi (Muriungi, 2016), the fermentation time was 5.1% (less than 6 hours), 9.1% (6-12 hours), 7.3% (12-18 hours), 16.4% (one day), 30.9% (one to two days), and 30.9% (more than two days), but a few producers fermented milk for less than 6 hours, predisposing consumers to potential food-borne-food-borne pathogens in partially fermented milk. Fermentation time influences the pH of milk because the pH study on Brucella survival in fermented milk results in a decrease in colony numbers at pH 4.4 or lower under laboratory conditions. Under normal conditions, this pH can only be achieved after 3-4 days of fermentation; however, consumer habits and the high demand for dairy products mean that fermented milk is consumed within 24-48 hours (Fane, 2003). When the milk comes from a *Brucella*-infected cow, the critical pH may not be reached, and thus the risk of infection to the consumer remains (Fane, 2003).

In our study, 63.8% fermented milk at room temperature, 3.7% fermented milk in the coldest part of the house, 5.6% in the shade, and 5.6% in the sun. Similar findings were found in Nairobi (Muriungi, 2016), where 41.8% of producers fermented milk at ambient room temperatures ranging from 26 to 36 ℃, 38.2% fermented milk in the coldest part of the house (below 25 ℃), and 20.0% kept in warm temperatures (higher than 25℃). In the case of the types of containers used to store the milk products, 65% used plastic containers, 9 % used metal containers, 3% used

a clay pot, 2 % used plant material (wood, calabash) and 21% those who did not ferment but bought the milk already fermented; they used plastic to store the milk, the total was 86% used plastic to store the milk product. In Nairobi, most respondents (71 %) stored traditional fermented milk in containers made of plastic material, 27 % stored fermented milk in materials made from plant materials, and 2% used metallic storage containers (Muriungi, 2016). Several authors have highlighted the risks of using plastic materials to store fermented dairy products, including health risks such as the rapid build-up of food-borne pathogens and cleaning difficulties (Swai & Schoonman, 2011; Omore et al., 2005).

5.2.2 Consumption of fermented milk

Most respondents (52.9%) drank 0.5 to 1 litre of sour milk per person daily, while 47.1% drank less than 0.5 litres. The average milk consumption per day per person in the three districts was predicted to be 0.765 L (90% 0.523 - 0.974) following the simulation of the input parameters in a Monte Carlo simulation using the ModelRisk® platform.

According to Rutamu (2008), the average milk consumption in Rwanda was 0.035 l per person per day (13 litres per person per year); the difference was that the annual milk production was insufficient to meet the population's needs and requirements. However, Southern province is one the largest cattle-producing areas in Zambia, and generally, milk consumption tends to be higher than in other areas of Zambia (Muma et al., 2013). In this study, most people (60.7%) drank sour milk 2-3 times per day, 35.6% (1-2 times) per day per person, and 3.7% consumed more than seven times per week as long as milk was available. Fermented (*Mabisi*) milk is a popular product in the Southern province. The product is either drunk directly or consumed with other food preparation such as maize meal (nshima), the staple food. Other mixed *Mabisi* with rice, for making porridge or for breakfast.

5.3 Exposure Assessment

Our study considered four scenarios to estimate the probability of Brucella exposure in fermented milk. We assumed that the milk met the boiling point required, similar to pasteurisation. We also assumed that pasteurised cheese is like fermented milk because fermented milk is used to make pasteurised milk. After simulation, the model predicted the first scenario:

i. Fermentation of milk from an infected cow without boiling, the probability of exposure from farm to consumption was 0.0191 (90% CI 0.059-0.022), and the number of people to be exposed was 17 (90% CI 4-24).
ii. Fermentation of milk boiled from an infected cow, the probability of exposure from farm to consumption was 0.018 (90%CI 0.013-0.037), and the number of people to be exposed was 21 (90% CI 11-39).
iii. Fermentation of milk without boiling from seronegative cow, the probability of exposure from farm to consumption was 0.012 (90%CI 0.0014-0.015), the number of people to be exposed was 15 (90% 1-16)
iv. Fermentation of milk boiled from seronegative cow, the probability of exposure from farm to consumption was 0.023 (90%CI 0.003-0.027), the number of people to be exposed was 24 (90%CI 2-29)

