A Quantitative Risk Assessment of Humans Exposure to Brucellosis Through the Consumption of Raw Cow Milk in Arusha, Tanzania

By

Enock Magoke Ndaki

(2019118963)

A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of the degree of Master of Science in Food Safety and Risk Analysis

The University of Zambia

School of Veterinary Medicine

COPYRIGHTS

DECLARATION

I, **Enock Magoke Ndaki**, do hereby declare that the contents of the dissertation being submitted herein are my original work, and has not been previously submitted to any University for the award of a degree or any other qualification.

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

APPROVAL

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

Dr Chisoni Mumba (PhD): …… ……..
Supervisor Signature Date

Examiner: …… ……..
Signature Date

Examiner: …… …….
Signature Date

Examiner: …… ……..
Signature Date

Chairman (Board of Examiners) ……. …….……
Signature Date

DEDICATION

I dedicate this work to my lovely father, the late Daniel Magoke Ndaki, my mother Penina Cosmas Ncheye, my brother Edwin Ndaki, my wife Euphrasia Hugo, our children Daniel and Bryan, my relatives and friends who love me.

ACKNOWLEDGMENT

I acknowledge the African Centre of Infectious Diseases for Humans and Animals (ACEIDHA), School of Veterinary Medicine at the University of Zambia, for the financial support. My principal supervisor Dr Chisoni Mumba and the co-supervisors, Professor John B Muma and Professor Esron Karimuribo, for their efforts in correction, guidance, and urgent response to assignments requested the timely completion of this work. I would also like to appreciate the effort made by Monduli District Veterinary Officer (DVO), Dr Yandu X. Marmo and the Head of Livestock Department Longido District Council, Mr Nestory Dagharo, for accepting and allowing me to conduct my research in their districts. I also appreciate their care throughout the period I was collecting my data. I would also like to thank the officers Makame Zuberi, Elihuruma Mollel, Mamus Toima, and Joel Ngangabale from the Livestock Department Monduli and Longido Districts for their facilitation of the process of data collection.

I also appreciate my employer (Livestock Training Agency-LITA) for the trust in me in which I was granted a study leave to pursue a master's degree at the University of Zambia. I also thank my friends in Tanzania and Zambia, who have assisted me in one way or another in accomplishing my study programme.

ABSTRACT

Brucellosis is a zoonotic disease that exists worldwide and is endemic in most sub-Saharan African countries. World Health Organisation has named Brucellosis among the neglected diseases. The disease causes substantial morbidity among humans and their livestock. Although it has a low mortality rate, the disease causes a heavy burden on public health and animal health.

The study aimed to assess the risk of exposure to various Brucella species through cow milk consumption in the Arusha region, Tanzania. This was a two-tier study; the first study was a cross-sectional descriptive survey involving the collection of primary data related to milk consumption. The second part was a risk modelling study based on the Codex Alimentarius commission framework and utilised data from the consumption survey and secondary data from the literature search. Both questionnaire surveys and data from previous studies were used to collect information for the input variables in the risk model. The obtained data were entered into IBM SPSS version 20, and descriptive statistics on average serving portions and consumption patterns were analysed. Stochastic Monte Carlo simulation in @risk software (version 8.1) platform was used for risk assessment.

Results revealed that 96.5% of the population consumes milk in three (3) serving portions in three serving time i.e., morning, afternoon and night, while 73% of the people in the area consume 500ml-1000ml of milk per day. People in the rural setting (about 71%) reported consuming raw milk, while 10% of people in the town setting reported consuming raw milk.
The results from the model described the probability of getting infected with Brucella through the consumption of raw milk was estimated at 0.64 (95%CI 0.333-0.861). The model also predicted the number of people likely to get infected with Brucella in the Arusha region in a one-year consumption period to be 1,084,358 (95%CI: 565,000-1458,000) out of 1,694,310 people following consumption of raw milk. The risk of exposure was estimated to be high when dairy cows were infected with Brucella at the farm and when the milk portions were consumed raw.

To reduce the risk of human exposure, there is a need to create awareness about Brucellosis in these communities concerning how the disease is transmitted to humans, its associated effects, and the preventive and control measures. Further studies are required to assess the risk of exposure to

Brucella species through other pathways such as soft cheese consumption and contact with tissues of infected cattle. The information in this study will help provide guidance in decision-making among stakeholders and the policymakers on control of Brucellosis.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Ahp:	Alkyl Hydroperoxide Reductase
BER:	Base Excision Repair
BPAT:	Buffered Plate Agglutination Test
BvfA:	Brucella Virulence Factor A
CDC:	Center for Disease Control
CFT:	Complement Fixation Test
CFU:	Colony Forming Unit
CI:	Confidence Interval
CβG:	Cyclic β-1-2-glucans
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme Linked Immunosorbent Assay
FAO:	Food and Agriculture Organization
FPA:	Fluorescence Polarization Assay
FPSR:	False-positive Serological Reactions
ID:	Infectious Dose
LPS:	Lipopolysaccharide
NaCl:	Sodium Chloride
NIMR:	National Institute for Medical Research
NorD:	Nitric Oxide Reductase
OIE :	World Organization for Animal Health
PCR:	Polymerase Chain Reaction
QRA:	Quantitative Risk Assessment
RBPT:	Rose Bengal Precipitation Test
RBT:	Rose Bengal Test
SAT:	Serum Agglutination Test
SPSS:	Statistical Package for Social Sciences
T4SS:	Type IV Secretion System
WHO:	World Health Organization.

DEFINITION OF TERMS

Prepared done milk: This is a milk portion cooked or boiled at a temperature of at least 80-85°C for 5 minutes and above.

Prepared half-done milk: This is milk that is cooked or boiled either at a lower temperature than 80-85°C or at a shorter time than 5 minutes.

Raw milk: Fresh milk from an animal which has not undergone any treatment.

CHAPTER ONE

1. INTRODUCTION

1.1.Background

Brucellosis is sometimes referred to as undulating fever, Malta disease, or Bang disease. Brucellosis is a zoonotic disease which is worldwide distributed and is more or less endemic within most sub-Saharan African countries (Assenga *et al*., 2015; John *et al*., 2010; Ntirandekura *et al.*, 2020). The World Health Organization (WHO) named brucellosis among neglected diseases (Elelu e*t al*., 2019) endemic in most developing countries in Africa and Asia. It affects a large group of pastoral societies and causes a burden on both public health and animal welfare health (Elelu *et al*., 2019). The disease causes substantial morbidity among humans and their livestock. The pastoralist societies, veterinary surgeons, people working in the meat industries, and animal health workers are at high risk; however, in the general view, every person in public is at risk (Carugati et al., 2018; Kunda *et al*., 2007).

The most common clinical presentation or symptoms of brucellosis in humans are not specific, which is a challenge in diagnosis and are easily confused with other fever causing diseases such as malaria, typhoid fever, rheumatic fever, and arthritis (Ntirandekura *et al.*, 2020). The clinical features in humans include fever, fatigue, headache, sweating, joint pain, loss of appetite, muscular pain, lumbar pain, weight loss, hepatomegaly, splenomegaly and arthritis (Kunda *et al*., 2007). Brucella infects humans following consumption of raw milk or infected soft cheese, contact with infected animals, the placenta, or aborted fetus. Brucellosis is poorly diagnosed among humans due to under-recognition by health care providers, lack of appropriate diagnostic laboratories, and difficult access to health care facilities among populations at risk (Carugati *et al.*, 2018; Olsen & Palmer, 2014). In some countries where the diagnostic tools are available, brucellosis is diagnosed following failure to respond to malaria, typhoid or tuberculosis treatments (Kunda *et al*., 2007). Some studies in some East African countries showed that flu-like conditions with similar manifestations commonly occur among various diseases and complicate the diagnostic procedures. For example, in Narok, Kenya,12% of flu-like patients were diagnosed by using Rose Bengal Precipitation Test (RBPT) as Brucellosis patients and 40% as typhoid patients, while in Kampala, Uganda, 73% of patients with joint pain, general malaise or constant headache were found to be suffering from malaria, and 13.3% were suffering from brucellosis (Kunda et al., 2007).

Brucellosis in animals was initially reported in Tanzania in 1927 in the Arusha region. In 2007, some studies estimated the prevalence of bovine brucellosis in Arusha to be 15.2% (Karimuribo *et al.*, 2007).

Twelve (12) *Brucella* species cause the disease in domestic animals, including *Brucella abortus, B. melitensis, B. ovis, B. suis, B. microti, B. ceti, B. inopinata, B. pinnipedialis, B. Neotomae, B. canis, Brucella papionis,* and *Brucella vulpis* (Figueiredo et al., 2015). Among all species, *Brucella ovis* and *B. neotomae* have not been reported to cause any disease in humans, but the rest are known to cause disease in humans (Galińska et al., 2013). If brucellosis is not treated correctly, it develops into a chronic infection that leads to severe health problems resulting in remarkable morbidity and economic loss in endemic areas (Ahmed et al., 2016).

In Tanzania, no study has been conducted to quantitatively assess the risk of exposure to Brucella species in humans through milk consumption. Therefore, this study aimed to quantitatively asses the risk of exposure to *Brucella* species through consumption of raw milk in the Arusha region. It is hoped that the results obtained will contribute to developing effective public health policies.

1.2. Statement of the Problem and Justification

Bovine brucellosis has been reported in all districts of the Arusha region for so many years now (Karimuribo et al., 2007) . In most rural areas in Arusha, there are pastoral societies whose diet entirely depends on milk and meat. In these communities, it is reported that, to a large extent, milk is consumed raw (Njarui *et al*., 2011). This consumption behaviour is likely to risk acquiring various foodborne zoonotic diseases, including brucellosis. Unfortunately, the risk of exposure to Brucella in Tanzania is not known despite its public health significance. In most African countries, brucellosis is overlooked or neglected, leading to the chronicity of the disease in the community. Therefore, this study was conducted to assess the risk of people being exposed to Brucella through the consumption of raw milk.

1.3.Research Questions

a) What is the consumption pattern of raw cow milk among the people of the Arusha region?
b) What is the risk of human exposure to Brucellosis through the consumption of raw cow milk in the Arusha region of Tanzania?

1.4.Study Objectives

1.4.1. General Objective

The study's objective was to quantitatively assess the risk of human exposure to Brucellosis through the consumption of raw cow milk in Arusha-Tanzania.

1.4.2. Specific objectives

a) To assess the consumption patterns of raw milk in the Arusha region of Tanzania.
b) To estimate the risk of human exposure to *Brucella* species through the consumption of raw cow milk in the Arusha region of Tanzania.

1.5. Scope of the study

The study focused on estimating the risk of people being exposed to Brucellosis through the consumption of raw cow milk in the Arusha region. The study did not identify the Brucella species in milk; hence limited to all Brucella species.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Introduction

Brucellosis is a neglected zoonosis of public health and economic significance in most developing countries (El-wahab *et al*., 2020). Clinical signs of Brucellosis are not specific, causing difficulties in its clinical diagnosis (Ducrotoy *et al.*, 2017). Abortion cases are prevalent manifestations of bovine brucellosis, which is also common in other reproduction-related infections such as leptospirosis, listeriosis, Q fever, bovine viral diarrhoea, trichomoniasis, mycotic abortion and neosporosis (Dereje *et al*., 2018). In humans, the disease presents as an acute to chronic illness characterized by intermittent fever, generalized pain, and influenza-like syndrome (Franc *et al*., 2018). Other constitutional symptoms include joint pains, fatigue, and muscle ache that vary with the stage of infection and body system affected (Bodenham *et al.*, 2020; Chota *et al.*, 2016; Muturi *et al*., 2018), loss of appetite, muscular pain, lumbar pain, weight loss, hepatomegaly, splenomegaly and arthritis (Kunda *et al.*, 2007).

Management-related factors such as the source of replacement stock, grazing strategy, breeding system, interaction with wildlife and herd size are significant risk factors for bovine brucellosis (Muma e*t al.*, 2007). Raw milk consumption, unpasteurised dairy products and assisting parturition without protective attire, direct contacts with aborted fetuses and slaughter practices are high-risk practices for this zoonosis (Akakpo *et al.*, 2010; Musallam et al., 2019).

Brucellosis was discovered in the 1850s in Malta, where it infected several British soldiers during the Crimean war (Wyatt, 2013) and now is distributed worldwide, except for a few countries such as Australia, Canada, Cyprus, Denmark, Finland, Netherlands, New Zealand, Norway, Sweden and the United Kingdom that have successfully eradicated the disease (Kiros *et al*., 2016). More than half a million human cases are reported annually globally (De Figueiredo *et al.*, 2015). Brucellosis incurs high costs by impacting agricultural production and human communities (Roy *et al*., 2011)**.** It causes significant direct and indirect economic losses for animal owners worldwide, such as abortion, reduction of milk yields, culling of positive reactors and the expense of disease control (Musallam *et al*., 2019). There are many reasons to believe in the re-emergence of brucellosis in the near future despite eradicating it from some developed countries (El-Sayed et al., 2018). According to El-Sayed *et al*. (2018), some of the reasons for re-emergence are the recent discovery of new atypical *Brucella* species with new genetic properties, the evolution of *Brucella*, urbanization, and changing human behaviour socio-demographics.

2.2. Aetiology

The disease is caused by bacteria of genus *Brucella*, phylum Proteobacteria, class Alphaproteobacteria, order Rhizobiales, and family Brucellaceae, which affects domestic animals, humans, and wildlife animals (Głowacka et al., 2018). There are twelve (12) *Brucella* species and various natural hosts: *Brucella melitensis* (biovar1-3) (Goat and sheep), *Brucella abortus* (biovar 1–6, 7, 9) (cattle), *Brucella suis* biovar (pig and wild pigs), *Brucella ovis* (sheep), *Brucella neotomae* (Desert rat), *Brucella canis* (dogs), *Brucella ceti*, *Brucella pinnipedialis* (Seals), *Brucella microti* (Wild voles), *Brucella inopinata* (Human), *Brucella papionis* (Baboons-*Papio spp.*), and *Brucella vulpis* in Red foxes *(Vulpes vulpes)* (De Massis et al., 2019; Mathew et al., 2015).

Among all *Brucella* species, the pathogenicity of five (5) species has been confirmed to cause infections in humans, namely *Brucella abortus, B. melitensis, B.suis, B. canis* and *B. marina* (Galińska *et al.*, 2013). Among them, *B. melitensis*, *B. abortus* and *B. suis* are the most frequently implicated species (Bodenham *et al*., 2020). *B. melitensis* is the most common pathogenic and virulent cause of human infections (Chota *et al*., 2016; Galińska *et al*., 2013; Kunda *et al.*, 2007; Mburu *et al.*, 2021; Muma *et al*., 2013). It is typically associated with sheep and goats and *B. abortus* with cattle. However, cross-species infections can occur (Bodenham *et al*., 2020; Bouley *et al*., 2012).

2.3. Brucellosis in humans

Brucellosis in humans is also known as Bang's disease (Kaden *et al*., 2018), Maltese fever, undulant fever, or Mediterranean fever, a zoonosis of widely varied clinical presentation caused by small coccobacilli Gram-negative anaerobic bacteria of genus *Brucella* (Galińska *et al.*, 2013). The disease is reported to be among the top ten (10) zoonoses in terms of effects on human health and economic destruction in the world, as well as ranking in the top five (5) globally in livestock losses (Bodenham *et al*., 2020). The main transmissions routes from animals to humans are the result of consuming raw milk or infected soft cheese or contact with infected animals or the placenta or aborted fetus (Akakpo *et al*., 2010; Bouley *et al*., 2012; Muma *et al*., 2013; Muturi *et al*., 2018), while consumption of un-boiled milk is suggested to be the main route (Muma *et al*., 2012), it was significantly ($p=0.004$) associated with seropositivity in Mbarara District (Nguna *et al*., 2019). Although potentially more prevalent in northern Africa, human brucellosis seroprevalence ranges from 3% to 8% in sub-Saharan Africa (Bouley *et al.*, 2012). Human cases are reported in 11 countries in Africa (Algeria, Eritrea, Guinea, Guinea-Bissau, Kenya, Morocco, Mauritania, Niger, Sudan, Tanzania and Tunisia), but the prevalence varies in animals and human beings from one country to another (Nguna *et al*., 2019). Globally, approximately 500,000 humans are infected per year (Figueiredo *et al*., 2015; Muturi *et al*., 2018; Nguna *et al*., 2019).

Brucellosis in humans presents as an acute to chronic illness characterized by fever and other constitutional symptoms such as joint pains, fatigue and muscle ache that vary with the stage of infection and body system affected (Bodenham *et al*., 2020; Chota *et al.*, 2016; Muturi *et al*., 2018), loss of appetite, muscular pain, lumbar pain, weight loss, hepatomegaly, splenomegaly and arthritis (Kunda *et al.*, 2007). If not treated, it can persist for a long time, approximately several months (Kaden *et al*., 2018). The most common clinical presentation or symptoms of brucellosis in humans are not specific (Bodenham *et al.*, 2020), which is a challenge for diagnosis. The symptoms are easily confused with other fever causing diseases such as malaria, typhoid fever, rheumatic fever, and arthritis (Bodenham *et al*., 2020; Chota *et al*., 2016; Ntirandekura *et al*., 2020). In Tanzania, human brucellosis is reported in various places such as Arusha, Manyara, Lake zones, western zone, Tanga and Morogoro, with a seroprevalence ranging from 0.7-20.5% (Assenga *et al.*, 2015; Chota *et al*., 2016). The acute and chronic symptoms of the disease can result in a significant loss of workdays and consequential disparity in the social-economic status of the infected persons and their families (Franc *et al*., 2018). This directly affects the individual livelihood, community social-economic development, and national development.

2.4. Brucellosis in animals

Brucellosis is a collective name given to a group of zoonoses caused by bacteria of the genus *Brucella* which is reported worldwide (Ducrotoy *et al*., 2017; Kunda *et al*., 2007; Mathew *et al.*, 2015). The bacteria infect various animals, including domestic animals, aquatic, wildlife animals and humans, but the seroprevalence is higher in cattle than in other animals (Assenga *et al.*, 2015; Muma *et al.*, 2007). In dairy animals, these bacteria localize in supra-mammary lymph nodes and mammary glands of nearly all infected animals. They are excreted in milk for the entire life of that animal (Hamdy & Amin, 2002). The sources of infection to the livestock animals include aborted materials, vaginal discharges, milk and semen from infected animals, which animals get infected through ingesting contaminated pastures, feedstuffs and water or licking infected placenta fetuses or genitalia of infected female animals soon after abortion or delivery (Assenga *et al*., 2015). The source of infections to the wild animals includes leftovers from the infected livestock, sustained natural infections, contaminated forages for wild ungulates and infected meat, aborted materials and placentas for carnivores (Assenga *et al*., 2015; Sambu *et al*., 2021). Several Brucella species with zoonotic potential have been isolated from wild animals, but *Brucella abortus, Brucella melitensis* and *Brucella suis* were frequently isolated from the wild animals (Sambu *et al*., 2021). Among the wild animals, 24% of buffaloes and 17% of wildebeests in Katavi national park reported being exposed to Brucella (Assenga *et al.*, 2015). Some studies conducted in various national parks in Tanzania estimated

seroprevalence ranging between 10.5-17% (Sambu *et al*., 2021). Most developed countries in Europe have managed to eradicate brucellosis while remaining an endemic problem in Africa, the Mediterranean, the middle east, part of Asia and Latin America (Asakura *et al*., 2018b). In Tanzania, the first outbreak of bovine brucellosis was reported in 1927 in the Arusha region (Assenga *et al*., 2015; Karimuribo *et al*., 2007), and since then, some studies in brucellosis have been conducted in various areas of the country and estimated the prevalence of bovine brucellosis in various areas as follows, Arusha to be 15.2% (Karimuribo *et al*., 2007), 14.3% in the Mikumi–Selous ecosystem, 6.8% in the Katavi–Rukwa ecosystem, 12.0% in Tanga city (Asakura *et al*., 2018b) in which the disease is more reported in pastoral systems and wildlife than in the other farms (Mathew *et al*., 2015; Swai & Schoonman, 2010). The disease in animals is characterized by abortion, infertility in adult animals, and reduced milk yield (Asakura *et al*., 2018a). Generally, the disease has no pathognomonic symptoms, making its diagnosis difficult (Ducrotoy *et al*., 2017). According to the studies conducted in the country, most farmers have little knowledge about brucellosis, the name of the disease, its symptoms/signs the risk factors and how it is transmitted from animal to human (Asakura *et al*., 2018a; Mburu *et al*., 2021). Brucella has been isolated from raw cow milk collected from dairy farms, street vendors, and milk shops (Assenga *et al*., 2015; Wainaina *et al*., 2020; Waiswa *et al*., 2010). Therefore, among others, the consumption of raw milk has been listed as a significant risk factor for brucellosis exposure in human beings (Muturi *et al*., 2018).

