

ORIGINAL ARTICLE

# Risk of human exposure to *Brucella* species through the consumption of milk among traditional cattle farmers in the western province of Zambia

MARY MUBIANA<sup>1,2\*</sup>, CHISONI MUMBA<sup>1</sup>, DOREEN SITALI<sup>1</sup>, MULENGA MALATA<sup>1,3</sup>, ISABEL NAMFUKWE LUAMBIA<sup>4</sup>, JOHN BWALYA MUMA<sup>1</sup>

## AFFILIATIONS:

<sup>1</sup>Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

<sup>2</sup>Malcolm Moffat College of Education, P/B 1, Serenje, Zambia

<sup>3</sup>Zambia Compulsory Standards Agency, Great North Road, P.O. Box 31302, Lusaka, Zambia

<sup>4</sup>School of Nursing and Midwifery and Health Sciences, Department of Nursing Sciences, Mulungushi University, P.O. Box 80415, Town Campus, Kabwe, Zambia

## CORRESPONDENCE:

Mary Mubiana  
Department of Disease Control,  
School of Veterinary Medicine,  
University of Zambia, P.O. Box  
32379, Lusaka, Zambia  
Email: mubsma@gmail.com

## ABSTRACT

### BACKGROUND:

Consumption of raw milk is a common practice among traditional livestock farmers. Recently, Zambia has experienced increased milk production.

### OBJECTIVE:

This study estimated the risk of human exposure to *Brucella* pathogens through the consumption of raw milk among traditional cattle farmers in the western province of Zambia.

### METHODS:

The research used a cross-sectional study design to collect the primary data. Risk estimation was modelled using the swift quantitative microbiology risk assessment (SQMRA) in the Excel platform using both primary and secondary data sources. The risk assessment was performed according to the Microbial Risk Assessment protocol of the Codex Alimentarius Commission (CAC), 1999. The SQMRA model which was developed by Evers and Chardon (2010) was used in the study.

### RESULTS:

The results from the questionnaire survey on milk consumption showed that 38.5% of the people consumed milk once a day; 36.9% consumed milk twice a day and 24.6% consumed milk once a week. Most respondents (77.9%) never boiled milk before consumption, with only 22.1% of the 122 respondents boiling their milk before consumption. The average serving portion of milk was 99mls/person/ day. Results of the SQMRA model indicated that at a serving portion of 99mls in households, an infectious dose of 100 (ID50) and a contamination concentration of 15 cfu/ g, 561 people out of the population of 219, 218 would get ill, representing a probability density of 0.0026%. At a serving portion of 99mls in households, a contamination concentration of 1000 cfu/g, and an infectious dose of 100 (ID50), 794 people out of 219 218 would get ill, representing a probability density of 0.0036%.

### CONCLUSION:

The study concludes that the risk of developing brucellosis through the consumption of milk among traditional cattle farmers is low, however, there is a need to raise awareness about milk pasteurization and good hygiene practices among traditional cattle farmers.

### KEYWORDS:

Risk assessment, *Brucella* species, Brucellosis, Raw milk, Traditional cattle farmer

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## INTRODUCTION

Brucellosis is a zoonotic disease affecting livestock caused by several bacteria in the genus *Brucella*.<sup>(1)</sup> The disease mostly affects livestock, and it is caused by among four *Brucella* species known to cause disease in humans (*Brucella abortus*, *Brucella melitensis*, *Brucella canis*, *Brucella suis*) and *Brucella melitensis*<sup>2</sup>. As a foodborne disease, human *Brucella* infections occur mainly by ingesting raw milk or milk products and contact with animals.

Brucellosis poses significant public health threats, especially in regions with suboptimal hygiene and food safety, and mainly affects livestock and wildlife with human contractions which occur by consuming contaminated animal products or interacting with infected animals<sup>(2)</sup>. *Brucella* can be transmitted from animals to humans through various routes such as in unpasteurized milk, dairy products, and undercooked meat from infected animals, exposure to diseased animals, and can be present in dust and other airborne particles where infected animals have been kept or slaughtered<sup>(3)</sup>.