In our study, cultured milk prepared from either raw or boiled milk obtained from an infected cow or seronegative was not free of Brucellosis, thus posing a potential health risk. We did not go further in modelling the numbers of people infected, as the uncertainty of the dose-response curve for *Brucella* was so large. If the infectious dose of *Brucella* organisms in which half of the exposed population will get ill (ID50) was 94-1885(Teske et al., 2011), the number of microorganisms to be ingested/per person/day in contaminated milk using Monte Carlo simulation model would be extreme (>108). In that case, this would be a very high level of exposure, and everyone exposed will be infected in practice even though a clinical infection remains uncertain or polymorphic.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The consumption of cultured milk in the three districts was high and frequent. Cultured milk was mainly prepared by using raw milk without boiling it. From the observation reported, the fourth probability (cultured milk prepared from boiled milk obtained from a seronegative cow) posed a high risk of exposure (0.023). The average number of people likely to be exposed under the fourth probability was 24 out of 1000. *Mabisi*, made from fermented boiled/pasteurised milk, was likely to be infected with *Brucella* because the pasteurisation methods were inadequate for destroying this pathogen. To eliminate *Brucella* within 30 to 60 minutes required temperatures approaching boiling (at 95°C).

Cultured milk or fermented milk from infected animals cannot be considered brucella-free. During fermentation, the pH decreases due to the acidity of lactic acid or organism. The current practice of milk fermentation to make *Mabisi* does not guarantee the complete elimination of Brucella since Brucella decreases colony numbers at a pH of 4.4 or lower. This pH can only be reached under normal conditions after 3-4 days of cultivation, which is not normally practised. The critical pH may not be reached; therefore, the risk of infection to the consumer still exists when milk comes from a *Brucella*-infected cow (Fane, 2003).

6.2 Recommendation

The implication of this study in brucellosis control: The risk of human exposure through consumption of cultured milk could be greatly reduced by routine testing animals at the farm against brucellosis; detecting contaminated milk and its removal from the food chain and lastly, boiling milk before fermentation and maintaining hygienic practices. Specifically, the following recommendations are made to farmers, milk collection centres, consumers, the government, decision-makers, and researchers:

- Farmers
    - Farmers must be aware that their products may pose a risk to consumers, a reason for livestock technicians to monitor herds; the primary infectious diseases of livestock must be prevented, and the rules of livestock hygiene and milking must be followed.

- Milk collection centres.
    - Because they receive milk from various sources for sale, hygiene rules, the cooling chain, and testing for major zoonotic diseases such as Brucellosis.
- Consumers from endemic areas
    - Should be aware that they can be infected by drinking fermented milk. It would be best to use milk tested and approved negative to Brucella, to boil before fermenting it and maintain hygienic practices.
    - The government should be involved in the following:
        - Raising awareness of zoonoses transmissible through consuming foodstuffs, particularly milk and its derivatives such as Brucellosis.
        - Training and sensitisation of the actors of the dairy sector. A permanent control of the quality of dairy products.
- The researchers
    - Studies should be carried out on the contamination levels and cross-contamination of milk and naturally fermented milk and the dose-response of *Brucella.*

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APPENDIX 1: INFORMED CONSENT FORM

Request: My name is……, and I am a student at the University of Zambia. I am requesting your participation in the study.

What is the project title: Risk of Human Exposure to Brucella Pathogen through consumption of cultured milk in the Southern Province of Zambia?

Brief description of the study

You may be aware that many diseases in humans are caused by germs that are directly or indirectly transmitted from animals to humans through handling infected animals or animal products.

For this study, we are doing research on Brucellosis, a bacterial disease that affects cattle and can be transmitted to humans through the handling of infected animals and drinking untreated milk.

This study aims to estimate the risk of human exposure to the Brucella pathogen through the consumption of cultured milk in the Southern Province of Zambia. The study will also determine the population's consumption patterns of cultured milk.

The information gained will help in designing intervention and control measures against Brucellosis.

Who is running the study: The study is conducted by a team of doctors from the University of Zambia.

Do I have to participate: It is not a must for you to participate in the study. You are free to make a voluntary decision about participation or not. There is no penalty for refusing to participate. In case you agree to participate, you are free to skip questions you may deem personal or otherwise and to withdraw from the study at any time without penalty.

What will happen to me if I participate in the study: If you agree to participate in the study, we will ask you a few questions about where you live, your educational background, the number of people living in your household, if your household produces and/or consume fermented milk, etc.....…

Are there any risks if I participate in the study: Asking questions will take some of your time.

Are there any benefits from the study: There is no direct benefit to participation in the study, but it will lead to a better understanding of the level of exposure to Brucellosis when consuming fermented milk. The findings of this study will help design intervention and control measures against Brucellosis.

Will there be any compensation for being in the study: There is no compensation to volunteers for your participation.