2.5. Brucellosis in milk

The quality of raw milk is important for humans and its technological processing on products, but milk may contain pathogenic microorganisms that can seriously affect consumers' health (Hanuš *et al*., 2021). Due to the shedding of the brucellae in milk, Brucella-contaminated raw milk and unpasteurized dairy products are the most important vehicles of human infection and a considerable public health risk even in non-endemic countries (Jansen *et al*., 2020). Most *Brucella* species can survive in fresh milk for up to 5 days at 4 °C and up to 9 days at − 20 °C (Bayramoglu et al., 2019). Studies conducted in Kenya (which is near Arusha) discovered that the prevalence of the pathogen in raw milk at the animal level (considering samples from individual animals) was 2.4% (95% confidence interval (CI) 1.1-4.5) (Wainaina *et al*., 2020). In endemic regions, findings of the current investigation revealed that the overall prevalence of *Brucella* species in dairy products remains high. In Tanzania, the brucellosis prevalence in cow milk was estimated to be around 8% (95% CI 6.5–10.2) (Alonso *et al*., 2016). In countries like Syria, Iraq, Tanzania, and Uganda, brucellosis still spreads as an endemic zoonotic disease without adequate control approaches in dairy animals, making a realistic prevalence estimation difficult. According to studies conducted in

endemic regions, there is a high prevalence of *Brucella* species contamination in raw milk (16.97%) compared with cheese (7.10%) (Dadar *et al.,* 2020).

2.6. Diagnosis in animals and Humans

Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens. Both human and animal brucellosis presents a great variety of clinical manifestations, making it challenging to diagnose clinically (Ducrotoy *et al*., 2017). Therefore, brucellosis diagnosis must be confirmed by laboratory tests (Seleem *et al*., 2010). All diagnostic methods have limitations concerning specificity and sensitivity, especially when testing individual animals (Gwida *et al*., 2010), hence a need to use combined serological tests to obtain more reliable results and to ensure optimal disease diagnosis and control in the absence of a gold standard test (Sanogo *et al*., 2013).

2.6.1 Bacteriological Isolation and Identification

The isolation and identification of *Brucella* offer a definitive diagnosis of brucellosis and may be helpful for epidemiological purposes (Corbel, 2006). The golden standard for diagnosing brucellosis is bone marrow cultures due to the high concentration of *Brucella* in the reticuloendothelial system. In contrast, blood culture is the method of choice for isolation of the causative agent despite some limitations (Kiros *et al*., 2016). The specimens of choice from animals include stomach contents, spleen and lungs from aborted fetuses, placentomes, fetal membranes, vaginal swabs, milk, semen and arthritis or hygroma fluids from adult animals (Corbel, 2006). Blood for culture is the material of choice, but specimens need to be obtained early in the course of the disease (Poester *et al*., 2015). The sensitivity of detection varies from 15% to 70% in acutely infected patients and even lower in chronically infected patients (Seleem *et al*., 2010).

2.6.2. Serological diagnosis

Serological diagnosis is presumptive evidence of infection (Poester *et al.,* 2015). The most often used serological tests include the RBT (or its equivalent, the card test), serum agglutination test (SAT) in a tube, the complement fixation test (CFT), the indirect enzyme-linked-immunoassays (ELISA) and competitive (cELISA) enzyme-linked-immunoassays, the fluorescence polarization assay (FPA) and the lateral flow immune chromatography test. These tests can be used for sero-epidemiological surveys and to monitor the disease once control (and vaccination) is implemented (Ducrotoy *et al*., 2017). Buffered Brucella Antigen Tests (the Rose Bengal Test and the buffered plate agglutination test [BPAT]), ELISA and FPA are considered as appropriate screening tests for the control of brucellosis at the national or local level (OIE, 2018). Traditionally, screening tests are inexpensive, fast and highly sensitive, but most of the time, they

lack specificity (Kaltungo *et al*., 2014). Rose Bengal test (RBT) is the most widely used of the buffered plate agglutination tests (Godfroid & Nielsen, 2010) and is recommended as the first test and, depending upon the titer; a positive result does not need confirmation by others when there is suspicion of brucellosis (Spickler & Rovid., 2018). Confirmation by other tests such as ELISA or CFT due to false-positive reactions has been critical after using RBT. The sensitivity is very high (>99%), but the specificity is disappointingly as low as 68.8% (Kiros *et al.*, 2016). False-positive serological reactions (FPSRs) occur due to lipopolysaccharide (LPS), an immunodominant antigen. The serum agglutination test (SAT) is the best technique used to detect acute infections and is suitable for herd testing rather than for testing individual animals (Kaltungo *et al.*, 2014). False-positive and false-negative results are other shortcomings of SAT (Poester *et al*., 2015).

2.6.3. Molecular methods

The Polymerase Chain Reaction (PCR) can identify Brucella DNA at genus, species, and even biovar levels; this has been extended to improve diagnostic tests. A diversity of methods has been developed (Ducrotoy *et al*., 2017). Applications for PCR methods range from diagnosing the disease to the characterization of field isolates for epidemiological purposes, including taxonomic studies (Poester *et al*., 2015). Molecular characterization techniques are very useful tools for differentiating Brucella spp. especially on phenotypic results of Brucella isolates, but these techniques in Brucella endemic areas need to be explored before they can be applied (Kiros *et al.*, 2016).

2.7.Prevention and control

Human exposure can be reduced by controlling brucellosis in livestock (Corbel, 2006). Test-and-slaughter is the recommended measure in the eradication program in animals. The eradication of brucellosis by the test-and-slaughter method is only recommended and feasible in countries where the individual animal prevalence is less than 2% (Bamaiyi *et al*., 2014). However, problems reported in implementing the test-and-slaughter policy were time, financial constraints, and farmer disillusionment with the compensations (Ducrotoy *et al*., 2017). In regions with a high disease prevalence, the only way to control and eventually eradicate this zoonosis is by vaccination of all susceptible hosts and eliminating infected animals (OIE, 2018). The most commonly used vaccines against bovine brucellosis are *B. abortus* strain 19 and the recently approved strain RB51 (Bouley *et al*., 2012). *Brucella melitensis* strain Rev 1 controls brucellosis in goats and sheep despite being highly infectious in humans, especially when administered in the conjunctival route (Kiros e*t al*., 2016). Although several successful vaccines are being used to immunise animals, no satisfactory vaccine against human brucellosis is available (Seleem *et al*., 2010). The prevalence of brucellosis in animals has a significant effect on the incidence of the disease in humans (Corbel, 2006). In the absence of a human brucellosis vaccine, prevention of human brucellosis depends

on the control of the disease in animals and adherence to the public health measures, especially putting on all personal protective equipment (PPE) when handling animals or animal products. Boiling milk thoroughly before consumption or processing to kill all Brucella bacteria is also cardinal (Corbel, 2006; Dadar et al., 2019; Makita et al., 2012).

2.8. Treatment of brucellosis in animals and humans

In animals, treatment of brucellosis is discouraged because, even when the organisms seem to have disappeared after antibiotics treatment, they might persist in lymph nodes or other tissues and re-emerge. For this reason, the treatment of domestic animals with antibiotics is not usually successful (Kiro *et al*., 2016). In humans, brucellosis is usually treated with dual-combination antimicrobial therapy such as doxycycline, rifampicin, cotrimoxazole and streptomycin that should be administered for several weeks, which the dosage and the duration should be prescribed by a physician (CDC, 2017). Monotherapy is reported to have a high relapse rate (Spickler 2018).

2.9. Risk assessment studies on Brucellosis

Risk analysis has demonstrated its ability to improve food safety decision-making processes and produce improvements in public health (FAO/WHO, 2005). Milk is one of the essential food sources of vital nutrients for human beings, but its composition favours the growth of microorganisms, including pathogenic ones such as Brucella (Berhe *et al*., 2020). Similar studies have reported the risk of getting infected with Brucella at 12.6% (6.8–18.9: 90%CI) in informally marketed milk in Uganda, and the annual incidence rate was estimated to be 5.8 (90% CI: 5.3–6.2) per 10,000 people (Waiswa et al., 2010). In another study in Kenya, exposure to *B. abortus* through milk consumption showed that the risk varies by production system and bulking if the milk is consumed raw (Arimi *et al*., 2005). Apart from milk consumption, exposure to Brucella can be through direct contact with the infected animal(s). Studies have been conducted to assess risk as occupational exposure assessment (Alzuheir *et al*., 2022), where it was reported that the probability of exposure for veterinary healthcare professionals through their daily activities was estimated to be 0.76 in Palestine. This was conducted following the increase of human Brucellosis within two decades.

2.10. CODEX Risk assessment

Codex Alimentarius Commission (CAC) is a standards-setting organization, and having an obligation to protect the health of the consumers from being exposed to food hazards provides a guideline for conducting risk assessment in food (Codex Alimentarius Commission, 1997). Risk assessment is done in four stages, namely hazard identification, exposure assessment, hazard characterization, and risk characterization

(FAO, 1999). Risk assessment can be either qualitative or quantitative; in which quantitative risk assessment provides the use of mathematical models to account for the probability of risk and the magnitude of the consequences, whereas qualitative risk assessment does not (Dufour et al., 2011).

2.11. Hazard identification

Hazard identification identifies, by a thorough review of available information, the pathogen that may be present in particular food and the adverse health effects that can potentially occur due to its presence. This step identifies the harmful agents (Hazards) that are present in food and the adverse health effects they can cause to food consumers (FAO & WHO, 2009). Hazards can be biological agents such as pathogenic bacteria, parasites, viruses, or fungi. However, also, they can be chemical agents such as toxins, poisons, and other chemicals that can harm the health of food consumers. Likewise, these hazards can be physical objects that can cause adverse health effects to the consumers. when taken through food

At this point, hazard identification hence evaluates the known or potential adverse health effects associated with a particular hazard aiming to provide a critical understanding of the hazard in question. For this reason, hazard identification provides a qualitative characteristic of the hazard of concern, whether the hazard has public health concerns and the linkage between the exposure pathways from farm to consumption and the adverse effect produced.

2.12. Hazard characterization

Hazard characterization (dose-response) assesses the relationship between the level of intake (dose) and the nature, severity, and frequency of illness or other adverse health effects (response). This assessment may be qualitative or quantitative, and one cannot discuss dose-response without mentioning the disease triangle, which is the three components, namely, pathogen, host, and environment (Miliotis & Buchanan, 2008).

2.13. Exposure assessment

This step aims to estimate the level of hazard or amount of microorganism consumers are exposed to at consumption by following a chain of events associated with food processing from farm to consumption. Potentially, this could involve multiple routes of entry (e.g., oral, respiratory, dermal) and vehicles (e.g., food, water, person-to-person) (Miliotis & Buchanan, 2008).

This is the qualitative and/or quantitative evaluation of the nature of the adverse health effect associated with hazard(s) that may be present in food by establishing the hazard consumer(host) interaction (FAO, 1999). This interaction between the host and the hazard reading to establish adverse health effects on the consumer is referred to as the dose-response relationship.

2.14. Risk characterization

Risk characterization integrates the previous steps (i.e., hazard identification, hazard characterization, and exposure assessment) to produce a risk estimate, the likelihood of illness or death from exposure to a particular microorganism. This estimates the probability of occurrence and severity of known potential adverse health effects that could affect a given population due to a particular hazard for a given period of consumption (FAO, 1999).

2.15. Research gaps in the literature reviewed

Since milk is a food source of essential nutrients, as was said by Berhe et al. (2020), also it has been observed that there is frequent consumption of milk in the Maasai community, as was reported by previous studies (Gibney & Burstyn, 1980; Gidel *et al*., 1976; Melubo, 2020). It has been reported that raw cow milk is important in Brucellosis transmission to humans (Akakpo *et al*., 2010; Bouley *et al*., 2012; Muma *et al*., 2013; Muturi *et al*., 2018), especially in the areas where the disease is endemic in animals like Tanzania (Karimuribo *et al*., 2007). Currently, there is no information about the risk of people being exposed to Brucellosis in Tanzania through the consumption of raw cow milk. Therefore, this study aimed to quantitatively assess the risk of exposure to Brucella species through the consumption of raw cow milk in Tanzania.

CHAPTER THREE

3.0.METHODOLOGY

3.1.Research Design

This was a two-tier study; the first study was a cross-sectional descriptive survey involving the collection of primary data related to milk consumption. The second part was a risk modelling study conducted based on the Codex Alimentarius Commission risk analysis framework. It utilised data from the consumption survey and secondary data from the literature search. A quantitative risk assessment method on data collection involving the four distinct steps of risk assessment, namely hazard identification, hazard characterization, consumer exposure assessment and risk characterization, were employed. A Quantitative Risk Assessment model (QRA) was developed right from the farm to exposure of humans to Brucella at the point of consumption following the conceptual model pathways described.

Secondary data were obtained from previous studies, peer-reviewed scientific papers, and grey literature based on the key search terms like quantitative risk assessment, brucellosis, and raw milk consumption. There was a gap in milk consumption patterns in the study area. Therefore, a survey was conducted to fill the information gaps using a structured questionnaire on milk consumption patterns (consumption survey).

3.2.The study area for the consumption survey

The study was conducted in two districts (Monduli and Longido) of the Arusha region in Tanzania shown in Figure 3.2. The region was selected due to the high number of cattle in Tanzania, which is estimated at 1,373,839 (Livestock Population Census of 2014). The area is also estimated to have a human population of approximately 1.7 million, according to the population census of 2012 (Levira & Todd, 2017). More than 80% of the land in the Arusha region is occupied by the Maasai community, which is famous for keeping livestock. The Maasai community is also known for maintaining the traditional culture. Their cultural food is mainly animal blood, meat and milk (Melubo, 2020). Also, the area is the hub of tourism in Tanzania because it is surrounded by wildlife habitats such as national parks, game reserves, and conservation areas, which are all potential reservoirs for the disease. The pastoral societies in this region are known for consuming raw milk, despite brucellosis disease being reported in the area for so many years now.

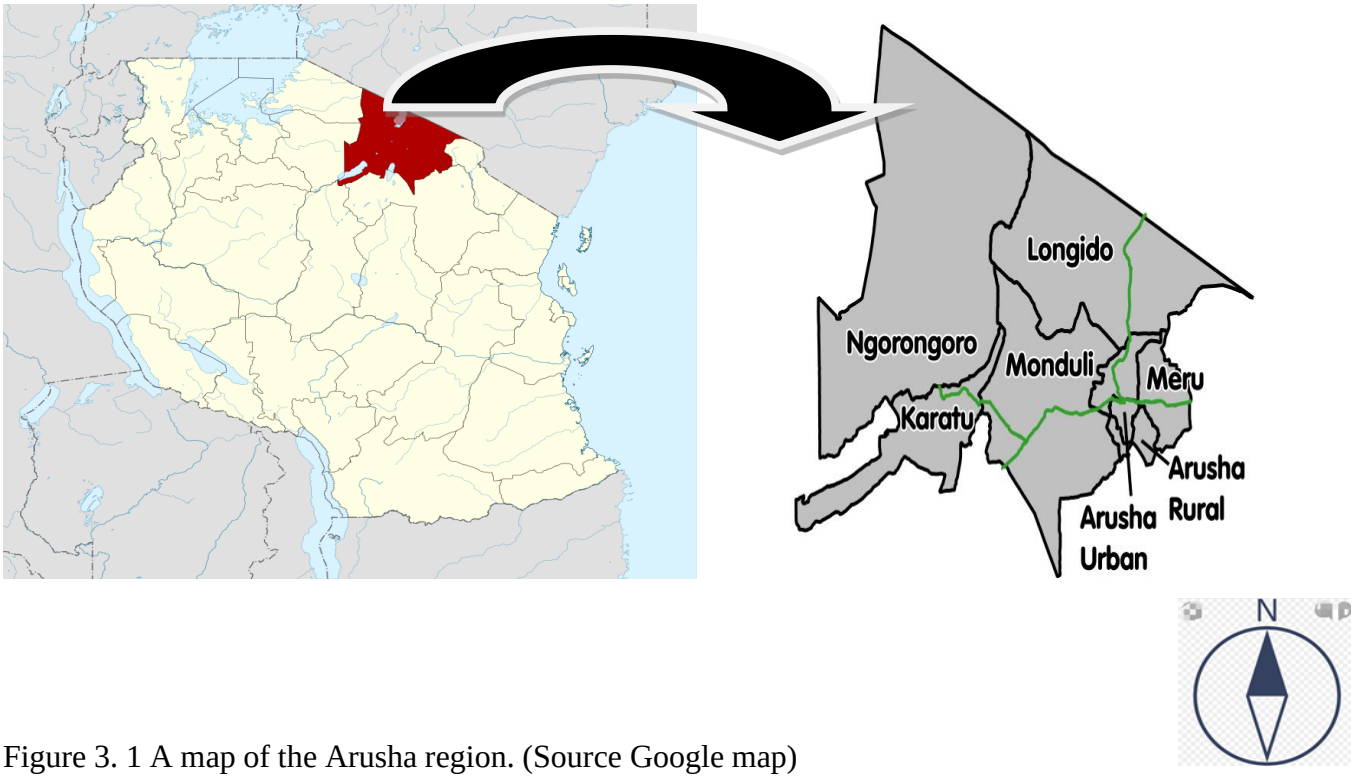


Figure 3. 1 A map of the Arusha region. (Source Google map)

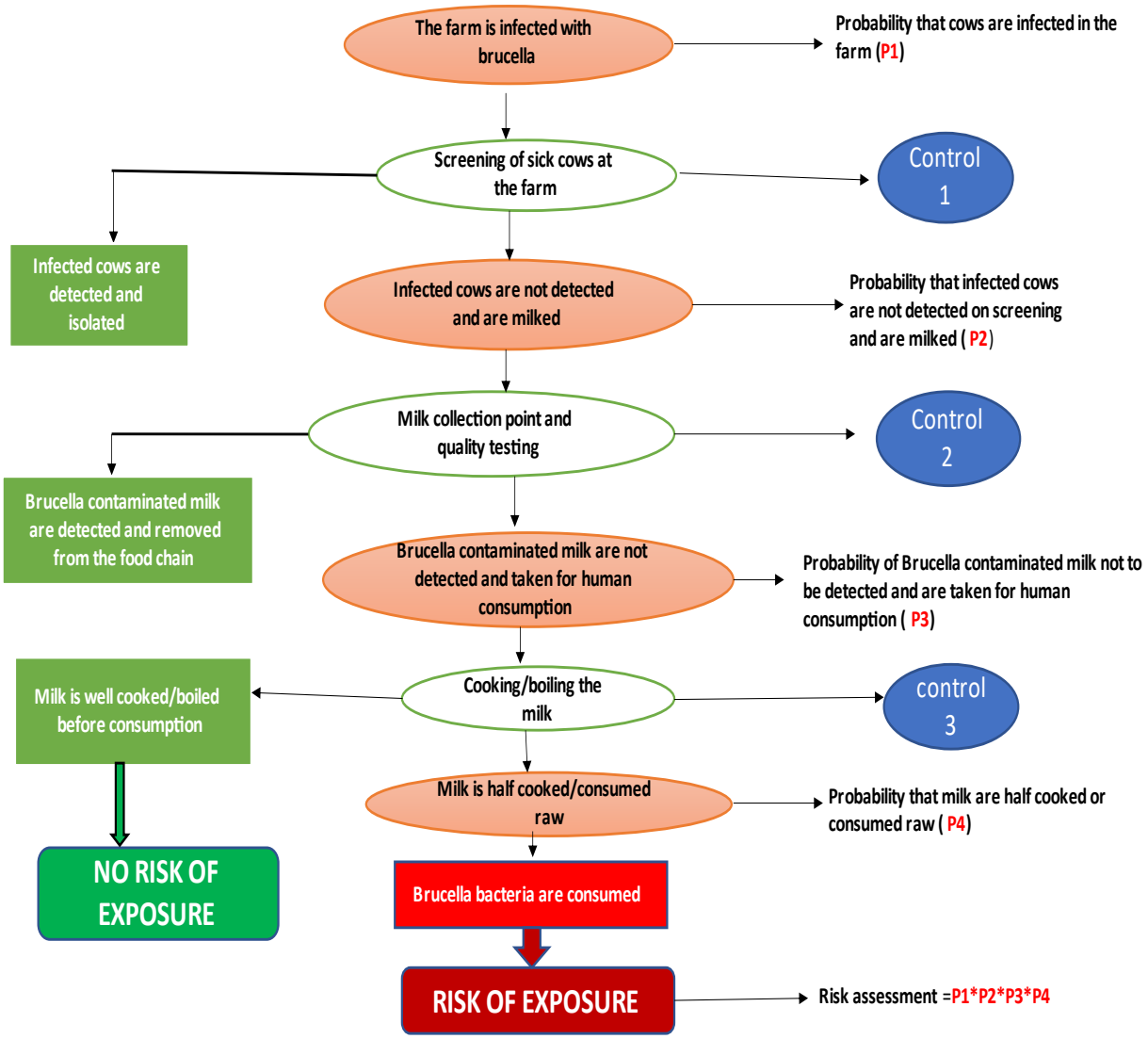


Figure 3. 2 The conceptual model pathways from the farm to consumption

3.3.Instruments for Data Collection

A pretested structured questionnaire was used to collect primary data. Secondary data was collected using a checklist guided by quantitative risk assessment model questions or parameters in line with the Codex Alimentarius Commission food safety risk assessment framework.