People at risk of exposure are farmers, shepherds, butchers, laboratory workers, veterinarians, and slaughterhouse workers who may contract the disease through inhalation of contaminated aerosols, contact with conjunctival mucosa, or entry of bacteria through cuts and abrasions in the skin as a result of contact with infected animals or their products. Human brucellosis represents itself in different ways, presenting various diagnostic difficulties because it mimics many other diseases such as malaria, typhoid, rheumatic fever, joint diseases, and other conditions causing pyrexia<sup>(4)</sup>.

Humans become susceptible to *brucella* species depending on how the immune system works, the size of the inoculum, and the species of *brucella*<sup>(5)</sup>. Brucellosis is recognized as an occupational health hazard and is termed a neglected zoonotic disease among agro-pastoral communities and is widely<sup>(6)</sup>. It mostly affects adult animals because susceptibility increases after sexual maturity and pregnancy when sex hormones and erythritol increase in concentration with age and sexual maturity<sup>(7)</sup>. Consumption of unpasteurized milk and milk-based products including

homemade milk products such as cultured milk can cause brucellosis, a highly contagious zoonotic disease<sup>(8)</sup>. The disease is a significant global public health concern, especially in regions where there are limited resources and poor management practices<sup>1</sup>.

The food safety risk analysis, used as a tool for controlling hazards in foods, is becoming internationally accepted as an effective strategy for assuring food safety<sup>1</sup>. It should be the role of official bodies to use risk analysis to determine realistic and achievable risk levels for food-borne hazards and to base food safety policies on the practical application of the results of these analyses<sup>(9)</sup>. Furthermore, the elements of food safety should form the basis on which the government agencies and industry establish food safety objectives<sup>9</sup>. Brucellosis is prevalent in Southern and Western provinces of Zambia and poses a significant public health risk to traditional farmers<sup>10</sup>.

A study conducted by Mubanga et al (2021) showed that 20.3% (95% CI: 14.6–27.5) of people showed serological evidence of *Brucella* spp exposure among traditional Zambian livestock farmers. The Seropositivity was mostly among herdsmen and abattoir workers was 14.4% (95% CI: 9.2–21.8) and 46.4%, (95% CI: 28.8–65.0), respectively<sup>7</sup>. Poverty and lack of awareness of what zoonoses in many people, especially in rural areas, makes them consume high-risk foods, such as unpasteurized milk or uninspected meat obtained in informal food markets<sup>7</sup>.

The One Health approach, which involves collaboration between experts in human and animal health and the environment including multiple other sectors is vital in the control and prevention of brucellosis<sup>(10)</sup>. The important question to answer is, “What is the risk of being exposed to *Brucella* spp. among traditional farmers who consume raw milk?” Despite evidence of the presence of *Brucella* species in raw milk from previous studies, very little is known about the risk of being exposed to brucellosis through consumption of raw milk in Western province and Zambia in general. Therefore, this study aimed to estimate the risk of human exposure to *Brucella* species through the consumption of milk among traditional cattle farmers in the Western province of Zambia.

## METHODS

### Study design and Data sources

The research used a cross-sectional study design to collect the primary data. Risk estimation was modeled using the SQMRA in the Excel platform using both primary and secondary data sources.

### Setting

The study was carried out in the areas of Zambia that are known to be endemic for brucellosis in livestock; these included Mongu and Senanga districts in the Western province. Western province covers an area of about 126, 386 km<sup>2</sup>. The choice of the province and districts was based on the fact that the Western province is one of the major livestock-producing provinces and brucellosis has been reported (Muma et al., 2012). The study was carried out between August 2022 and January 2023.

After going through most of the published literature, there was still an information gap on consumption patterns and serving portions of milk in Zambia. A survey questionnaire was conducted to fill these information gaps. The structured questionnaire was developed and administered to address the information gaps on consumption patterns and consumption levels of raw milk amongst traditional farmers in Western province. The questionnaire also helped to collect information on portions consumed and portion sizes in ml/ L. It also guided on the risks associated with the consumption of raw milk.

The questionnaire included socio-demographic factors (sex, age, marital status, religion, education level, and occupation), knowledge of brucellosis, contact with animals and animal products, and participant's involvement in milking and assisting animals to give birth. The traditional farmers were interviewed under different scenarios: as they brought their milk for sale at the milk collection centers, at the kraals when milking, and also from selected households.