How long does the study last: This study requires only the completion of a short questionnaire. There is no follow-up or further information needed. The questionnaire will take about 30 minutes.

Who will be able to see my information: Any information about you and your household will be kept very confidential. Only the people directly involved in the study will be able to see your information. Your name will not be used in any report resulting from this study. Any report from this study will refer to you only by a study identification number and not by a name.

Who can I contact about the study or my rights as a volunteer in this research study: If, during the course of this study, you have questions concerning the nature of the research, you should contact:

Lysa Thendji Muila

Master of Science student in Food Safety and Risk Analysis

Department of Disease Control

School of Veterinary Medicine

University of Zambia

What if you have questions about your rights as a research participant: All research on human volunteers is reviewed by ERES Converge IRB, a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research participant, you may contact:

The Chairperson

ERES Converge IRB

Joseph Mwilwa Road, Lusaka, Lusaka, 10100

Landline Telephone: 0211-230-581

IF THERE IS ANY PORTION OF THIS CONSENT AGREEMENT THAT YOU DO NOT UNDERSTAND, PLEASE TALK TO SOMEONE FROM THE STUDY TEAM BEFORE SIGNING.

APPENDIX 2: INFORMED CONSENT FORM

Kulomba: Ndime Lysa …….Kuzwa ku Chikolo Chipati Ku University of Zambia (UNZA) mwalombwa kutola lubazu muchibela eechi.

Muntwe wamakani: (Risk of Human Exposure to Brucella pathogen through consumption of cultured milk in Southern Province of Zambia).

Kulipandulula:

Inga kamuzyi makani abulwazi bwabantu buzwa kutuuka tuzwa kubanyama kuya kubantu kwiinda mukujata banyama bajisis bulwazi na mukupa, na nyama, bulanganizingwa muchibela eeci.

Mu cibeela eci tulanganya bulwazi bwa kusowa na (Brucellosis) bulwazi bwa Ngo'mbe bukonzya kusika kubantukwiinda mukujata banyama bajisis bulwazi obu kwiinda mukulya na kunywa mukupa kuzwa kubanyama bajisis bulwazi oobu.

Muzezo Mupati: Muzezo mupati ngwakulanga bubi bulikubantu baswangana a kazunda kakonzya kuleta bulwazi obu kuzwa ku ngombe zyinjisi kazunda aka, kwiinda mukulya na kujata banyama bajisi bulwazi oobu. Echi cibela chiya kulanganya kulya kwamukupa utajikidwe abantu bakumusanza a Zambia. Mulumbe uyakujanwa kwiinda muchibela echi uyakubeleshegwa kukulanga kuyandilila kwakazunda ka brucellosis muchibaka echi.

Nguni ulangana chibela echii: Oku kuhungulula kulalanganizingwa abamayi abasikichikolo kuzwa ku (UNZA).

Heena Ndelede Kutola lubazu: Muntu uliwonse ulijisis nguzu zyakusala na ulayanda kutola lubazu kunyina mulandu na mwalimvwa kuti tamuyandi kutola lubazu kubuvuntaunzi obu. Kwina

mulandu mukutatola lubazu mubuvuntauzi obu, mulijisis nguzu zyakusiya mubuzyo ngomutalimasimpe kubwinguzyi. Na mwayanda kuchileka kwingula muchibela echi inga mwaleka kunyina mulandu pe.

Heena Nchinzi Chinga Chachitika na Ndatola lubazu: Na mwalisungula kutola lubazu mubuvuntauzi obu tulamubuzya mibuzyo**,** mbuli nkomukkala, lwiiyo lwanu, bantu mbomulela, na mukwashi wanu ulalya mabisi.

Heena kulibubi buliko na Ndatola lubazu: Kunyina bubi buliko kwiinda mukutola lubazu

Heena mbubotu nzi bwakutola lubazu: Kunyina bubotu buliko pesi tuliwano akubamba nseba zyabantu amusashi wenu alimwi akulanga bulwazi bwa brucellosis mbobuyandilinde muchibaka chanu kwiinda mukulya mabisi. Zyonse zituyojana ziyobikwa mubulembo kutengwa nseba zyesu zibe kabotu.

Heena kuyakuba kulumbulwa kwinda mukutola lubazu: Kunyina kulumbulwa kuliko kwinda mukutola lubazu mubuvuntauzi oobu pele tulanganya buyo nseba nzyabantu muchibela cheenu.

Heena chitola ciindi chilamfu buti: Chibela ecchi chilli chilamfu pele chiyanda buy kumvila mibizyo illembendwe kwama Tanzania tuli marumi rotate na (30 min).