3.4. Secondary data:

The literature search was conducted on major electronic databases such as Google Scholar, PubMed, Science direct and research gate. The grey literature, like reports from government institutions and non-governmental organizations, was obtained online using a "Google search engine" using a search of key terms such as Brucella, raw milk consumption, and quantitative risk assessment. The literature related to the study was included, while unrelated ones were not included.

3.5.Primary data

A structured individual questionnaire addressed the knowledge gap about milk consumption patterns. Four hundred (400) samples were conveniently sampled from two districts of Arusha, namely Monduli (176 samples) and Longido (224 samples). The respondents were categorized into two groups, those who stay in rural and urban areas.

The sample size of 400 was based on a statistical calculation by using the formula: -

$$n = Z^2pq / E^2$$

Where: n= Required sample size; Z is Z-score from the Z table; E is the desired level of precision (margin of error); P is the estimated proportion of the population Q=1-p, therefore p=0.5. Assuming that we needed to be 95% confident and at least plus or minus 5% of precision. The Z-score was 1.96 at a 95% confidence level. This gives a sample size of 384; since the study intended to get samples from ten (10) streets (from three different towns) and ten (10) villages in each district, we had to make it 400, with which 100 samples from town and another 100 from villages of each district.

3.6. The quantitative risk assessment process

The quantitative risk assessment was prepared following the guidelines by Miller *et al*. (1993):

a) State the question and the scope of the risk assessment
b) Identify the hazard of interest
c) Develop a scenario tree that outlines the pathway of expected events and all the failures which could occur, culminating in the occurrence of the identified hazard

d) Label the scenario tree and assign units
e) Gather and document evidence (Data collection and management)
f) Assign values to the branches of the scenario tree
g) Perform the calculations to summarize the likelihood of the hazard occurring
h) Consider risk management options

3.7.Risk question

What is the risk of exposure to *Brucella* species for people in the Arusha region through consuming raw cow milk?

3.8.Scope of the risk assessment

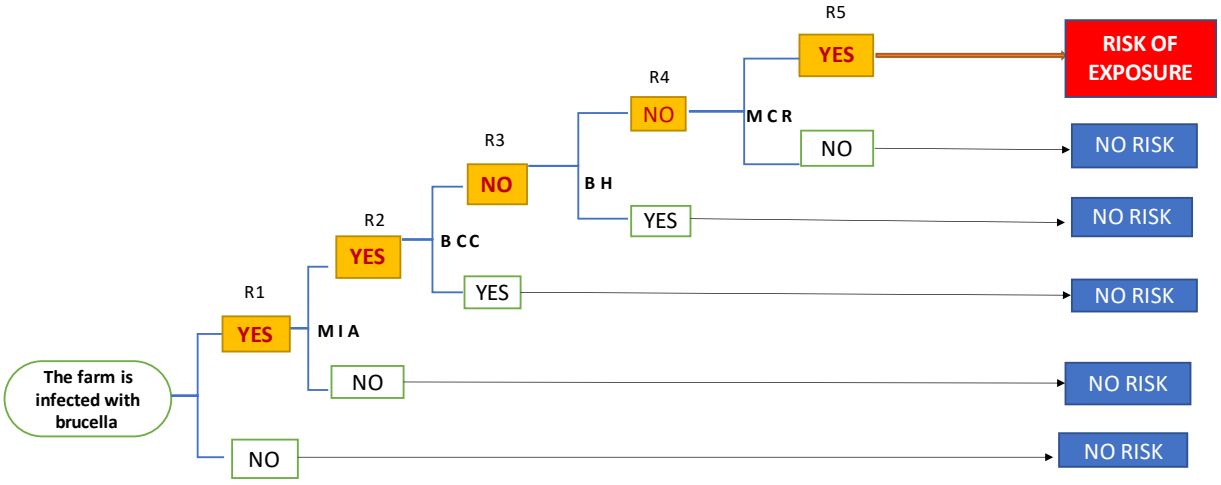
The risk assessment was limited to assessing the risk of people being exposed to Brucella species through the consumption of raw cow milk in Arusha.

3.9.Scenario trees

3.10. Risk assessment model

Risk assessment is a systematic and science-based process which estimates the likelihood and severity of a particular outcome given a well-defined scenario (FAO/WHO, 2005). There are several ways of conducting food safety risk analysis that depends on the type of risk itself, the questions to be answered, and the model used (FAO/WHO, 2005). It is a scientifically based process consisting of the following steps: i) hazard identification; ii) hazard characterization; iii) exposure assessment; and iv) risk characterization (FAO, 2006).

The risk assessment model was described using information that could answer the risk question, "What is the risk of exposure to brucella for people in the Arusha region through consumption of raw cow milk?" A total of four probabilities were considered through which milk consumers could be exposed to Brucella species in the milk food chain. These are the probability that the cows are infected at the farm, the probability that infected cows are not detected on screening and are milked, and the probability that Brucella contaminated milk is not detected on quality control testing at collection points and are taken for human consumption and the probability that milk is consumed half-cooked or consumed raw. The probability of exposure to Brucella was obtained as a product of all of the four probabilities mentioned above. Figure 3.4 shows scenario pathways.



KEY:
MIA-Milk from infected animal(s)

BCC-Boiling at collection center(s).

BH-Boiling at the households

MCR-Milk consumed raw

Figure 3.3 The scenario/risk pathways for exposure assessment to Brucella through milk consumption.

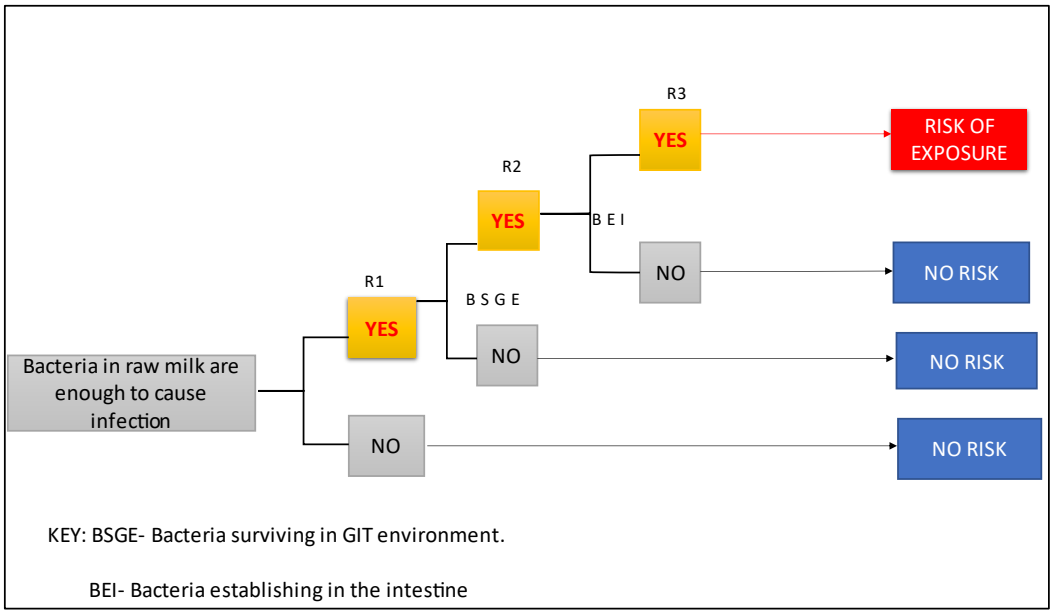


Figure 3. 3 The scenario/risk pathways for Brucella hazard characterization

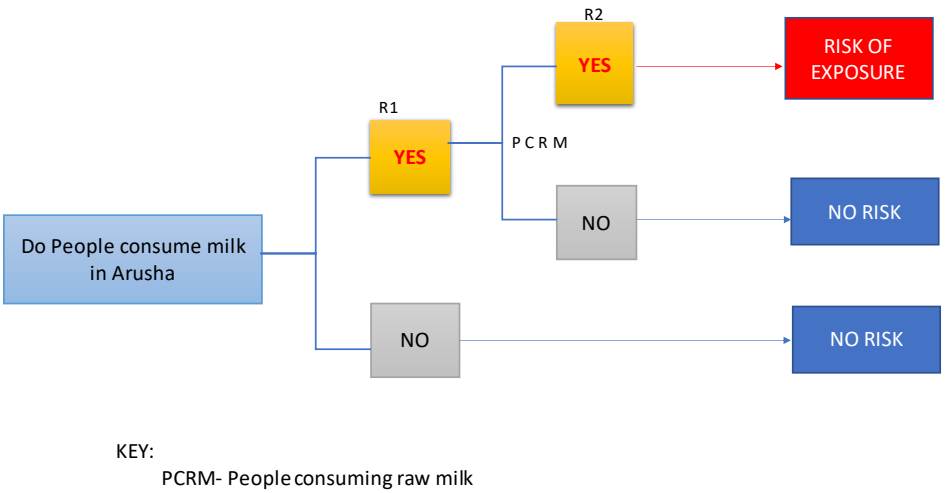


Figure 3. 4 The scenario/risk pathways for risk characterization.

3.11. Data management

Data were entered into *@risk*® software embedded into an Excel® spreadsheet containing four (4) attributes, the parameter number, Input parameter description, source of the information and their probability/proportion required. The obtained information was entered into a specific row and column with their minimum, most likely, and maximum probability values or proportions with a respective probability distribution for continuous and discrete data. Risk Pert and Risk Uniform were the only function distribution used for continuous variables, while Risk Poisson, Risk Binomial and Risk Uniform were used for discrete variables. Information from the questionnaire surveys was entered into *IBM SPSS® Statistics Version 21*, where they were coded for descriptive statistical analysis. The secondary data were used to formulate a model parameter input description and risk assessment model.

3.12. Data analysis

The survey data collected were analyzed using IBM SPSS® version 21, where descriptive statistics on average serving portions and consumption patterns were analyzed. Also, the stochastic Monte Carlo simulation with the aid of @risk software version 8.1 (Version), an add on in Microsoft Excel, was used for exposure assessment, hazard characterization and risk characterization.

3.13. Input Parameters for the Exposure assessment

These calculations involved inputs associated with the cow milk chain from farm to consumption. The input parameters used for this assessment were the amount of milk consumed per day per person, the amount (portions) of milk consumed in the study area in one year, the probability that the cows are infected at the farm, the probability that the infected cows are not detected on screening and are milked, probability of Brucella contaminated milk not detected during quality testing at the collection point and are taken for human consumption and the probability that milk is consumed raw or half-cooked.

3.14. Input Parameters for Hazard characterization

This involved establishing the dose-response relationship in the milk portion, considering the number of microorganisms consumed in various milk preparations with their respective effects. For this assessment, the input parameters used were the following: - colony-forming unit (cfu) per gram of the contaminated product, cfu surviving when milk is prepared done, cfu surviving when milk is prepared half done, cfu surviving when milk is prepared raw. Others were the probability of getting an infection from consuming prepared done milk, probability of getting an infection from consuming prepared half-done milk, probability of getting an infection from consuming prepared raw milk and the infectious dose at which half of the exposed population gets infected (infectious dose 50) for Brucella species.

3.15. Input Parameters for Risk characterization

Monte Carlo simulation was conducted to model the risk of exposure to Brucella species through milk consumption. In this case, the number of people exposed to Brucella per year and the number infected per year through milk consumption in the Arusha region were estimated. In this study, risk characterization employed the following input parameters; the total population of the Arusha region, a population which consumes milk in Arusha, the proportion of a portion prepared raw, the proportion of a portion prepared half done, the proportion of a portion prepared done, probability of exposure along the milk food chain. Other inputs are the number of people who can get infected from consuming prepared half-done milk, the number of people who can get infected from consuming prepared raw milk, and the number of people infected who are likely to get ill in a given population Table 3.1.

Table 3. 1 Probability distribution of input parameters.

Parameter	Input parameter descriptions	Source of information	Probability distribution	Level of confidence
n1	Population size	Tanzania Bureau of Standard	RiskNormal (1694310)	1694310
n2	Milk consumed per day/person(L)	Survey	RiskPert (0.5;1;2)	0,619-1,652
n3	Amount (portions) of milk consumed in the study area in one year	Survey	RiskUniform (865792410;1298680000)	1080000000
p1	Probability that the cows are infected at the farm	(Chota et al., 2016; Id et al., 2021; Karimuribo et al., 2007; Li et al., 2021; Mathew et al., 2015; Ukita et al., 2021)	RiskPert (0.65;0.588;0.152)	0.4803
p2	Probability that the infected cows are not detected on screening and are milked	(Bricker et al., 2003; Fosgate et al., 2002)	RiskPert (0.28;0.11;0.068)	0.1313
p3	Probability of Brucella contaminated milk not detected during quality testing at collection point and are taken for human consumption	(Dadar et al., 2020; Waiswa et al., 2010)	Riskuniform (0.127;0.169)	0.14785
p4	Probability that milk is consumed raw or half-cooked	(Kai & Aotearoa, 2009; Kouamé-Sina et al., 2012)	Riskpert (0.516;0.42;0.349)	0.424
n4	Number of colonies forming unity (cfu) per gram contaminated product	(Hamdy & Amin, 2002; Kaden et al., 2018)	RiskPoisson (100;100000)	50050
n7	Number of colons forming unity surviving when milk is prepared done	(M. Corbel, 2006; Dadar et al., 2019; Davies & Casey, 1973; DUMUȚA-CODRE et al., 2010; Makita et al., 2012)	RiskPoisson (0)	0
n8	Number of colons forming unity surviving when milk is prepared half done	(Celebi et al., 2013; Méndez-González et al., 2011)	RiskUniform (0.35;0.5)	0.4
n9	Number of colons forming unity surviving when milk is prepared raw	(Makita et al., 2012; Saber Marouf et al., 2021)	RiskPoisson (1)	1
p5	Probability of getting infection from consuming prepared done milk	Product of n7*p10	RiskBinomial (0;0.6)	0
p6	Probability of getting infection from consuming prepared half-done milk	Product of p9*n8	RiskBinomial (1;0.4)	0.4
p7	Probability of getting infection from consuming raw milk	Product of p8*n9	RiskBinomial (0.64;1)	0.64
n10	The infectious dose at which the half of the exposed population gets infected (infectious dose 50)	(Teske et al., 2011)	RiskUniform (94;1885)	989.5
p8	Proportional of a portion of raw milk	Survey, (Njarui et al., 2011; Prakashbabu et al., 2020; Rock et al., 2016)	RiskPert (0.1;0.71;0.9)	0.64
p9	Proportional of a portion prepared half done	Survey	RiskUniform (0;2)	1
p10	Proportional of a portion prepared done	Survey (Pappas et al., 2006; Rock et al., 2016)	RiskPert (0.27;0.68;0.9)	0.64833
n12	Number of people who can get infected from consuming prepared half-done milk	(Mangen Otte et al., 2002; Pappas et al., 2006)	RiskUniform (34000;1459000)	677724
n13	Number of people who can get infected from consuming raw milk	(Ngasala et al., 2015; Njarui et al., 2011; Rock et al., 2016)	RiskUniform (565000;1458000)	1084358
n14	Number of people infected who are likely to get ill in a given population.	(Spink, 1954)	RiskUniform (96404;269147)	189762.7

3.16. Ethical Considerations

Ethical approval was sought from The Tanzania National Institute for Medical Research (NIMR), reference number Q0003006. In each district, permission to conduct data collection was issued by the District Executive Director through the head of departments responsible for livestock development. A verbal informed consent was sought before administering a questionnaire to the respondents. A respondent did not put his/her name on a questionnaire, and through a consent form, respondents were given a chance not to respond to a question(s) that there are not willing to answer.

CHAPTER FOUR.

4.0. RESULTS

4.1. Demographic information

There were 305 males and 95 females in the study, representing 76.3% and 23.8%, respectively. The majority of respondents had secondary (44.8%) and primary (27%) levels of education. About 7.3% had never attended any level of education, but they could understand and respond to the questions in the Kiswahili language. The results are presented in Table 4.1

Table 4. 1 The demographic information of respondents to the questionnaire survey (n=400).

Characteristics		Frequency	Percentage
Gender	Male	305	76.2
	Female	95	23.8
Level of education	None	29	7.25
	Primary	108	27
	secondary	179	44.75
	Tertiary	83	20.75
	Others	1	0.25
Number of people living in a household	1-2	54	13.5
	3-12	339	84.75
	13-18	7	1.75
Meals prepared with milk per day	1-2	13	3.25
	3-4	387	96.75
Source of Milk	Own cattle	270	67.5
	Neighbour`s	33	8.3
	Local market	95	23.8
	others	2	0.4
Amount of milk consumed in a day/person	<0.5	24	6
	0.5-1	291	72.8
	1-2	68	17
	>2	17	4.2

Characteristics		Frequency	Percentage
Gender	Male	305	76.2
	Female	95	23.8
Level of education	None	29	7.25
	Primary	108	27
	secondary	179	44.75
	Tertiary	83	20.75
	Others	1	0.25
Number of people living in a household	1-2	54	13.5
	3-12	339	84.75
	13-18	7	1.75
Meals prepared with milk per day	1-2	13	3.25
	3-4	387	96.75
Source of Milk	Own cattle	270	67.5
	Neighbor`s	33	8.3
	Local market	95	23.8
	others	2	0.4
Amount of milk consumed in a day/person	<0.5	24	6
	0.5-1	291	72.8
	1-2	68	17
	>2	17	4.2

4.2.Milk consumption information

4.2.1. Number of milk meal servings per day

The majority (96.5%) of the respondents had 3-4 serving portions of milk meals per day, including tea, lunch and dinner. About 3.3% of the respondents had 1-2 meals made of milk for lunch and dinner per day, as shown in Table 4.1.

4.2.2. Methods of preparing milk meals

About 90% of respondents in urban areas consumed their milk well cooked (fully done), and 10% consumed raw milk in their households. Among the rural respondents, 27% consumed their milk meals well cooked, while 71 % consumed them raw. About 2% of the respondents partially cooked their milk before consumption. This was also called half-cooked, which was interpreted as just warming milk, as shown in Table 4.3.

Table 4. 2 Milk preparation methods in a rural and urban setup.

		MILK PREPARATION (%)		
		Raw	Half-done	Done
Area of residence	Town	10	0	90
	Village	71	2	27

4.2.3. Source of milk

Table 4.1 also shows that most (67.5%) of the respondents got their milk from their cattle, while 23.8% got their milk from the local markets around their areas. About 8.3% of all respondents got or purchased their milk from their neighbours, while 0.5% of all respondents got their milk from other sources apart from the mentioned ones.

From the results obtained from the administered questionnaire, most people, 72%, drank 0.5-1L of milk in a day, whereas 17% of all respondents took 1-2L in a day. Those who drank milk less than 0.5L were 6% of all respondents. A group of people who took milk greater than 2L was 6% of all respondents (Table 4.1).

4.2.4. Size of milk portion per day

The majority (72.8%) of the households consumed 500ml to 1000ml per person per day, followed by 17% who consumed between 1000-2000ml per person per day, 6% consumed less than 500ml, and 4.2% consumed more than 2000ml per person per day.

4.3. Risk assessment

This section presents the risk assessment results following the four steps guided by the Food safety risk assessment Codex Alimentarius Framework. It begins with the definition of risk assessment, followed by hazard identification and ends with risk characterization.

4.3.1. Hazard Identification

Brucellosis is caused by bacteria of the genus *Brucella*, phylum Proteobacteria, class.