Heena Nguni uukonzya kubona bwinguzi bwangu: Kufumbwa bwinguzyi mbumwapa kunyina ukonzya kububona kamulimvwa kwanguluka mubwinguzi bweenu, mbuli namba yamukwashi weenu. Pele swebo tulanganya bwinguzyi bwama peepa woonse. Izina lyenu talikalembwi pee.

Heena Nguni ngwenga ndatumina: Na mwayanda kubuzya inga mwatuma kumanamba aya alembedwe.

APPENDIX 3: PARTICIPANT INFORMATION SHEET

Good day, participant!

I appreciate your taking part in the study. My name is Lysa Muila Thendji, and I am a post-graduate student at the University of Zambia's (UNZA) School of Veterinary Medicine. The study that we are now working on is **Risk of Human Exposure to Brucella Pathogen Through Cultured Milk in the Southern Province of Zambia**. This research partially satisfies the master's degree requirement in food safety and risk analysis.

A questionnaire will be utilised that will cover knowledge about Brucellosis, milk consumption habits, information on fermented milk production methodology and household food safety

practices. In order to enhance communication and allow the responder the opportunity to express his/her point of view, the questionnaire will be translated into the local language.

To obtain a representative estimate of portion sizes and consumption patterns, the study will be conducted at random by interviewing farmers or vendors who drink and deliver milk for sale to the centres and chosen families.

You have been selected as one of the study's participants. It will take you around 25 minutes to complete this, and I would appreciate it if you would take a moment to share your opinion on the above-mentioned topic. Your involvement in this study will be kept in strict confidence and used solely for research.

Participating in this study is voluntary. You have the right to withdraw your agreement at any moment to take part in this study or to refuse to respond to any question that makes you uncomfortable.

Regarding this study, there is no financial reward or other immediate benefit associated with your participation in this study, but you will get the researcher's gratitude for your involvement.

Please feel free to get in touch with us if you have any questions about this study, contact:

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APPENDIX 4: PARTICIPANT INFORMATION SHEET

Kulumba kupati:

Ndipa kulumba kupati kulibabo boonse bachikonzya kutola lubazu mubuvuntauzi obu. Ndime Lyasa Muila Thendji, sichikolo waku University of Zambia (UNZA) kuchiko chilanganya Nseba zyabanyama na Veterinary medicine. Mubuvuntauzi oobu tulanganya: ***(Bubi bwabantu kwiinda mukazunda ka bulwazi bwa brucellosis kwinda mukulya mabisi cisi nkochili kumusanza) The study that we are now working on is : Risk of Human Exposure to Brucella Pathogen Through Cultured Milk in Southern Province of Zambia.***

Mibizyo eyi iyakubeleshegwa kuluhibo lwabu lwazi bwa brucellosis kwinda mukulya mabisi muminzi. Kukomenzya bwambauzi kulibabo batola lubazu mibuzo iyo pandulula muchikuwa na chingisis (English) kutengwa boonse bwinguzi bukendelane.

Kubweza namba yeledde kutola lubazu mukabunga aka, buvuntaunzi oobu buyobwezengwa kufumbwa kulibabo bayakusalwa kukutola lubazu, basambala mabisi, mumuhika amikwashi iyakusalwa.

Mwasalwa kukutola lubazu mubuvuntaunzi oobu, mibizyo ila toola mawoola ali 25 minutes, mwasalwa kutola lubazu amwingule mibuzyo njomukozya. Inga ndalumba na mwayuungizya kuyeya kwanu kwendelana amutwe wabuvuntauzi oobu. Bwinguzi bwanu buyokwabililwa kunyina uyakubona bwinguzyi bwanu.

Kutola lubazu mubuvuntauzi oobu nkulisungula kunyina kusinikizingwa pee na mwayanda kuchileka inga mwaleka kunyina bubi ninguzu zyenu kamulimwa kwanguluka mulizyoonse.

Amulimvwe kwanguluka mukutola lubazu mubuvuntauzi oobu alimwi akututumina luwaile namwayanda kubuzya.

APPENDIX 5: QUESTIONNAIRE

A. RESPONDENT DEMOGRAPHIC INFORMATION

1.Sex :

□Male □Female

2.Age in years:

3.Marital status :

□Married □Single, □Divorced/ separated □, Widowed

4.Did you go to school? :

□Yes □No • Non-information

5. if yes, in Q4,please indicate your highest level of education that you completed:

□ Kindergarten □Primary school □High school □ Tertiary

□University □Others □Don't know

6. Religion:

□Christian □Muslim □Other Specify

7. Structure of your nuclear family unit in your household:

□How many persons live in the household ? □Write a total number of persons :

□Number of persons aged 5-18 years: □Number of persons below age 5 years:

□Number of people above 18 years:

8. Do you follow specific dietary habits?

9. If yes, in Q.8, please indicate which dietary habit(s):

B. METHODOLOGY OF FERMENTED MILK PRODUCTION / Ask only if fermented/soured milk/cultured milk is produced within the household; if not produced, to question C.

10.which type of raw milk do you use for this production:

□Whole milk □Powdered milk □ Mix of whole milk and powdered milk

□Other □Don't know

11. which animal milk is used for making this fermented milk product?

□Cow milk □Goat milk □Camel milk □ Mix of cow and goat milk

□ Mix of cow and camel milk □ Mix of goat and camel milk

12. Do you boil the milk before starting the fermentation?

□Yes □No □ Don't know

13.How do you initiate the fermentation?

□Addition of a part of a previous fermented milk □Spontaneous, no addition

□Addition of yoghurt from commercial sources □Continuous addition of fresh milk to fermented milk □Addition of mala from commercial source □Addition of commercial starter culture

□ Other □ Don't know

14.For how long do you do the fermentation?

□ Less than 6 hours □ More than half a day (12-18 hours) □ Almost half a day (6-12 hours) □ About 1 day □ One to two days □ More than two days

□ Other □ Don't know

15.At which temperature do you do the fermentation?

• Keep in the coldest part of the house, below 25°C □Ambient temperature, 25-35°C

□Warm, higher than 35°C □Other □Don't know

16. At which temperature do you store the final product? □

□Fridge, below 10°C □Ambient temperature, 25-35°C □Warm, higher than 35°C

□ Other □ Don't know

17.which type of material is the container for the milk storage mode of?

□Plant material (wood, calabash) □ Plastic □Don't know

□ Metal □ Other

18.Describe the milking process

C. Milk consumption (consumption of cultured milk)

18.Do you drink milk in your household?

□Yes □No

19.How much milk do you consume per day

☐ Less than 0.5 litres ☐ 0.5- 1 litre ☐ 1- 2 litres ☐ More 2 litres ☐

20. How often do you consume sour milk in a week

☐ 1-3 times ☐ 4-7 times ☐ More than 7times

21.Where do you store the store milk before consumption

☐In the room temperature ☐ In the refrigerator ☐In deep freezer

☐Others, specify

22.Have you developed a symptom after consuming milk

☐Yes ☐No

24.How often does this symptom occur per year

☐ Once a year ☐ Twice per year ☐3-4 times per year

☐Several times per year

25.What was the diagnosis(if you can remember)

☐Malaria ☐Typhoid ☐ Brucellosis ☐Others (specify)

26.Is there any other members of your family has developed a symptom after consuming milk,

☐ Yes ☐No

if yes,specify the symptom

D. Brucellosis factors

27.Do you know what Brucellosis is

☐ Yes ☐No

28.If yes,what is the source of Brucellosis?

29. What are the symptoms of Brucellosis

30.Have you ever been infected with Brucellosis

31.Is there anyone who has been infected by Brucellosis

E. Household food safety Pratices

32.I wash my hands before and during handling,preparation and consumption

☐Always ☐ Most times ☐Sometimes

☐Not often ☐Never

33.I clean thoroughly with soaps, surfaces and equipment used for milk handling, preparation and drinking before reusing on other food.

☐Always ☐Most times ☐Sometimes ☐Not often

☐Never

34.There are key moments when you need to wash your hands to prevent germs from reaching food. What are these key moments?

□ After going to the toilet/latrine □After cleaning something □ Before preparing /handling food

□ Before feeding a child/eating □ After handling raw food □ After handling garbage □Don't know

35. Remove faeces from home & surroundings (latrine, use cat method, clean up animal faeces

□Yes □ No □ Don’t know

36. Can you please describe how you maintain personal health and hygiene when handling food, i.e. during food preparation and consumption?

□ 1- Keeping short fingernails, taking a daily shower, keeping short hair or gathered into a cap or a scarf.

□ 2- Refraining from preparing/handling food when showing symptoms of diseases such as skin rash, boils and cuts, running nose, eye and ear infections and diarrhoea.

□ 3- Avoids bad habits during preparation/serving food, such as smoking or chewing tobacco, nose picking, coughing and sneezing, spitting over food, tasting food with your fingers

□ 9- Don’t know/no answer