Alphaproteobacteria, order Rhizobiales, family Brucellaceae. They are small (0.5 to 0.7 by 0.6 to 1.5 µm), Gram-negative bacteria, nonencapsulated, nonmotile, facultatively intracellular coccobacilli (Mathew *et al*., 2015). These microorganisms have several virulence factors such as Lipopolysaccharide, Type IV secretion system (T4SS), Superoxide dismutase and catalase, Cyclic β-1-2-glucans (CβG), Urease, Cytochrome oxidase, Alkyl hydroperoxide reductase (AhpC, AhpD), Nitric oxide reductase (NorD), Brucella virulence factor A (BvfA), Base excision repair (BER) (Głowacka *et al.*, 2018). Brucella cells can survive for a prolonged time in aborted fetuses, dairy products, water, dust, soil, meat, and dung (Kaden *et al*., 2018). There are almost ten (10) species of Brucellosis, and among them, *B. melitensis* is the most common pathogenic and virulent cause of human infections (Galińska *et al*., 2013; Mburu *et al*., 2021; Muma *et al*., 2013) In humans, the disease presents as an acute to chronic illness that is characterized by intermittent fever, generalized pain and influenza-like syndrome (Franc *et al*., 2018). Other symptoms include joint pains, fatigue and muscle ache that vary with the stage of infection and body system affected (Bodenham *et al.*, 2020; Chota *et al.*, 2016), loss of appetite, muscular pain, lumbar pain, weight loss, hepatomegaly, splenomegaly and arthritis (Kunda *et al.*, 2007). The disease is reported worldwide in almost 500 000 cases are reported in a year (Muturi *et al*., 2018). Additionally, in both human and animal hosts, Brucella may be encountered within bones, joints, and male reproductive organs while in the placenta and fetus of females (Ahmed *et al*., 2016). They are intracellular parasites within the host organism and show environmental persistence outside the host. However, the pathogenicity of these bacteria depends on their ability to multiply and survive within macrophages (Głowacka *et al*., 2018). The foods that can be contaminated with Brucella are meat and milk. Humans are infected mainly through Brucella contaminated unpasteurised dairy products, assisting parturition without protective attire and direct contact with aborted fetuses from the infected animals (Akakpo *et al.*,2010). Brucella species can contaminate milk in two ways, direct from an infected animal or the environment during or after milking/production (Dadar *et al*., 2020).

Brucella species can grow at temperatures between 6-42°C, pH of 4.5-8.8, and to a maximum NaCl content of 4% when other conditions are almost optimum and can survive in frozen storage conditions. However, pasteurization of milk at 72°C for 15 seconds is sufficient to inactivate Brucella species (Kaonga, 2006).

4.3.2. Hazard Characterization

Hazard characterization is a description of 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) (Tesson *et al*., 2020). This step provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from the ingestion of a microorganism or its toxin in food (FAO/WHO, 2005). The lowest infectious dose for most Brucella species ranges from 10- to 100 microorganisms (De Figueiredo *et al*., 2015; Grützke et al., 2021; Kaden *et al*., 2018). The dose at which 50% of the population is exposed shows clinical signs as infectious dose 50 (ID50). This infectious dose (ID 50) on Brucella has been estimated to be the minimum dose is 94cfu and the maximum dose considered to be 1885 cfu (Teske et al., 2011), basing the dose on *Brucella melitensis*.

4.3.3. Consumer exposure assessment

This section gives the route source of exposure to the bacteria of the genus Brucella in individuals who consume milk. The pathway considered is from farm to consumption at the household level. This includes the results of the household survey questionnaire survey on serving portions, preparation methods and consumption patterns.

4.3.4. Case definition.

The population of people in the Arusha region, which is used in this model, was estimated to be 1 694,310 according to the national census of 2012 (Tanzania National Bureau of Statistics 2012). The study considered Arusha because it is the region with a large number of cattle in the country and the presence of Maasai people whose food entirely depends on milk and meat. The pathogen of interest is a bacterium of the genus Brucella, while the food is milk, and a portion was considered to be 500ml because it is the minimum amount consumed by the majority of people. For a person to get ill, he has to consume raw or partially cooked milk, which is contaminated with at least the minimum dose of the pathogen (Brucella). The consumption period considered in this study is one year (365 days), and then assessment of the number of people who will get ill during this consumption period of one year.

4.3.5. Serving portions and consumption pattern

Milk consumption input parameters were entered into a Monte Carlo simulation model in @ risk. The defined outputs were simulated at 1000 iterations and predicted the average milk consumption per person per day in Arusha to be 1.08L (95%CI 0.62-1.68), as shown in Fig. 4.1.

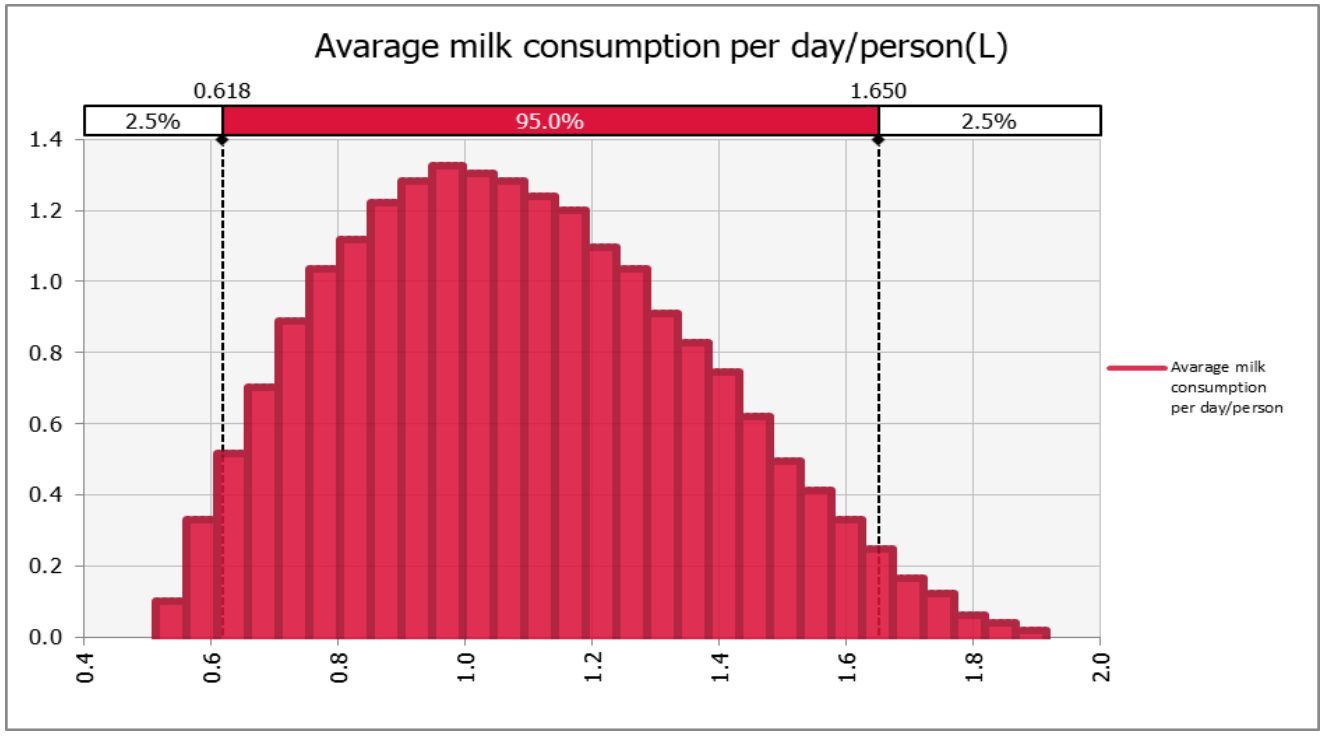


Figure 4. 1: Average milk consumption(L) per day per person

4.3.6. Kitchen preparation

In this study kitchen preparation method meant how milk was prepared before consumption in the kitchen or households, i.e., whether raw, half-done or well done. Section 4.4 provides results of kitchen preparation of milk. The Monte Carlo simulation in @risk after simulation of kitchen preparation data at 5000 iterations predicted the estimation of the average proportion of milk portions prepared done to be 0.648 (95%CI 0.411-0.846), as shown in **Figure 4.2**. At the same time, the model predicted the average proportion of raw milk to be 0.64 (95%CI 0.333-0.861), as is shown in **Figure 4.3**.

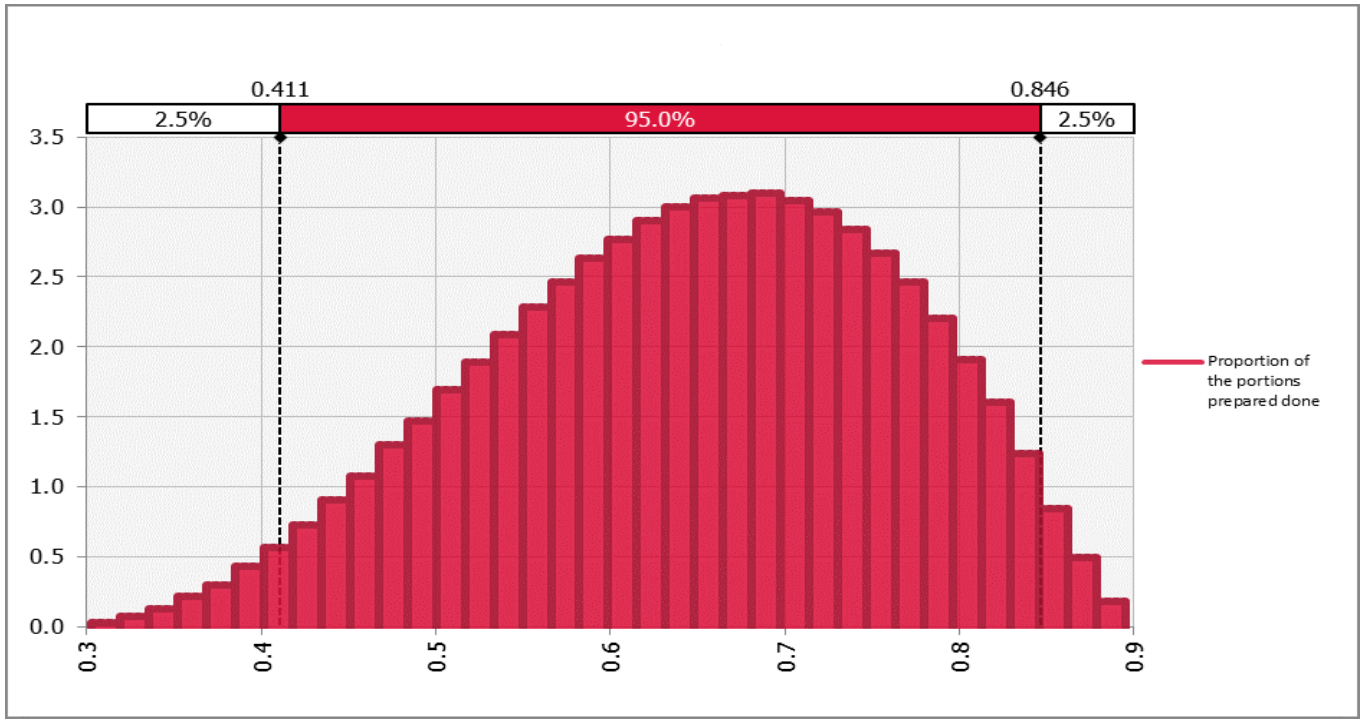


Figure 4. 2: Proportion distribution of the portion of milk prepared done

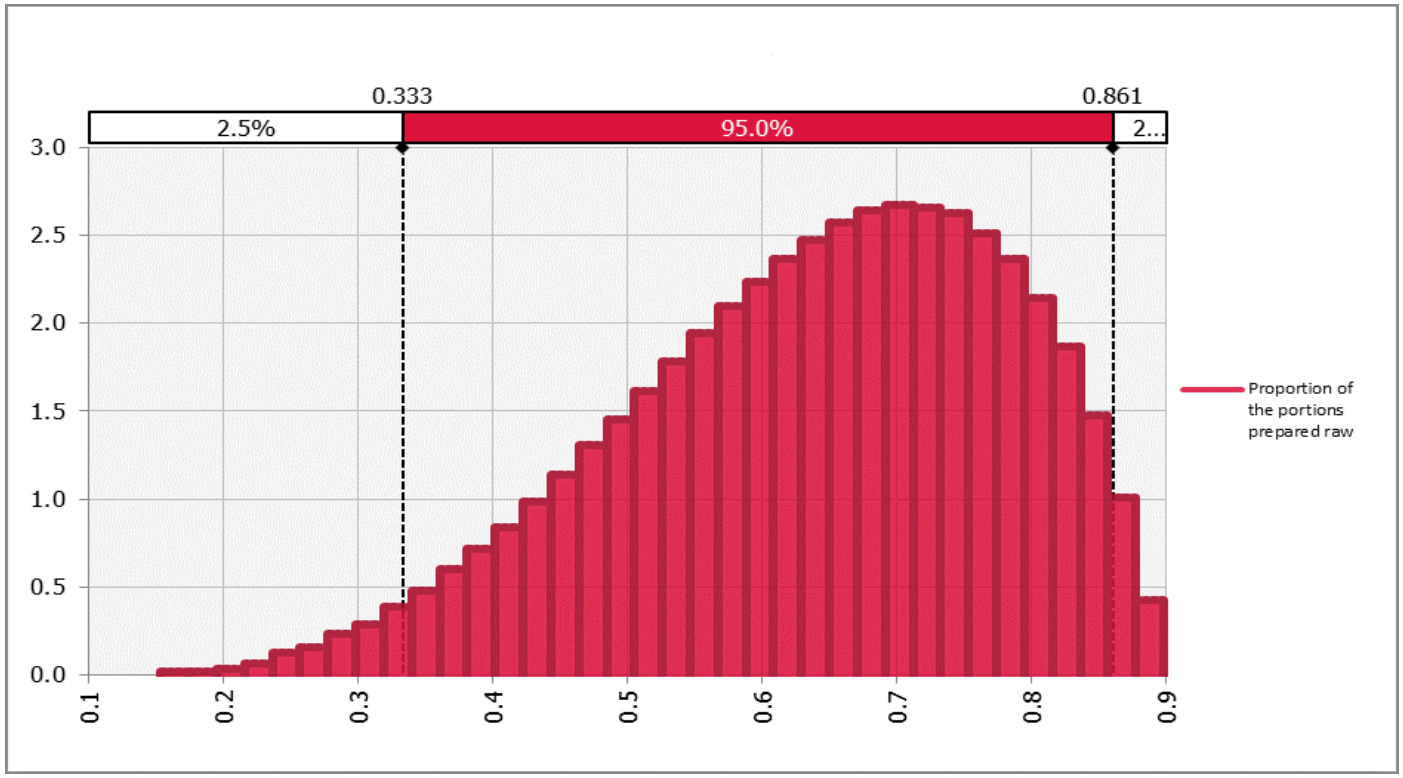


Figure 4. 3: The proportion distribution of cattle raw milk

The conceptual model pathways of bacteria species from farm to consumption (**Figure. 3.1**) show several probabilities that can lead people to be exposed to Brucella species and their likely control points. The input parameters for the four probabilities are shown in **Table 3.1.** The parameters were entered into the Monte Carlo simulation model in @risk, and their outputs were defined accordingly and simulated at 100,000 iterations to predict the probability of each output. The results show that the probability that cows are infected at the farm is 0.48 (95% CI 0.29-0.62) shown in **Figure 4.12**.

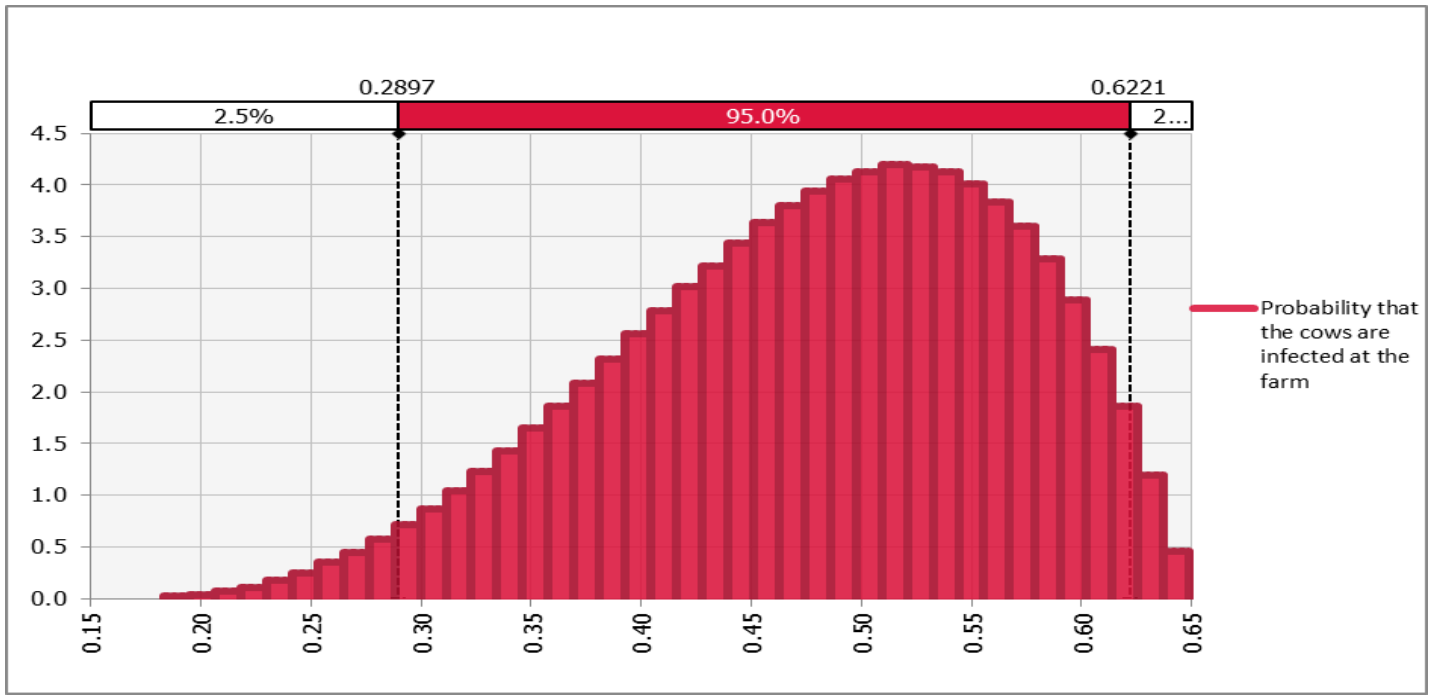


Figure 4. 4: The probability distribution that the cows are infected at the farm.

The probability that infected cows are not detected on screening and are milked was predicted to be 0.30 (95% CI 0.27-0.32), as is shown in **Figure 4.13**.

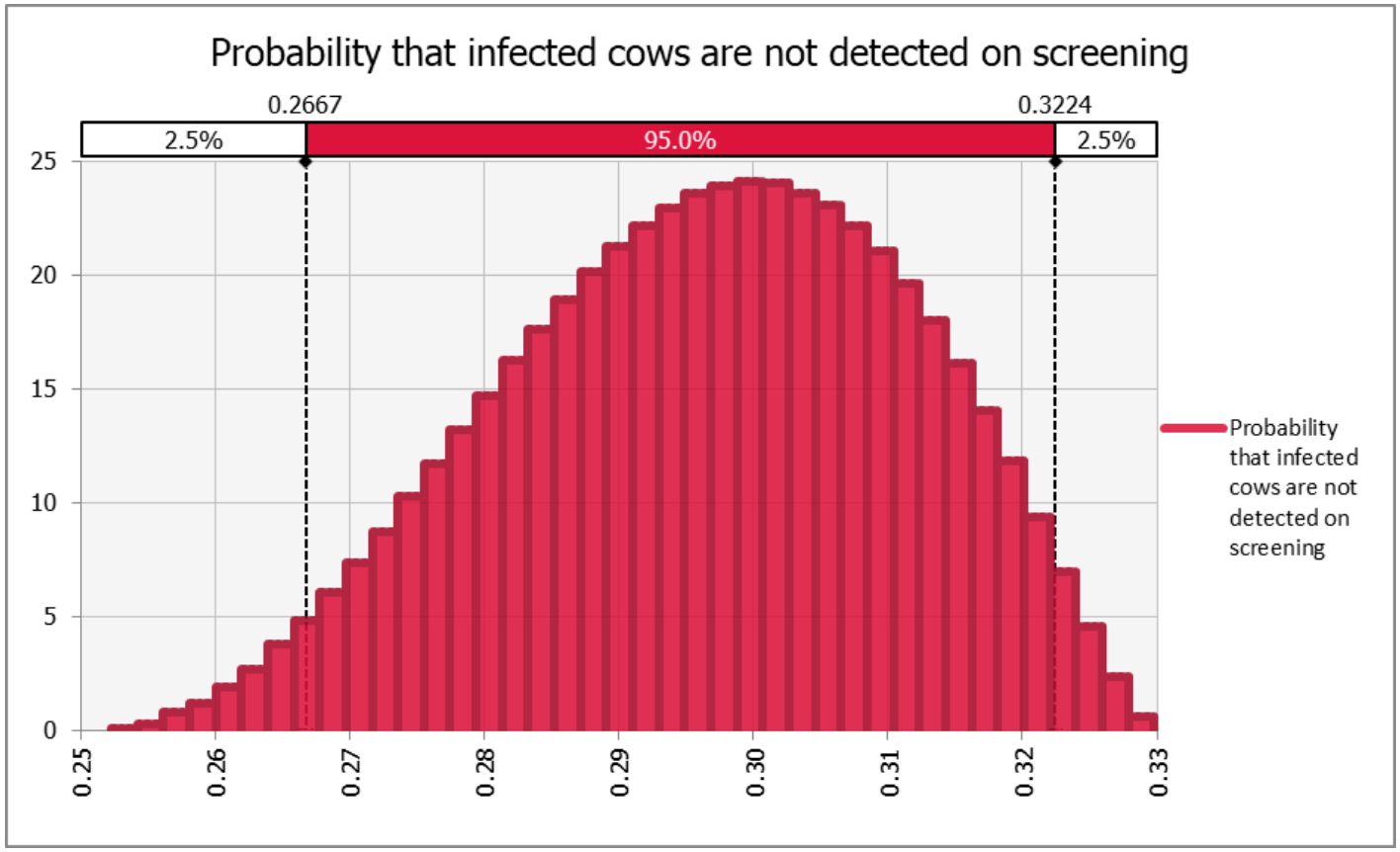


Figure 4. 5: The Probability distribution that infected cows are not detected on screening.

The probability that Brucella contaminated milk was not detected and was taken for human consumption was predicted to be 0.15 (95% CI 0.13-0.17) as shown in **Figure 4.15.** The probability that milk was consumed raw was predicted to be 0.424 (95% 0.37-0.49), as shown in **Figure 4.14**.

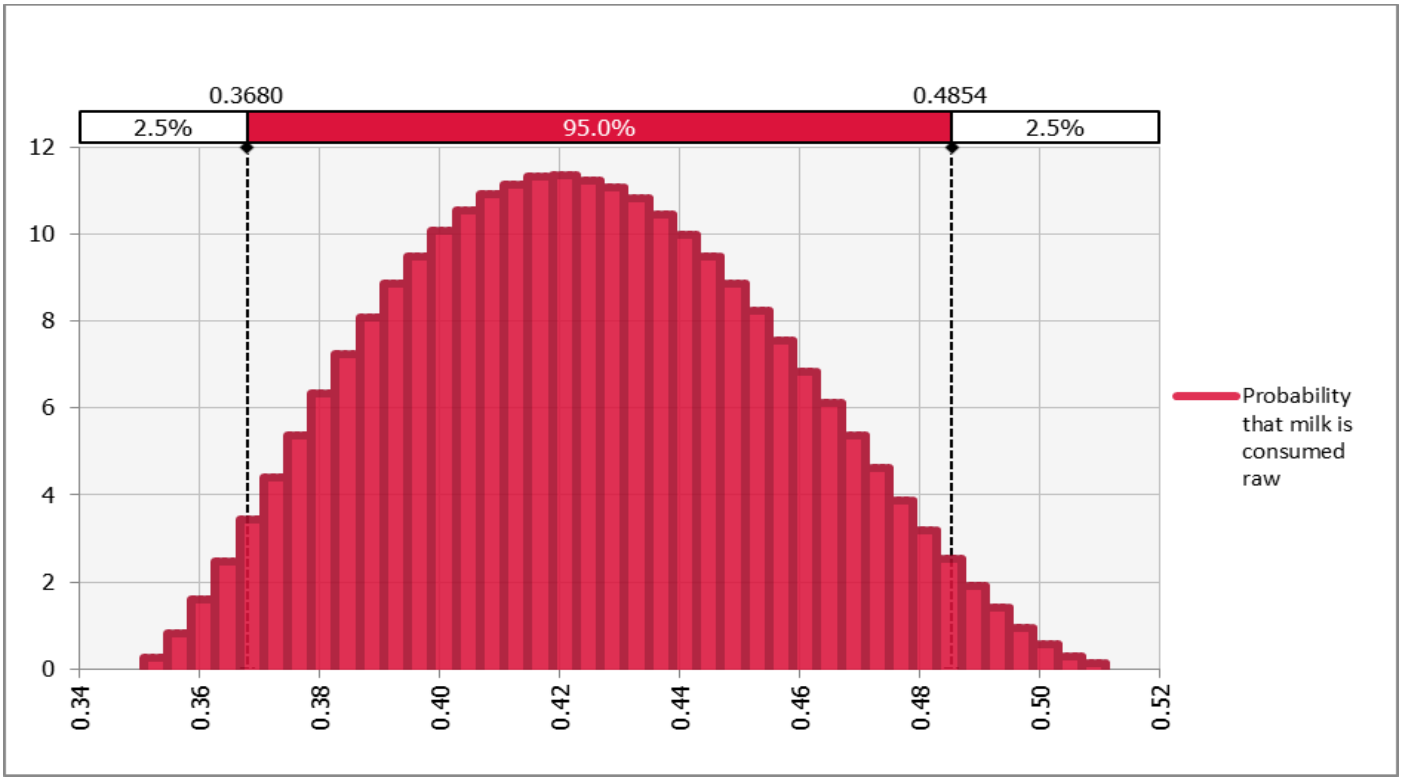


Figure 4. 6: The probability distribution of raw milk consumption.

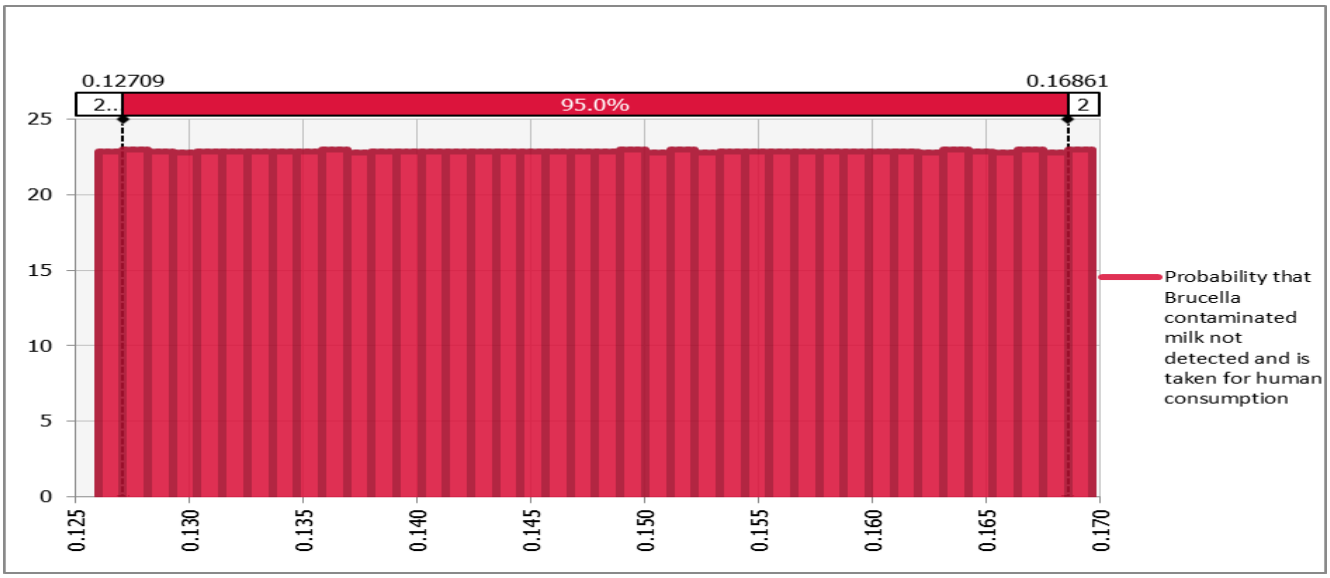


Figure 4. 7: The probability distribution that contaminated milk was not detected and taken for human consumption.

The model also estimated the risk of exposure from farm to consumption, the product of all possible exposure probabilities along the milk food chain. The product was defined as output and simulated once at 100,000 iterations. The probability of exposure was estimated to be 0.0089 (95% CI 0.0058-0.012), as shown in **Figure 4.16.**

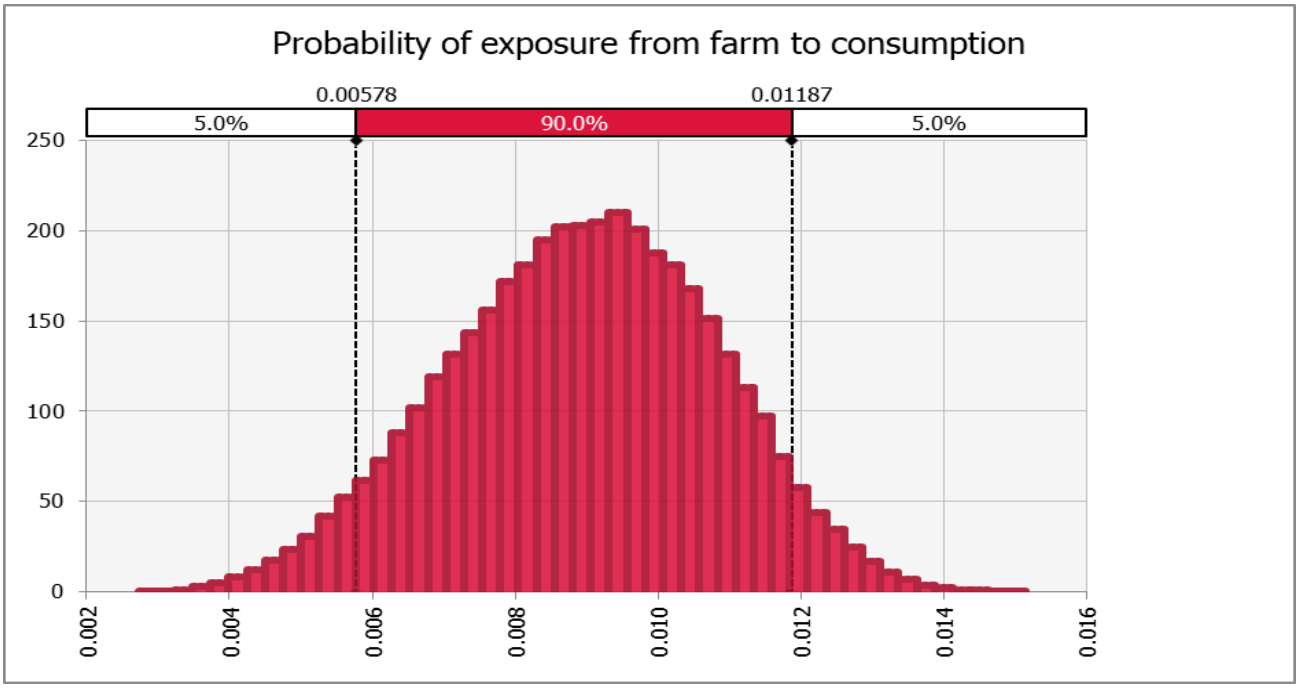


Figure 4. 8 Probability distribution of human exposure from farm to consumption

4.4.Risk characterization

4.4.1. Probability of infection

The probability of one getting infected with brucellosis following consumption of milk after various preparation was also calculated in the model based on the input parameters shown in **Table 3.1**.

The results were entered into the @Risk Software, and the risk model was estimated using Monte Carlo simulation, and the defined output was simulated at 100,000 iterations. After simulation, the model predicted the probability of getting an infection with brucella through consumption of prepared half-done milk, which was estimated to be 0.4 (95%CI 0.32-0.48), as show**n in Figure 4.5**. The probability of one getting a brucella infection following consumption of raw milk was predicted to be 0.64(95% CI 0.54-0.73), as described in **Figure 4.4**.

From **Figure 4.6**, the model estimated no probability of a person getting infected with Brucella by consuming prepared, done milk.

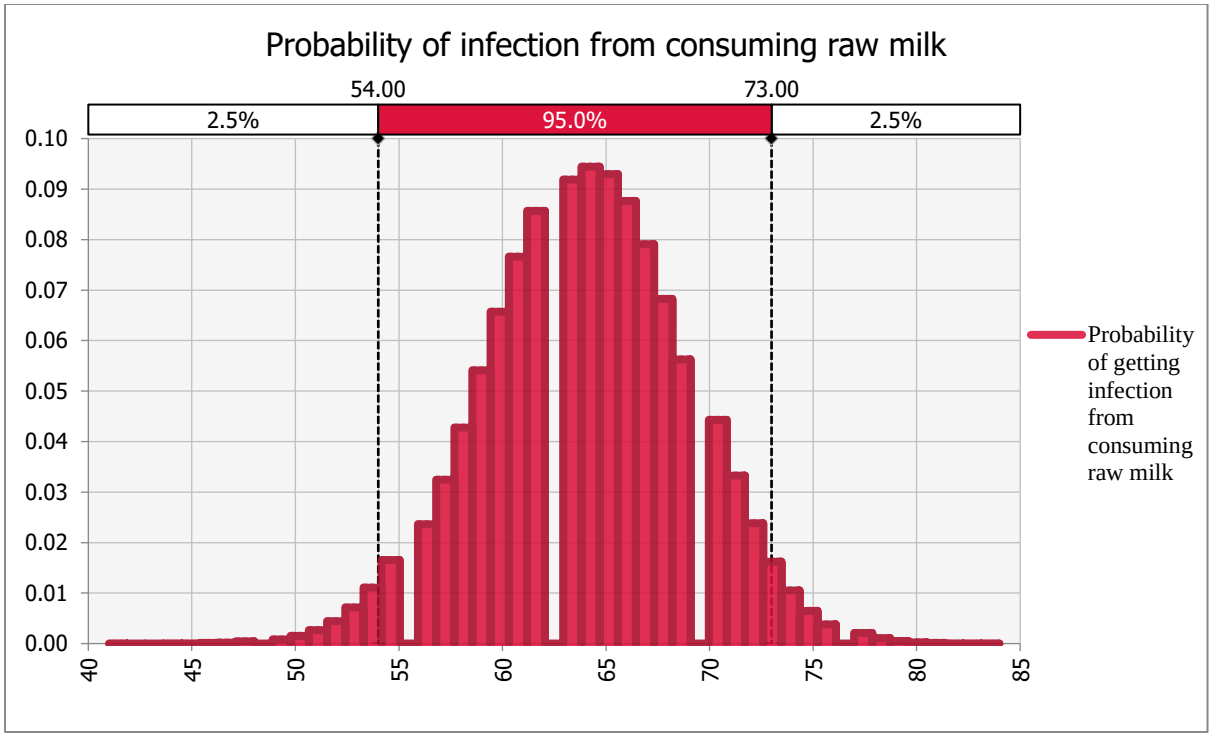


Figure 4. 9: Probability distribution of getting the infection from consuming raw milk.

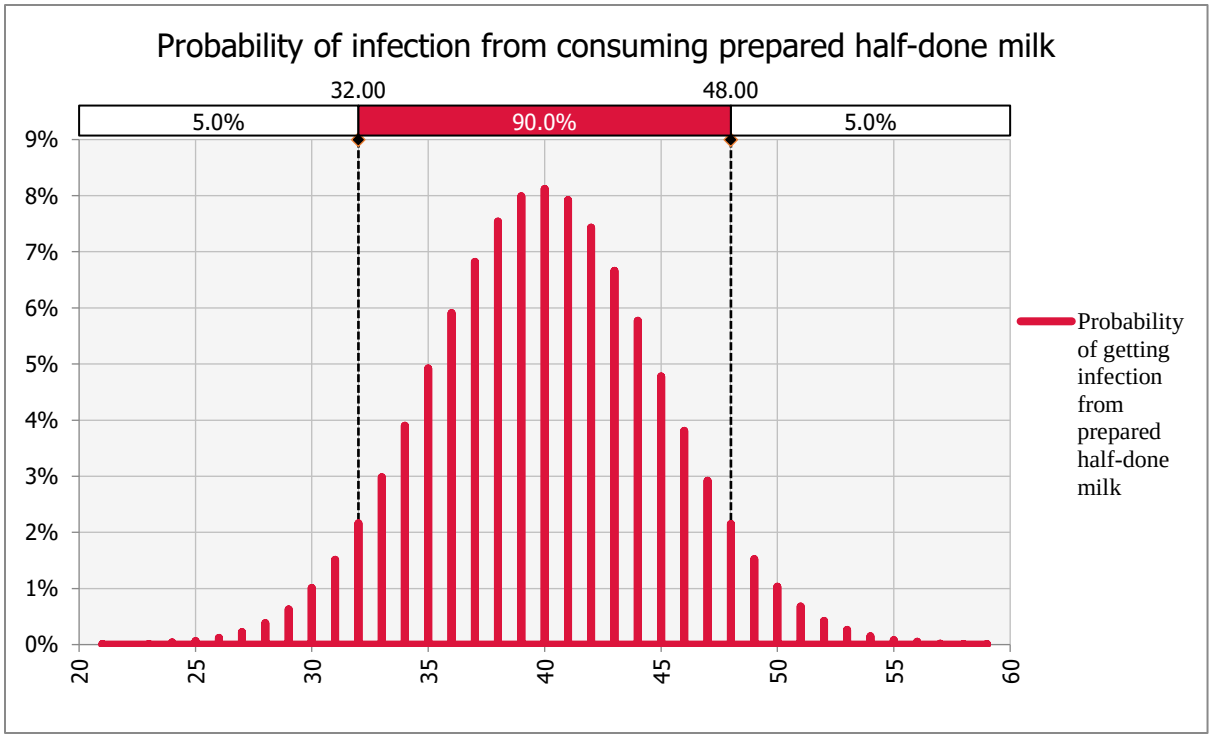


Figure 4. 10: Probability distribution of getting the infection from consuming prepared half-done milk

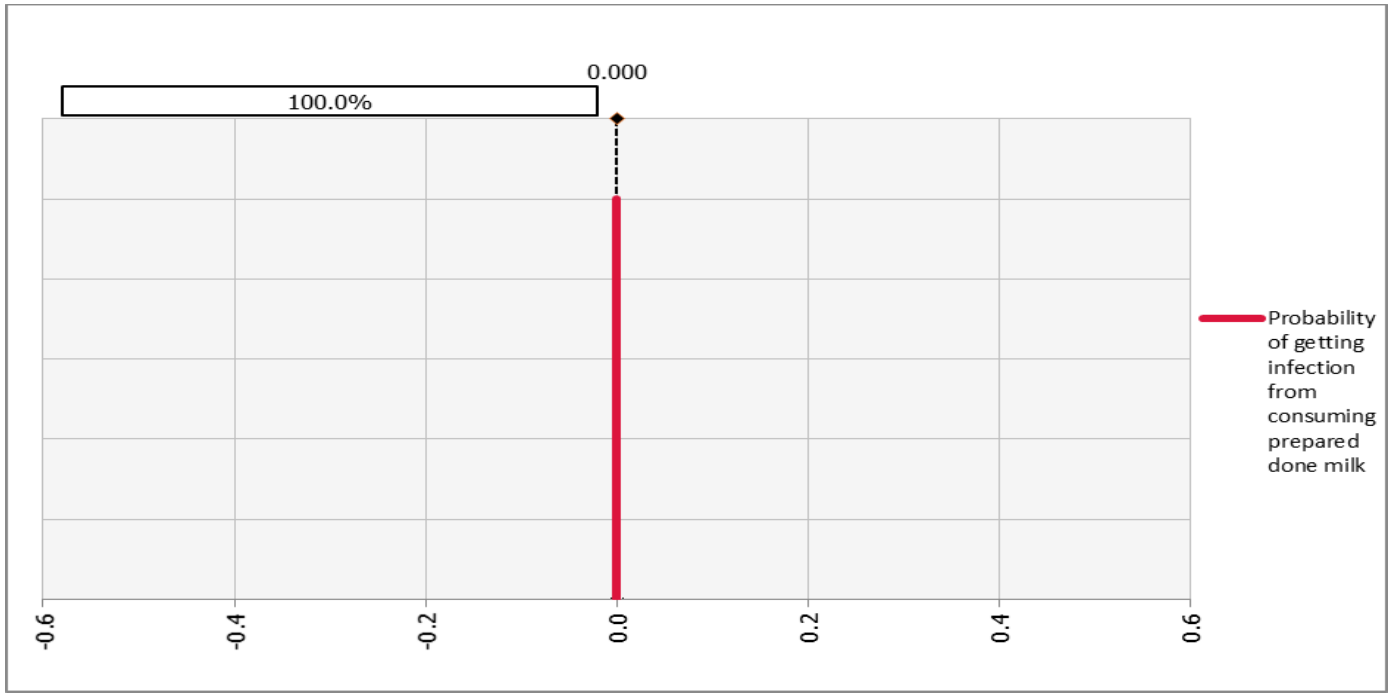


Figure 4. 11: Probability of getting the infection from consuming prepared, done milk.

4.4.2. Infection and illness

In this study, the infectious dose for Brucella in human beings was estimated to range from 10-to 100 microorganisms (De Figueiredo *et al.*, 2015; Grützke *et al*., 2021; Kaden *et al*., 2018). Since *Brucella melitensis* is among the species which have been confirmed to cause infections in humans (Galińska *et al*., 2013), the model assumed the infectious dose in which half of the exposed population will get ill (ID50) for *Brucella melitensis* from the human vaccine trial in which the minimum dose is 94cfu and the maximum dose considered to be 1885cfu (Teske *et al*., 2011) to be considered as an infectious dose 50 for the Brucella species. After that, the input parameters were put in a Monte Carlo simulation model, and the outputs were defined. The infection and illness outputs were simulated at 100,000 iterations. The model predicted the number of people likely to get infected with Brucella in the Arusha region in a one-year consumption period of 677,678 (95%CI 33,000-1460,000) following consumption of prepared half-done milk, as shown in **F igure 4.7**.

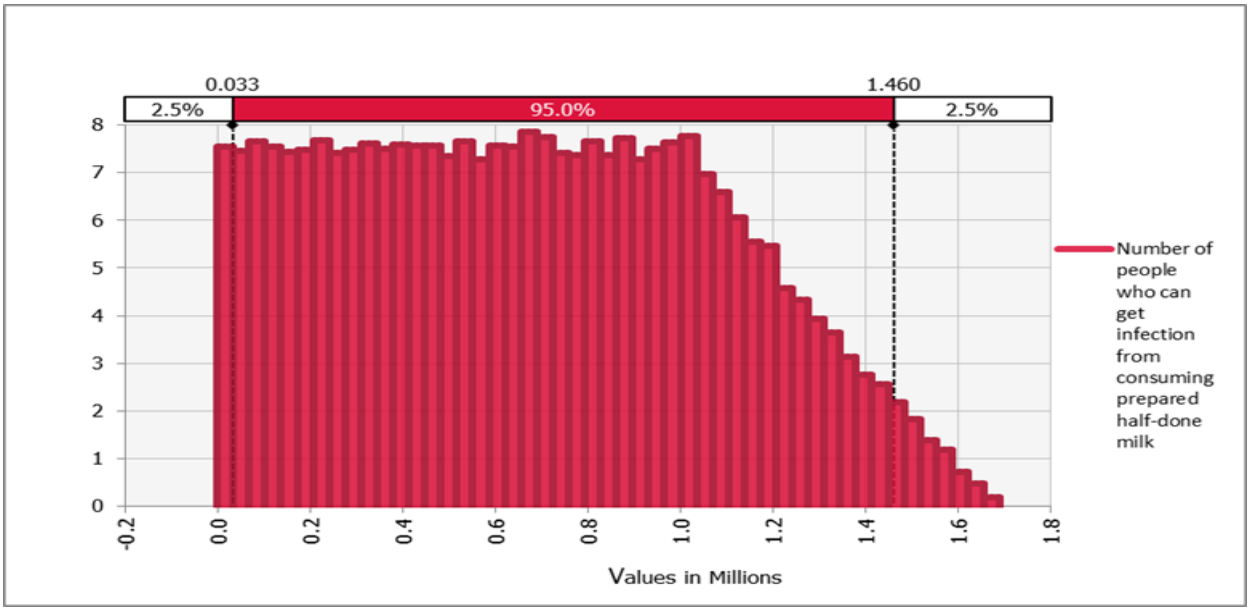


Figure 4. 12: Distribution of the number of people who can get the infection from consuming prepared half-done milk

The number of people likely to get infections from consuming raw milk in one year was estimated to be 1,084,358 people (95%CI 565,000-1458,000), as shown in **Figure 4.8**.

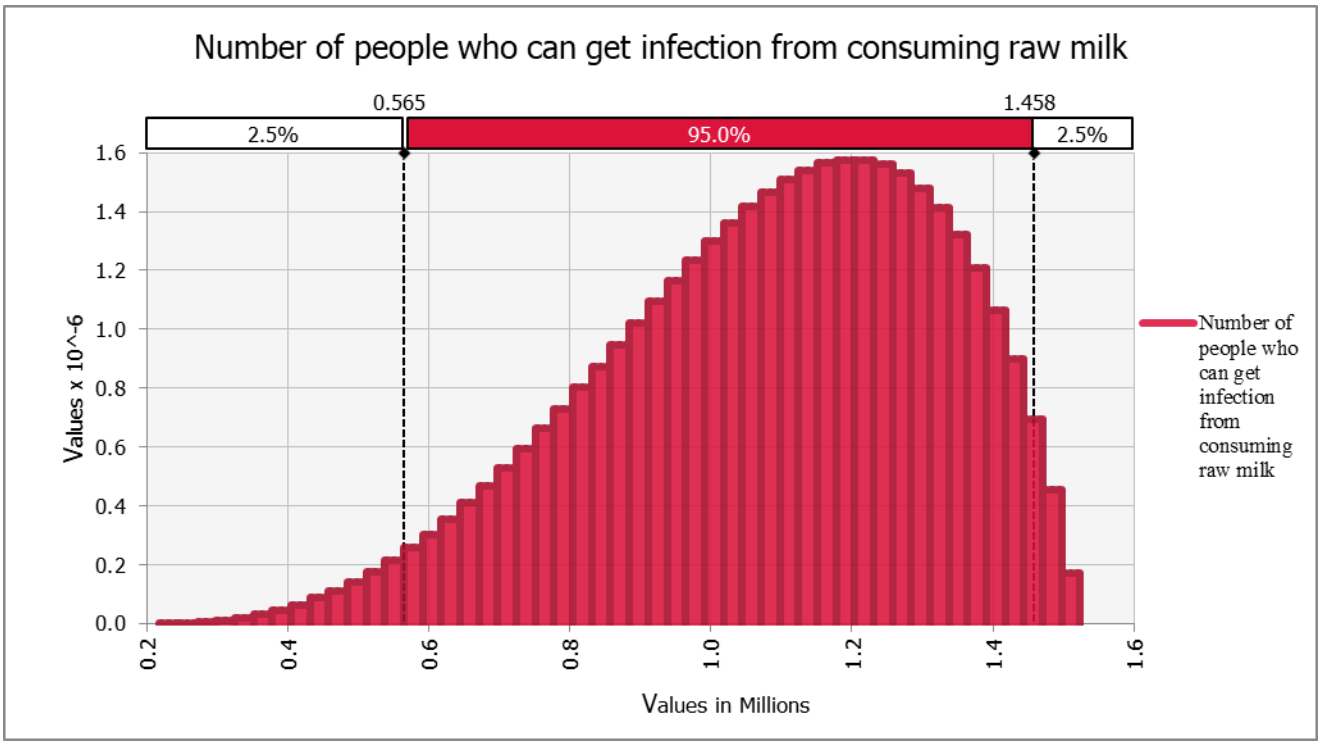


Figure 4. 13: Distribution of the number of people who can get the infection from consuming prepared raw milk

Figure 4.9 Summarizes the estimates of the number of people likely to get brucellosis through cow milk consumption in different preparations. It shows that no person is likely to be infected by brucellosis following the consumption of cow milk that is prepared well done, as stated earlier.

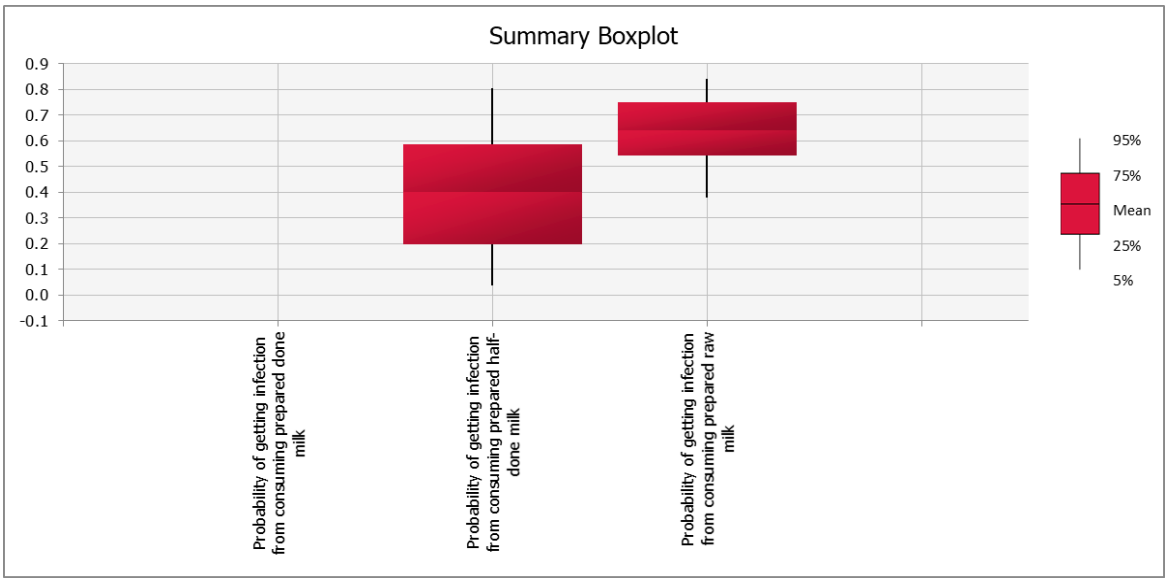


Figure 4. 14: Box and whisker plots of the probability of someone getting infected with brucellosis by consuming cow milk in different preparations.

Spink (1954) reported that not all people who get Brucella infections are likely to develop and show clinical signs; instead, only 15-20% of infected people have that chance. All of these possibilities were entered into the Monte Carlo simulation model in @risk, and the output was defined as the number of brucella infected people who are likely to get ill. This was simulated at 100,000 iterations. The model predicted the number of people who are likely to get ill after being infected with *brucella* to be 189,760 (95%CI 96,696-269,804), and the results are presented in **Figure 4.10.** The number of people infected with Brucella through consumption of different preparation, i.e., raw, half-done and done milk portions, together with the number of people who are likely to develop clinical signs, are summarized in Figure 4.11.

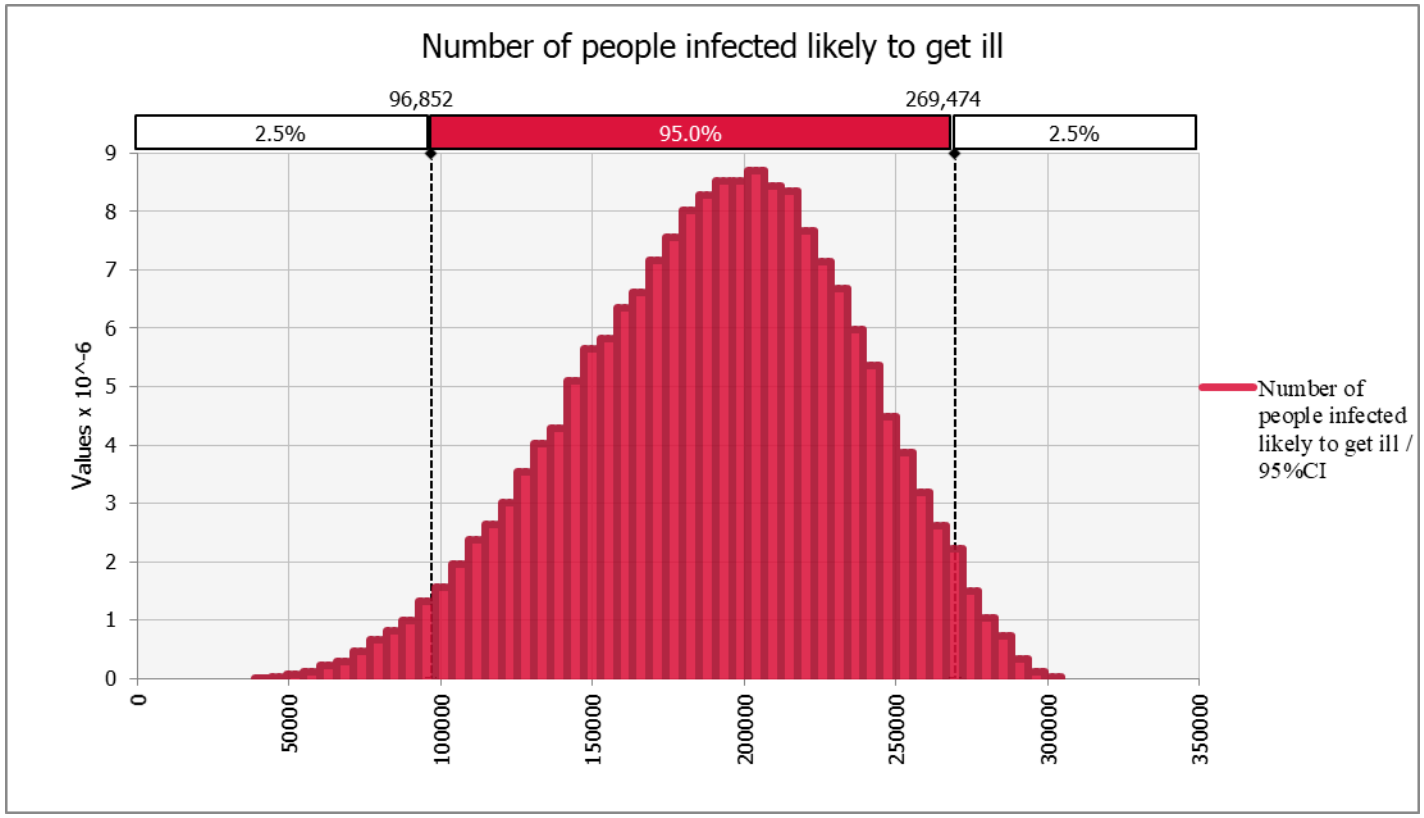


Figure 4. 15 The distribution of the number of people infected with Brucella spp. who are likely to get ill.

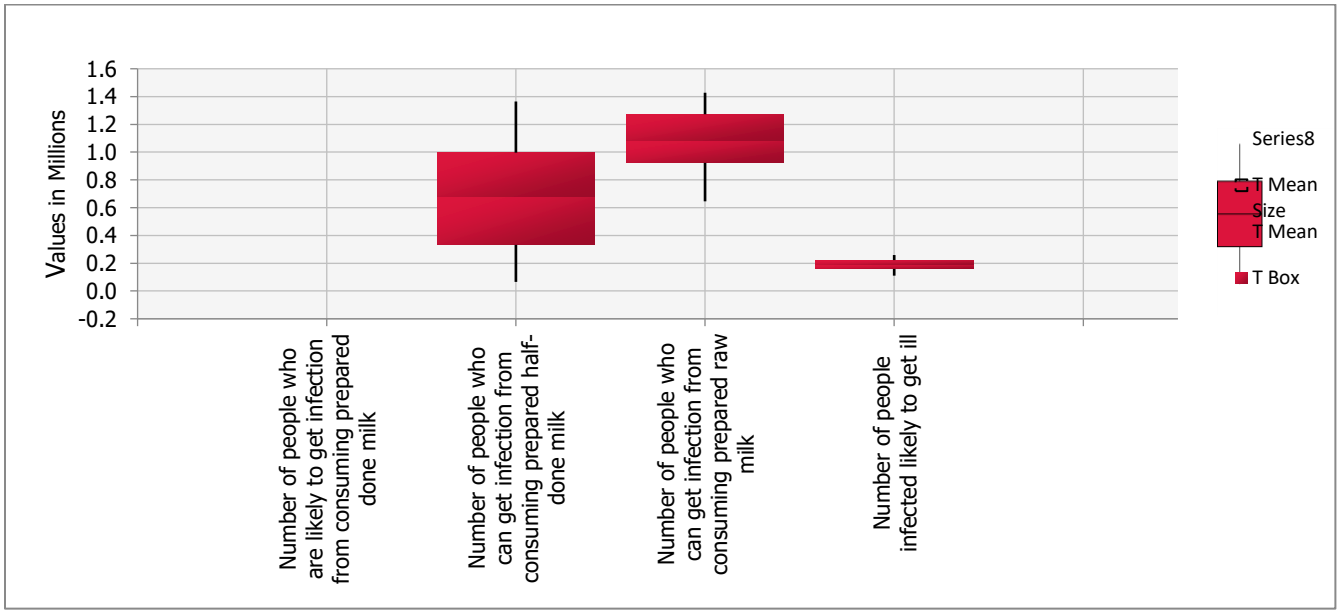


Figure 4. 16 Box and whisker plots of the number of people who can get the infection through consumption of various milk preparation.

4.4.3. Sensitivity analysis

The model also estimated the influence of each probability and preparation factor on the risk of exposure through a sensitivity analysis. The results on a Tornado graph in **Figure 4.17** show that 83% of the risk of exposure was contributed by the probability that cows were infected at the farm, 36% the contaminated milk was not detected and was taken for human consumption, 31% probability that milk was consumed raw, and 22% the probability that infected cows were not detected on the screening and are milked.

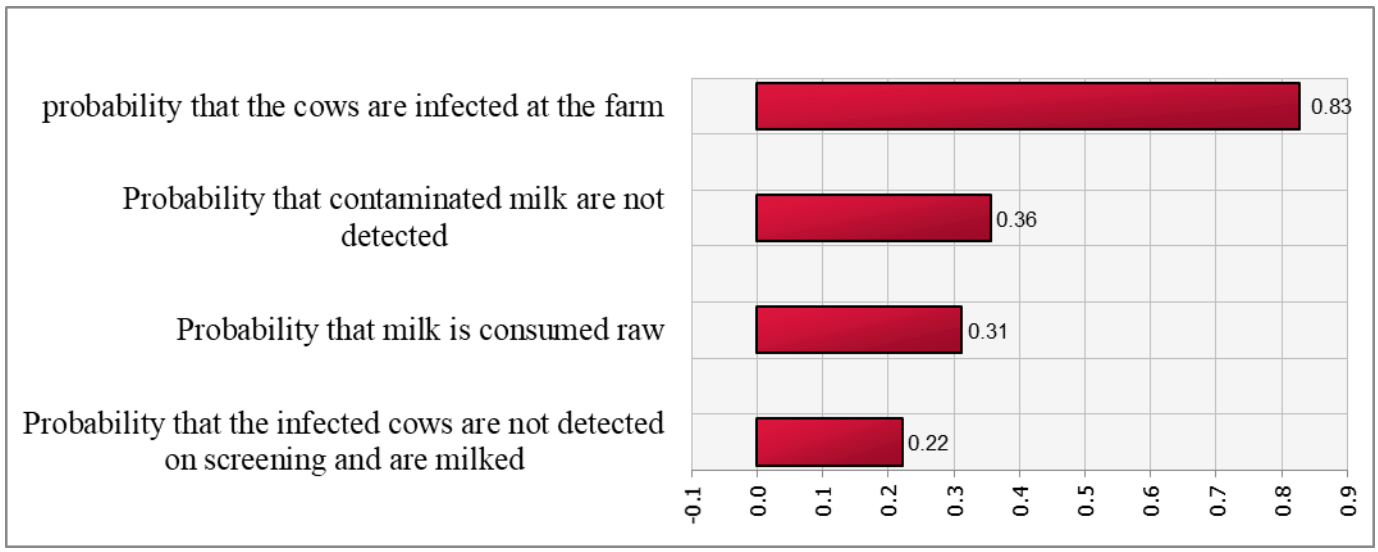


Figure 4. 17: A Tornado graph ranking the factors that facilitated the risk of exposure.

The Tornado graph shows a 100% chance of the consumers getting exposed to brucella when milk is prepared raw, 3% when milk is prepared half-done, and no chance when milk is prepared done (**Figure 4.18**).

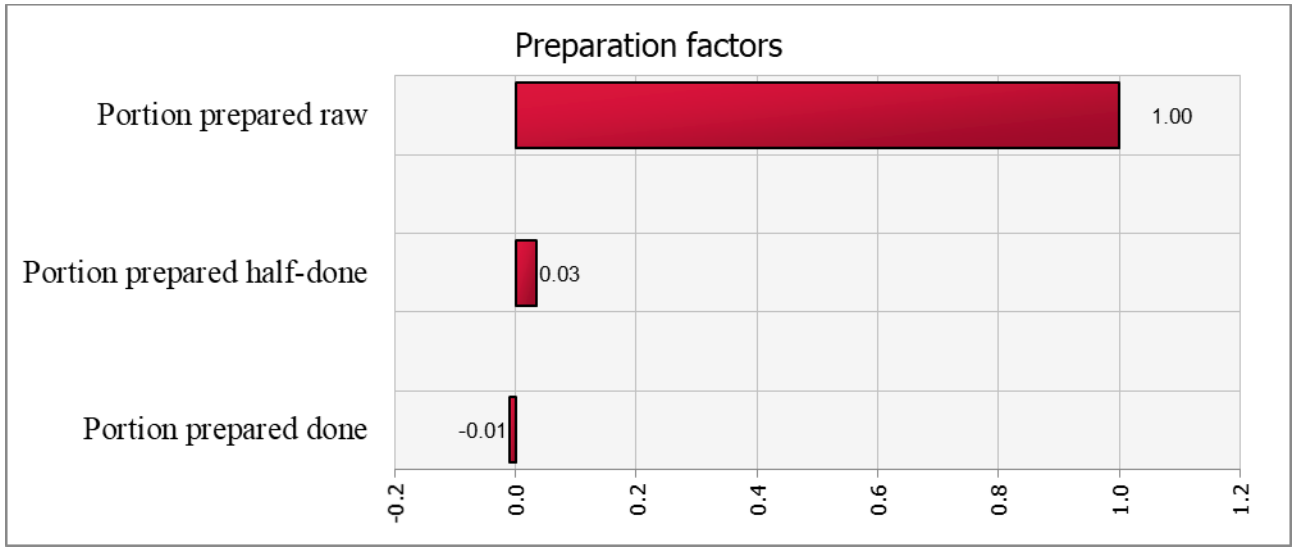


Figure 4. 18 The ranking of the preparation factors

CHAPTER FIVE.

5.0. DISCUSSION.

This study was conducted to assess the risk of developing brucellosis through milk consumption in the Arusha region of Tanzania. The key questions were to find out the milk consumption scenario among people in the Arusha region and the risk of exposure to Brucella species through the consumption of raw milk in the Arusha region.

5.1. Demographic information

The survey comprised mostly male respondents, constituting 76.2% of the respondents, while female respondents were 23.8%. The finding is contrary to Njarui *et al*. (2011), who reported that 11.9% of the respondents were males while 88.1% were female. This difference could be attributed to the nature of the tribes from which the people were interviewed, Kamba in Kenya, compared to Maasai in Tanzania. In the Maasai tribe, women are not supposed to respond to anything before men, which influenced the number of male respondents observed in this study.

5.2.Serving portions kitchen preparation and consumption pattern

The study observed that 6% consumed less than 500ml or 500g of milk a day while 4.2% consumed more than 2L or 2000g per day. The large population in Arusha, about 72.8%, consumed milk between 0.5-1L per day, whereas 17% of the population consumed between 1-2L per day per person. Following simulation of the input parameters in a Monte Carlo simulation platform, the average milk consumption per day per person in the Arusha region was predicted to be 1.0834L (95%CI 0.618-1.6850). The value is slightly similar to that reported earlier by Njarui *et al*. (2011), which reported the average milk consumption per day per person in the Maasai community in Morogoro to range between 0.84-1.75L and by Kouamé-Sina et al. (2012) in Abidjan where the average milk consumption per day per person was estimated to be 0.5L. This might be facilitated by the similar traditions of Maasai people, whose diet depends much on meat and milk, as previous studies described it (Gibney & Burstyn, 1980; Gidel et al., 1976; Melubo, 2020). The survey also showed that most people (99.5%) in the study area consumed milk daily, whereas the preparation methods varied depending on residency. This is contrary to the study done in Abidjan, Côte d'Ivoire by Kouamé-Sina et al. (2012), who reported that only 28% consumed milk every day. This difference might be caused by the affordability of milk in these areas. People who stay in town, 90% consume prepared done milk (well-cooked/boiled) either during breakfast as tea, freshly boiled milk or mixed with other ingredients to make their food and 10% consume raw milk. According to the survey, among people in village settling, 27% consumed their milk while well done, mostly during tea and

traditional food. About 71 % consume raw milk in their households, while 2% do partially cook their milk before consumption which is referred to as half-done. When all of the input data about kitchen preparation were simulated by using a Monte Carlo simulation in @risk, it gave the estimation of the average proportion of milk portions that are prepared done to be 0.648 (95%CI 0.411-0.846), the proportion of the portions which were prepared raw to be 0.64 (95%CI 0.333-0.861). This is in line with Kouamé-Sina et al. (2012), who reported that 51.6% of residents in urban and peri-urban consumed raw milk, whereas 48.6% of residents in urban and peri-urban consumed boiled/pasteurized milk. Studies conducted in Kenya (which is near Arusha) discovered that the prevalence of the pathogen in raw milk at the animal level (considering samples from individual animals) was 2.4% (95% confidence interval (CI) 1.1-4.5) (Wainaina *et al.*, 2020) but also it was reported that only 1 cfu/ ml is enough to initiate the brucellosis infections (Bhankole, 2013). Therefore, there is a high risk of getting infections in the population that consumes more milk than those who consume less.

5.3.Quantitative risk assessment

5.4. Exposure assessment

Different milk preparation gives different implications for the transfer of the diseases from the results obtained from the Monte Carlo simulation. There was a high risk of exposure to Brucella infection, which was 0.64(95%CI 0.54-0.73) through consumption of prepared raw milk. These findings agree with Bhankole (2013) report, which reported 0.63 as the risk of getting Brucella infection from consuming contaminated dairy products. This is because the contaminated brucella bacteria in raw milk are active and have the potential to cause disease in comparison with other preparations. That is why the ingestion of fresh milk or other dairy products prepared from unheated/un-boiled milk is said to be the significant source of Brucella infection in most communities all over the world, as it was described by Corbel *et al*. (2006) John *et al.*, (2010) as well by Massis *et al*. (2019). Other researchers described the consumption of raw milk as the leading risk factor for human brucellosis and are strongly conditioned by the geographical situation, which impacts the number of infected animals and the occurrence of Brucella contamination in dairy products (Dadar *et al*., 2019). Also, the model showed that the consumption of half-cooked/half done milk also has a risk of getting brucellosis which was described as the probability of getting an infection being 0.4 (95%CI 0.32-0.48). This might be because not all Brucella bacteria will die on the partial heating or boiling of the milk, making them the potential for disease transmission. This agrees with the study conducted by Méndez-González *et al*. (2011), in which *Brucella melitensis* survived in goat milk at a combination of various temperatures and times. A similar observation was reported by Davies & Casey (1973) on *Brucella abortus* in which the viable cells count were visible after heating treatment of milk in

161-162°F (71.7°c) for 5 seconds. The model also assessed the risk of getting a disease for those who consumed prepared or well-cooked/boiled milk. It showed that there was no risk of getting infected with any brucella species by consuming prepared well-done milk. This is because all bacteria are killed by heating or high-temperature pasteurization. A similar observation was made by Corbel et al. (2006), who reported that heating milk at a temperature between 80-85°c for several minutes destroys all brucella bacteria. Also, a report from Davies & Casey (1973) on *Brucella abortus* in which after heat treatment of milk in 161-162°F (71.7°c) for 5 minutes, there was no observed viable *Brucella abortus* cell. Manyori *et al*. (2017) also demonstrated that the risk of developing salmonellosis in Zambia was low due to overcooking.

5.5.Sensitivity analysis

The factors that influence the risk of exposure from the farm to the time of consumption were ranked using a Tornado correlation coefficient, which showed that the high risk of exposure (83%) could be attributed to cows getting infected at the farm. This agrees with John *et al*. (2010), who reported on the cow's infection in the herd as an influential risk factor for human brucellosis. If the contaminated milk is not detected and is released for human consumption, there is a 36% chance of contributing to people's exposure to brucellosis. The risk of exposure at 31% can be influenced when milk is consumed raw, and there was a 22% chance of people being exposed to various Brucella species when the infected cows were not detected during screening and were milked at the farms. Also, on the preparation of portions, when someone consumed prepared raw milk, the chance of being exposed to various species of Brucella was as high as 100%. There was a 3% chance of exposure when consuming prepared half-done milk. When the milk portion is prepared well done, there is no influence on the exposure to Brucella species. This is in line with Corbel (2006) and Davies & Casey (1973), who reported that a high risk of exposure to brucella is when milk is consumed raw, there is a reduced risk when partially boiled, and no risk when milk is well boiled before consumption

5.6.Risk characterization

As it was previously described by (Wainaina et al., 2020) that the prevalence of the pathogen (Brucella) in raw milk at the animal level (considering samples from individual animals) is 2.4% (95% confidence interval (CI) 1.1-4.5), but also it was reported by Bhankole (2013) that only 1 cfu/ ml is enough to initiate the brucellosis infections. Based on these findings, 71% of the population from the villages are at higher risk of getting the infection because do consumes raw milk, while 10% of the town population are also at the risk of getting brucellosis.

The conceptual model pathways of Brucella species from farm to consumption show four probabilities that could lead people to be exposed to Brucella species along the milk food chain from the farm to the table. These are the probability that cows are infected at the farm, the probability that infected cows are not detected on screening and are milked, the probability that Brucella contaminated milk is not detected and is taken for human consumption and the probability that milk is consumed raw**.**

Generally, the probability of exposure from farm to consumption is estimated by taking the product of all of the probabilities. It is sometimes referred to as a probability of release. Individual probability was taken as an output defined in a Monte Carlo simulation during simulation, and the given probabilities were obtained. The overall probability of exposure was calculated to be 0.0089. Among all four probabilities, the highest probability observed on the probability that cows are infected at the farm is 0.48. This is similar to findings from Mathew *et al*. (2015), who reported a herd prevalence of 48% (95% CI 41-55). This similarity might be attributed to the similar highland climatic conditions of the Mbeya and Arusha regions. The probability that milk was consumed raw was 0.42, slightly similar to the report from Kai & Aotearoa (2009), who reported the probability of 0.35 that milk was consumed raw. The lowest probability observed on the probability that Brucella contaminated milk is not detected and is taken for human consumption was 0.1478, while the probability that infected cows are not detected on screening was 0.296.

After running and integrating the results from hazard identification, hazard characterization, and consumer exposure assessment, the Monte Carlo simulation predicted the number of people who are likely to get infected by various species of Brucella through consumption of milk at an average consumption of 1.08L per day per person on different milk preparations. The model predicted that through consumption of half-done milk, many people are likely to be infected with various Brucella species in a year in both town and village settings. This number is high and has an economic impact on individuals, households, and national levels. On the other hand, the model predicted that 1,084,358 people (95%CI 565,000-1458,000) out of 1,694,310 would get infected with various species of Brucella through consumption of prepared raw milk. Then, in most cases, brucellosis goes asymptomatic, and it is reported that at least 15-20% of people who get infected with brucella are likely to develop the disease and show clinical signs (Spink, 1954). Therefore, the model also predicted the number of brucellae infected people who were likely to get ill and showed the clinical signs to be 189,760 (95%CI 96,696-269,804) out of 1,084,358 who were likely to get infections in one year. Most of these affected people, especially in east Africa, are reported from the low economic societies, as was reported in previous studies by Wainaina *et al.* (2020) and Pappas *et al*. (2006).

CHAPTER SIX.

6.0. CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

The milk consumption in the Arusha region is high and frequent. Generally milk consumption was mainly prepared as half-cooked, well cooked and raw. This posed a high risk of developing human brucellosis, especially among those who prepared half-done/cooked or prepared raw before consumption. From the observation reported, raw milk consumption is said to pose a high risk of exposure in which the probability of getting infected with brucella through consumption of raw milk is estimated to be 0.64 (95% CI 0.54-0.73), which is high but also 1,084,358 (95%CI 565,000-1,458,000) people in a population of about 1.7 million are likely to get infected with various species of Brucella in one year. There is no risk of getting exposure to brucellosis for people who consume prepared milk.

6.2. RECOMMENDATION

For better public health and prevention of human brucellosis, the communities have to be made aware of the risk of exposure to raw milk and its effects and encouraged to cook or boil their milk thoroughly before consumption to minimize or eliminate the particular risk. Vaccination of the animal population will also, to a large extent, prevent people from being infected with Brucellosis. Although the elimination of an infected livestock population and adequate surveillance system in animals is a building block for the prevention and control of this zoonotic disease, the challenge is poor economies in most African countries that cannot afford the compensation to the farmers. Also, the development of improved diagnostic tests for diagnosing brucellosis in cattle so that no infected animals are missed, milked, and infected milk is passed on for consumption.

For this reason, there is a need for a one health approach to fighting human brucellosis. Further research is needed to assess the risk of developing brucellosis through other potential pathways like meat consumption and direct contact with the infected animal(s).

REFERENCES

Ahmed, W., Zheng, K., & Liu, Z. F. (2016). Establishment of chronic infection: Brucella's stealth strategy. *Frontiers in Cellular and Infection Microbiology*, *6*(MAR), 1–12. https://doi.org/10.3389/fcimb.2016.00030

Akakpo, A. J., Têko-Agbo, A., Koné, P., & others. (2010). The impact of brucellosis on the economy and public health in Africa. *Compendium of Technical Items Presented to the OIE World Assembly of Delegates or to OIE Regional Commissions, 2009, file:///E:*, 71–84.

Alonso, S., Dohoo, I., Lindahl, J., Verdugo, C., Akuku, I., & Grace, D. (2016). *Prevalence of tuberculosis , brucellosis and trypanosomiasis in cattle in Tanzania : a systematic review and meta-analysis*. *17*(1). https://doi.org/10.1017/S146625231600013X

Asakura, S., Makingi, G., Kazwala, R., & Makita, K. (2018a). Herd-level risk factors associated with Brucella sero-positivity in cattle, and perception and behaviours on the disease control among agro-pastoralists in Tanzania. *Acta Tropica*, *187*(July), 99–107. https://doi.org/10.1016/j.actatropica.2018.07.010

Asakura, S., Makingi, G., Kazwala, R., & Makita, K. (2018b). Brucellosis Risk in Urban and Agro-pastoral Areas in Tanzania. *EcoHealth*, *15*(1), 41–51. https://doi.org/10.1007/s10393-017-1308-z

Assenga, J. A., Matemba, L. E., Muller, S. K., Malakalinga, J. J., & Kazwala, R. R. (2015). Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. *BMC Veterinary Research*, *11*(1), 1–11. https://doi.org/10.1186/s12917-015-0504-8

Bamaiyi, P. H., Hassan, L., Khairani-Bejo, S., & Zainal, M. a. (2014). Updates on brucellosis in Malaysia and southeast Asia. *Malaysian Journal of Veterinary Research*, *5*(1), 71–82.

Bayramoglu, G., Ozalp, V. C., Oztekin, M., & Arica, M. Y. (2019). Rapid and label-free detection of Brucella melitensis in milk and milk products using an aptasensor. *Talanta*, *200*(March), 263–271. https://doi.org/10.1016/j.talanta.2019.03.048

Bhankole, A. . (2013). *Brucellosis risk assessment*. 29–31.

Bodenham, R. F., Lukambagire, A. S., Ashford, R. T., Buza, J. J., Rubach, M. P., Sakasaka, P., Shirima, G. M., & Swai, E. S. (2020). Prevalence and speciation of brucellosis in febrile patients from a pastoralist community of Tanzania. *Scientific Reports*, 1–11. https://doi.org/10.1038/s41598-020-62849-4

Bouley, A. J., Biggs, H. M., Stoddard, R. A., Morrissey, A. B., Bartlett, J. A., Afwamba, I. A., Maro, V. P., Kinabo, G. D., Saganda, W., Cleaveland, S., & Crump, J. A. (2012). Brucellosis among

hospitalized febrile patients in northern Tanzania. *American Journal of Tropical Medicine and Hygiene*, *87*(6), 1105–1111. https://doi.org/10.4269/ajtmh.2012.12-0327

Carugati, M., Biggs, H. M., Maze, M. J., Stoddard, R. A., Cash-Goldwasser, S., Hertz, J. T., Halliday, J. E. B., Saganda, W., Lwezaula, B. F., Kazwala, R. R., Cleaveland, S., Maro, V. P., Rubach, M. P., & Crump, J. A. (2018). Incidence of human brucellosis in the Kilimanjaro Region of Tanzania in the periods 2007-2008 and 2012-2014. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *112*(3), 136–143. https://doi.org/10.1093/trstmh/try033

CDC. (2017). *BRUCELLOSIS REFERENCE GUIDE : EXPOSURES , TESTING , AND PREVENTION*.

Chota, A. C., Magwisha, H. B., Stella, B., Bunuma, E. K., Shirima, G. M., Mugambi, J. M., Omwenga, S. G., Wesonga, H. O., Mbatha, P., Gathogo, S., Complex, V., & Muguga, C. (2016). *PREVALENCE OF BRUCELLOSIS IN LIVESTOCK AND INCIDENCES IN HUMANS IN EAST AFRICA Brucellosis is among the most widely distributed zoonoses of economic importance in developing countries . Most of the zoonotic diseases are poorly controlled , and endanger ec*. *24*, 45–52.

Corbel, M. et al. (2006). Brucellosis in humans and animals. *World Health Organization 2006 All*, 89. https://doi.org/10.2105/AJPH.30.3.299

Dadar, M., Fakhri, Y., Shahali, Y., & Mousavi Khaneghah, A. (2020). Contamination of milk and dairy products by Brucella species: A global systematic review and meta-analysis. *Food Research International*, *128*, 108775. https://doi.org/10.1016/j.foodres.2019.108775

Dadar, M., Shahali, Y., & Whatmore, A. M. (2019). Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention. *International Journal of Food Microbiology*, *292*(November 2018), 39–47. https://doi.org/10.1016/j.ijfoodmicro.2018.12.009

Davies, G., & Casey, A. (1973). The survival of Brucella abortus in milk and milk products. *The British Veterinary Journal*, *129*(4), 345–353. https://doi.org/10.1016/S0007-1935(17)36436-9

De Figueiredo, P., Ficht, T. A., Rice-Ficht, A., Rossetti, C. A., & Adams, L. G. (2015). Pathogenesis and immunobiology of brucellosis: Review of Brucella-host interactions. *American Journal of Pathology*, *185*(6), 1505–1517. https://doi.org/10.1016/j.ajpath.2015.03.003

De Massis, F., Zilli, K., Donato, G. Di, Nuvoloni, R., Pelini, S., Sacchini, L., D'Alterio, N., & Giannatale, E. Di. (2019). Distribution of Brucella field strains isolated from livestock, wildlife populations, and humans in Italy from 2007 to 2015. *PLoS ONE*, *14*(3), 1–16. https://doi.org/10.1371/journal.pone.0213689

Dereje, T., Benti, D., Feyisa, B., & Abiy, G. (2018). Review of common causes of abortion in dairy cattle in Ethiopia. *Journal of Veterinary Medicine and Animal Health*, *10*(1), 1–13. https://doi.org/10.5897/jvmah2017.0639

Ducrotoy, M., Bertu, W. J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A. M., Welburn, S., Ocholi,

R., Blasco, J. M., & Moriyón, I. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica*, *165*, 179–193. https://doi.org/10.1016/j.actatropica.2015.10.023

Dufour, B., Plée, L., Moutou, F., Boisseleau, D., & Chartier, C. (2011). *A qualitative risk assessment methodology for scientific expert panels*. *30*(3), 673–681.

El-Sayed, A., Awad, W., De Massis, F., Zilli, K., Donato, G. Di, Nuvoloni, R., Pelini, S., Sacchini, L., D'Alterio, N., & Giannatale, E. Di. (2018). Brucellosis: Evolution and expected comeback. *PLoS ONE*, *6*(3), 1–16. https://doi.org/10.1371/journal.pone.0213689

El-wahab, E. W. A., Hegazy, Y., El-tras, W. F., Mikheal, A., & Kabapy, A. F. (2020). *A multiple risk model for brucellosis at the human-animal interface in Egypt*. https://doi.org/10.1111/tbed.13295

Elelu, N., Aiyedun, J. O., Mohammed, I. G., Oludairo, O. O., & Odetokun, I. A. (2019). *Neglected zoonotic diseases in Nigeria: role of the public health veterinarian*. *8688*, 1–12. https://doi.org/10.11604/pamj.2019.32.36.15659

Fao/Who. (2005). *Food Safety Risk Analysis Part I An Overview and Framework Manual Provisional Edition*. *June*, 1–76.

FAO. (1999). Principles and Guidelines for the Conduct of Microbiological Risk Assessment. *FAO: Agriculture and Consumer Protection*, *63*, 68. http://www.fao.org/docrep/004/y1579e/y1579e05.htm

FAO, R. A. (2006). *Risk Assessment Outputs to Develop Practical Risk Management Strategies : April*.

FAO, & WHO. (2009). Risk assesment and its role in risk analysis. In *Principles and Methods for the Risk Assessment of Chemicals in Food* (pp. 2–1). World Health Organization.

Figueiredo, P. De, Ficht, T. A., Rice-ficht, A., Rossetti, C. A., & Adams, L. G. (2015). Pathogenesis and Immunobiology of Brucellosis Review of Brucella e Host Interactions. *The American Journal of Pathology*, *185*(6), 1505–1517. https://doi.org/10.1016/j.ajpath.2015.03.003

Franc, K. A., Krecek, R. C., & Häsler, B. N. (2018). *Brucellosis remains a neglected disease in the developing world : a call for interdisciplinary action*. 1–9. https://doi.org/10.1186/s12889-017-5016-y

Galińska, E. M., Zagórski, J., Em, G., & Brucellosis, Z. J. (2013). 233-238 in Humans-Etiology, Diagnostics, Clinical Forms. *Annals of Agricultural and Environmental Medicine*, *20*(2), 233–238. www.aaem.pl

Gibney, M. J., & Burstyn, P. G. (1980). Milk, serum cholesterol, and the Maasai. A hypothesis. *Atherosclerosis*, *35*(3), 339–343. https://doi.org/10.1016/0021-9150(80)90131-8

Gidel, R., Albert, J. P., Le Mao, G., & Retif, M. (1976). [Epidemiology of human and animal brucellosis in western Africa. The results of six studies in the Ivory Coast, Upper Volta, and Nigeria]. *Developments in biological standardization*, *31*, 187–200.

Głowacka, P., Zakowska, D., Naylor, K., Niemcewicz, M., & Bielawska-Drózd, A. (2018). Brucella – Virulence factors, pathogenesis and treatment. *Polish Journal of Microbiology*, *67*(2), 151–161. https://doi.org/10.21307/pjm-2018-029

Godfroid, J., & Nielsen, K. (2010). *Diagnosis of Brucellosis in Livestock and Wildlife*. 296–305. https://doi.org/10.3325/cmj.2010.51.296

Grützke, J., Gwida, M., Deneke, C., Brendebach, H., Projahn, M., Schattschneider, A., Hofreuter, D., El-Ashker, M., Malorny, B., & Dahouk, S. Al. (2021). Direct identification and molecular characterization of zoonotic hazards in raw milk by metagenomics using brucella as a model pathogen. *Microbial Genomics*. https://doi.org/10.1099/MGEN.0.000552

Gwida, M., Al Dahouk, S., Melzer, F., Rösler, U., & Neubauer, H. (2010). Brucellosis – Regionally Emerging Zoonotic Disease? *Croatian Medical Journal*, *51*(4), 289–295. https://doi.org/10.3325/cmj.2010.51.289

Hamdy, M. E. R., & Amin, A. S. (2002). Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. *Veterinary Journal*, *163*(3), 299–305. https://doi.org/10.1053/tvjl.2001.0681

Hanuš, O., Kučera, J., Samková, E., Němečková, I., Čítek, J., Kopec, T., Falta, D., Nejeschlebová, H., Rysová, L., Klimešová, M., & Elich, O. (2021). Raw cow milk protein stability under natural and technological conditions of environment by analysis of variance. *Foods*, *10*(9). https://doi.org/10.3390/foods10092017

Jansen, W., Demars, A., Nicaise, C., Godfroid, J., de Bolle, X., Reboul, A., & Al Dahouk, S. (2020). Shedding of Brucella melitensis happens through milk macrophages in the murine model of infection. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-65760-0

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G. S., MacMillan, A., & Cleaveland, S. (2010). Quantifying risk factors for human brucellosis in Rural Northern Tanzania. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0009968

Kaden, R., Ferrari, S., Jinnerot, T., Lindberg, M., Wahab, T., & Lavander, M. (2018). Brucella abortus: Determination of survival times and evaluation of methods for detection in several matrices. *BMC Infectious Diseases*, *18*(1), 1–6. https://doi.org/10.1186/s12879-018-3134-5

Kai, T. M. K., & Aotearoa, A. (2009). Microbiological Risk Assessment of Raw Cow Milk. *Fsanz*, *December*.

Kaltungo, B. Y., Saidu, S. N. A., Sackey, A. K. B., & Kazeem, H. M. (2014). *A review on diagnostic techniques for brucellosis*. *13*(1), 1–10. https://doi.org/10.5897/AJB2013.13442

Kaonga, K. (2006). *Imported food risk statement Raw milk cheese and Brucella spp* .

Karimuribo, E. D., Ngowi, H. A., Swai, E. S., & Kambarage, D. M. (2007). Prevalence of brucellosis in

crossbred and indigenous cattle in Tanzania. *Livestock Research for Rural Development*.

Kiros, A., Asgedom, H., & Duguma, R. (2016). *A Review on Bovine Brucellosis : Epidemiology , Diagnosis and Control Options*. *2*(3), 8–21.

Kouamé-Sina, S. M., Makita, K., Costard, S., Grace, D., Dadié, A., Dje, M., & Bonfoh, B. (2012). Hazard identification and exposure assessment for bacterial risk assessment of informally marketed milk in Abidjan, Côte d'Ivoire. *Food and Nutrition Bulletin*, *33*(4), 223–234. https://doi.org/10.1177/156482651203300402

Kunda, J., Fitzpatrick, J., Kazwala, R., French, N. P., Shirima, G., MacMillan, A., Kambarage, D., Bronsvoort, M., & Cleaveland, S. (2007). Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health*, *7*. https://doi.org/10.1186/1471-2458-7-315

Levira, F., & Todd, G. (2017). Urban Health in Tanzania: Questioning the Urban Advantage. *Journal of Urban Health*, *94*(3), 437–449. https://doi.org/10.1007/s11524-017-0137-2

Makita, K., Kurwijila, L., & Grace, D. (2012). *Boiled milk, food safety and the risk of exposure to milk borne pathogens in informal dairy markets in Tanzania □*. *November 2012*, 4–8.

Manyori, C. I., Mumba, C., Muma, J. B., Mwale, M. M., Munyeme, M., Bwanga, E. K., Häsler, B., Rich, K. M., & Skjerve, E. (2017). Quantitative risk assessment of developing salmonellosis through consumption of beef in Lusaka Province, Zambia. *Food Control*. https://doi.org/10.1016/j.foodcont.2016.10.027

Massis, F. De, Zilli, K., Di, G., Id, D., Nuvoloni, R., Pelini, S., Sacchini, L., Alterio, N. D., & Giannatale, E. Di. (2019). *Distribution of Brucella field strains isolated from livestock , wildlife populations , and humans in Italy from 2007 to 2015*. 1–16.

Mathew, C., Stokstad, M., Johansen, T. B., Klevar, S., Mdegela, R. H., Mwamengele, G., Michel, P., Escobar, L., Fretin, D., & Godfroid, J. (2015). First isolation , identification , phenotypic and genotypic characterization of Brucella abortus biovar 3 from dairy cattle in Tanzania. *BMC Veterinary Research*, 1–9. https://doi.org/10.1186/s12917-015-0476-8

Mburu, C., Bukachi, S. A., Id, K. H. T., Id, G. F., Id, K. S., Ezekiel, M., Id, B. B., Id, R. K., Kreppel, K., Rica, C., & Rica, C. (2021). *Lay attitudes and misconceptions and their implications for the control of brucellosis in an agro-pastoral community in Kilombero district , Tanzania*. 1–22. https://doi.org/10.1371/journal.pntd.0009500

Melubo, K. (2020). *Why are wildlife on the Maasai doorsteps ? Insights from the Maasai of Tanzania*. https://doi.org/10.1177/1177180120947823

Méndez-González, K. Y., Hernández-Castro, R., Carrillo-Casas, E. M., Monroy, J. F., López-Merino, A., & Suárez-Güemes, F. (2011). Brucella melitensis survival during manufacture of ripened goat cheese at two temperatures. *Foodborne Pathogens and Disease*, *8*(12), 1257–1261.

https://doi.org/10.1089/fpd.2011.0887

Miliotis, M. D., & Buchanan, R. L. (2008). Microbial Risk Assessment. *Microbiologically Safe Foods*, 377–394. https://doi.org/10.1002/9780470439074.ch19

Muma, J. B., Samui, K. L., & Oloya, J. (2007). *Risk factors for brucellosis in indigenous cattle reared in livestock – wildlife interface areas of Zambia*. *80*, 306–317. https://doi.org/10.1016/j.prevetmed.2007.03.003

Muma, J. B., Syakalima, M., Munyeme, M., Zulu, V. C., Simuunza, M., & Kurata, M. (2013). Bovine tuberculosis and brucellosis in traditionally managed livestock in selected districts of Southern Province of Zambia. *Veterinary Medicine International*, *2013*. https://doi.org/10.1155/2013/730367

Muma, Pandey, G. S., Munyeme, M., Mumba, C., Mkandawire, E., & Chimana, H. M. (2012). Brucellosis among smallholder cattle farmers in Zambia. *Tropical Animal Health and Production*, *44*(4), 915–920. https://doi.org/10.1007/s11250-011-9987-x

Musallam, I., Prisca, A., Yempabou, D., Ngong, C. C., Fotsac, M., Mouiche-mouliom, M., Marc, J., Feussom, K., Bosco, J., Ntakirutimana, D., Fane, A., Dembele, E., Doumbia, A., Akpemdo, A., Ayih-akakpo, P. S., Pato, P., Pali, M., Tapsoba, A. S. R., Minougou, G., … Guitian, J. (2019). Acta Tropica Brucellosis in dairy herds : A public health concern in the milk supply chains of West and Central Africa. *Acta Tropica*, *197*(May), 105042. https://doi.org/10.1016/j.actatropica.2019.105042

Muturi, M., Bitek, A., Mwatondo, A., Osoro, E., Marwanga, D., Gura, Z., Ngere, P., Nganga, Z., Thumbi, S. M., & Njenga, K. (2018). Risk factors for human brucellosis among a pastoralist community in South - West. *BMC Research Notes*, 1–6. https://doi.org/10.1186/s13104-018-3961-x

Nguna, J., Dione, M., Apamaku, M., Majalija, S., Mugizi, D. R., Odoch, T., Drago, C., Tumwine, G., Kabaasa, J. D., Curtis, K., Graham, M., Ejobi, F., & Graham, T. (2019). *seropositivity in cattle , goats and humans in Iganga District , Uganda*. *8688*, 1–10. https://doi.org/10.11604/pamj.2019.33.99.16960

Njarui, D., Gatheru, M., Wambua, J., Hospital, L. C., & Nguluu, S. (2011). *Consumption Patterns and Preference of Milk and Milk Products among Rural and Urban Consumers in Semi-Arid Kenya This article was downloaded by : On : 31 May 2011 Access details : Access Details : Free Access Publisher Routledge Ecology of Food and Nutrit*. *June 2016*. https://doi.org/10.1080/03670244.2011.568908

Ntirandekura, J. B., Matemba, L. E., Kimera, S. I., Muma, J. B., & Karimuribo, E. D. (2020). Association of brucellosis to abortions in humans and domestic ruminants in Kagera ecosystem, Tanzania. *Transboundary and Emerging Diseases*, *67*(5), 1879–1887. https://doi.org/10.1111/tbed.13516

OIE. (2018). Brucellosis (Brucella Abortus, B Melintesis and B. suis). *OIE Terrestrial Manual 2018*, *1*(4), 355–398.

Olsen, S. C., & Palmer, M. V. (2014). Advancement of Knowledge of Brucella Over the Past 50 Years. *Veterinary Pathology*, *51*(6), 1076–1089. https://doi.org/10.1177/0300985814540545

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). *The new global map of human brucellosis*. *6*(February), 91–99.

Poester, F. P., Nielsen, K., Samartino, L. E., & Yu, W. L. (2015). *Diagnosis of Brucellosis Diagnosis of Brucellosis*. *May 2010*. https://doi.org/10.2174/1874318801004010046

Roy, S., Mcelwain, T. F., & Wan, Y. (2011). *A Network Control Theory Approach to Modeling and Optimal Control of Zoonoses : Case Study of Brucellosis Transmission in Sub-Saharan Africa*. *5*(10). https://doi.org/10.1371/journal.pntd.0001259

Sambu, R. M., Mathew, C., Nonga, H. E., Lukambagire, A. S., Yapi, R. B., Akoko, J., Fokou, G., Keyyu, J. D., Bonfoh, B., & Kazwala, R. R. (2021). *Circulating Brucella species in wild animals of the Serengeti ecosystem , Tanzania*.

Sanogo, M., Thys, E., Achi, Y. L., Fretin, D., Michel, P., Abatih, E., Berkvens, D., & Saegerman, C. (2013). Bayesian estimation of the true prevalence, sensitivity and specificity of the Rose Bengal and indirect ELISA tests in the diagnosis of bovine brucellosis. *Veterinary Journal*, *195*(1), 114–120. https://doi.org/10.1016/j.tvjl.2012.06.007

Seleem, M. N., Boyle, S. M., & Sriranganathan, N. (2010). Brucellosis: A re-emerging zoonosis. *Veterinary Microbiology*, *140*(3–4), 392–398. https://doi.org/10.1016/j.vetmic.2009.06.021

Spickler, & Rovid., A. (2018). *Brucellosis: Brucella melitensis*. Centers for Disease Control and Prevention (CDC). Brucellosis. Http://Www.Cdc.Gov/Brucellosis.

Spink, W. (1954). *Family studies on Brucellosis*. 127–140.

Swai, E. S., & Schoonman, L. (2010). The use of rose bengal plate test to asses cattle exposure to Brucella infection in traditional and smallholder dairy production systems of Tanga Region of Tanzania. *Veterinary Medicine International*, *2010*. https://doi.org/10.4061/2010/837950

Teske, S. S., Huang, Y., Tamrakar, S. B., Bartrand, T. A., Weir, M. H., & Haas, C. N. (2011). Animal and Human Dose-Response Models for Brucella Species. *Risk Analysis*. https://doi.org/10.1111/j.1539-6924.2011.01602.x

Tesson, V., Federighi, M., Cummins, E., Mota, J. de O., Guillou, S., & Boué, G. (2020). A systematic review of beef meat quantitative microbial risk assessment models. In *International Journal of Environmental Research and Public Health*. https://doi.org/10.3390/ijerph17030688

Wainaina, M., Aboge, G. O., Omwenga, I., Ngaywa, C., Ngwili, N., Kiara, H., Wamwere-Njoroge, G., & Bett, B. (2020). Detection of Brucella spp. in raw milk from various livestock species raised under pastoral production systems in Isiolo and Marsabit Counties, northern Kenya. *Tropical Animal Health and Production*, *52*(6), 3537–3544. https://doi.org/10.1007/s11250-020-02389-1

Waiswa, C., Eisler, M. C., Welburn, S. C., Makita, K., & Fe, E. M. (2010). *How Human Brucellosis Incidence in Urban Kampala Can Be Reduced Most Efficiently ? A Stochastic Risk Assessment of Informally-Marketed Milk*. *5*(12). https://doi.org/10.1371/journal.pone.0014188

Wyatt, H. V. (2013). *Lessons from the history of brucellosis Goats and the Royal Navy : the silent service Goats and Themistocles Zammit*. *32*(1), 17–25.

APPENDICES.

A questionnaire on cow milk consumption in the district of Monduli and Longido in Arusha region.

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF DISEASE CONTROL

INTERVIEWER-ADMINISTERED QUESTIONNAIRE

Research Study on

"**A quantitative risk assessment of exposure to Brucella species through consumption of raw cow milk in Arusha -Tanzania**"

Questionnaire serial number: ----------

Introduction and consent section:

INFORMED CONSENT

Greetings. My name is Enock Magoke Ndaki, I am a post-graduate student of the School of Veterinary Medicine-University of Zambia (UNZA), Lusaka Zambia.

We are conducting a study that asks questions about milk consumption scenario in pastoral households around this area so as we can easily asses the present risk of exposure to the disease called Brucellosis. This study is taking place in October 2021 and we would very much appreciate your participation in this study. This information will help the government to plan better health care services for the people of Tanzania. Whatever information you provide will be kept strictly confidential and participation in this study is voluntary, if we should come to any question you don't want to answer, just let us know and we will go on to the next question. However, we hope that you will fully participate in this study since your views are important. Would you like to participate in this study? YES. ◯ NO. ◯

Signature of respondent: …….

Name of interviewer ……

Date of interview (DD/MM/YY) ……..

Start time……..… End time…….

SECTION A: HOUSEHOLD CHARACTERISTICS

Instruction: Kindly circle the option that applies to you

1 Gender of respondent

a) Male
b) Female

2 Age (in years) of respondent

a) 18 – 20
b) 21-30
c) 31-40
d) 41-50
e) 51-60
f) Above 60

3 Education level of respondent

a) None
b) Primary
c) Secondary
d) Tertiary
e) Others (Specify)……..

4 Employment status of respondent

a) Formal employment (Government/ private salary worker)
b) Self employed
c) Allowance (remittance)
d) Unemployed
e) Others specify

5 Marital Status of respondent

a) Married
b) Single

c) Divorced/ separated

d) Widowed

6 Number of people living in the household (indicate the number of people)

a) 1-2

b) 3-12

c) 13-18

d) Above 18

7 Estimated household monthly income range (include all sources of income e.g. salary, bonus, housing allowance?

a) Less than 500,000 Tsh

b) 500,000 Tsh –1,000,000 Tsh

c) Above 1,000,000 Tsh,

SECTION B: MILK CONSUMPTION

Instruction: Kindly select and circle the option that applies to you

1. Do you drink milk at your household?
    a) Yes
    b) No

2. How often do you drink milk in your household?
    a) Every day
    b) Once every two weeks
    c) Once every week
    d) Once a month

3. How many litres of milk have you brought/produced?

4. How many meals with milk are normally prepared from the amount of milk brought?

5. How is the milk you brought often consumed?
    a) Raw
    b) Half cooked
    c) Well cooked
    d) Others (specify)……

6. Where is the source of milk you consumed in your household?
    a) Your own cattle
    b) Neighbour's
    c) Local market
    d) Others (specify)……

7. How many litres of milk do you consume in a day?
    a) Less than 0.5
    b) 0.5-1
    c) 1-2
    d) Above 2 ……

8. How often do you consume milk in a week?

a) 1-3 times
b) 4-6 times
c) 7-10 times
d) 11-13 times
e) 14-16 times
f) 17-20 times
g) More than 20 times, (specify)……..

9. What is usually the average time (in hours) from milk production/purchase to consumption?
    a) Less than 1 hour
    b) 1-3 hours
    c) 3-5 hours
    d) More than 5 hours, specify……

10. Where do you store the milk before consumption?
    a) In the room temperature
    b) In the refrigerator
    c) In deep freezer
    d) Others, specify……

11. Have you been to hospital due to fever like condition?
    a) Yes
    b) No

12. How often does this condition occur?
    a) Once a year
    b) Twice per year
    c) 3-4 times per year
    d) Several times per year

13. What was the diagnosis (if you can remember)?

    a) Malaria
    b) Typhoid
    c) Brucellosis
    d) Others (specify)……..

14. Is there any other member of your family has been to hospital due to fever like condition?

    a) Yes
    b) No

15. What was the diagnosis (if you can remember)?

    a) Brucellosis
    b) Malaria
    c) Typhoid
    d) Others (specify)……..

16. How far (in kilometre) is the hospital from here?

    a) Below 1 km
    b) 1-5 km
    c) 5-10 km
    d) 10-20 km
    e) More than 20km, (specify)…….

17. What is the average amount of money costs that illness?

    a) Below 10,000 Tsh
    b) 10,000-50,000 Tsh
    c) 50,000-100,000 Tsh
    d) Above 100,000 Tsh, specify……

SECTION C: HOUSEHOLD FOOD SAFETY PRACTICES

Instruction: Kindly select the option that applies to you

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

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

2. I clean thoroughly with soap, surfaces and equipment used for milk handling, preparation and drinking before re-using on other food.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

3. I use utensils that are recommended for milk handling and storage.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

4. I separate raw and cooked milk during storage.

a) Always
b) Most times
c) Sometimes
d) Not often

e) Never

5. I boil the milk until it is very hot throughout.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

6. I check and throw away spoiled milk.

a) Always
b) Most times
c) Sometimes
d) Not often
e) Never

THANK YOU VERY MUCH FOR YOUR PARTICIPATION

Principal Investigator

Name: Enock Magoke Ndaki

Phone number: +260770552833 & +255755940274

Address: The University of Zambia School of Veterinary Medicine

Email address: enockndaki5@gmail.com