### Participants and study size

The study used a convenient sampling method. To obtain estimates on the serving portions and consumption patterns, the sample size of 122 respondents was used from the two districts (Mongu

and Senanga) in Western province. Sixty (60) respondents were from Mongu while sixty-two (62) were from Senanga. The survey was done by interviewing farmers who brought milk for sale at milk collection centers, at the kraals as they were milking their animals, and from selected households.

### Data sources

Secondary information was obtained through a literature review to collect data for the input parameters in the model. The desktop research was done which depended on scientifically peer-reviewed papers (secondary data). The literature review was guided by research questions based on the Swift Quantitative Microbiological Risk Assessment model as shown in Table 1. The literature search was conducted on major databases such as Web of Science and Pub Med, "Google Search Engine" and "Google Scholar". In addition, some literatures were also obtained from government reports and Non-Governmental Organisations. The search of key terms such as milk consumption, brucellosis, milk hygiene, the population of Senanga and Mongu districts, and brucella contamination of milk and milk value chain were used. This cross-sectional study was designed to quantitatively assess the risk of consuming raw milk contaminated with *Brucella* from locally kept dairy animals using SQMRA and to determine the percentage of people likely to be infected. The risk assessment was done according to the Microbial Risk Assessment protocol of the Codex Alimentarius Commission (CAC), 1999. The SQMRA model which was developed by Evers and Chardon (2010) was used in the study. Quantitative Microbiological Risk Assessment (QMRA) is a methodology to evaluate microbiological public health risks, which can be food, direct contact, or environmental-related. In SQMRA, mathematical modelling is usually applied to describe a food production chain. These models, together with available data, were used to calculate the presence and propagation of *Brucella* in a specific production chain, and the exposure of *Brucella* species ('dose') to consumers. Dose-response models were subsequently used to estimate the number of cases of illness. Some risk inputs were required for the exposure assessment; a quantitative household chain model, information on handling of milk, contamination rates, and concentrations of milk/ milk products with *Brucella*.

These inputs were collected from the secondary data by desktop review of some scientifically written papers.

### Risk Assessment Framework

The present risk assessment includes the steps: (i) hazard identification, which searches to identify the risk of brucellosis associated with *Brucella* in milk; (ii) hazard characterization, which focuses on evaluating the nature of adverse health effects associated with

food-borne *Brucella* species and how to quantitatively assess the relationship between the magnitude of the food-borne exposure and the likelihood of adverse health effects; (iii) exposure assessment, in which the likelihood and magnitude of exposures to *Brucella* as a result of consumption of raw milk is estimated; and finally, (iv) risk characterization, which estimates the risk of brucellosis in a given population for a given set of input data.

**Table 1.** Literature Review Guide

<p><b>Case definition,</b></p> <ul style="list-style-type: none"> <li>• What is the pathogen of interest?</li> <li>• What is the food product of interest?</li> <li>• What is the population size?</li> <li>• What are the population characteristics?</li> <li>• What is the consumption period?</li> </ul>	<p><b>Consumption data,</b></p> <ol style="list-style-type: none"> <li>1. How many portions of milk are consumed in the population per consumption period?</li> <li>2. What is the average size of one portion of milk?</li> <li>3. What percentage of portions of milk is contaminated at retail?</li> <li>4. What is the concentration in log<sub>10</sub> of colony-forming units (cfu) per gram in contaminated portions of milk?</li> </ol>
<p><b>Kitchen cross-contamination</b></p> <ol style="list-style-type: none"> <li>5. given contaminated portions of milk, what percentage of the portions of milk will contaminate the environment (Such as hands and kitchen equipment)?</li> <li>6. Given contaminated portions, what percentage of cfu's in a portion will contaminate the environment? e.g. hands and kitchen equipment?</li> <li>7. Given cross-contamination, what percentage of cfu's in the environment ends up being ingested?</li> </ol>	<p><b>Kitchen preparation,</b></p> <ol style="list-style-type: none"> <li>8. What percentage of portions of milk is prepared; cooked, half done (roasted), raw?</li> <li>9. What percentage of cfu's on a portion will survive during preparation? Cooking, frying, and roasting?</li> </ol>
<p><b>Infection and illness</b></p> <ol style="list-style-type: none"> <li>10. at what dose (number of cfu's) per portion will half of the exposed population get infected?</li> <li>11. What percentage of infected people will get ill?</li> </ol>	

### Ethical clearance

Ethical clearance was obtained from Excellence in Research Ethics (ERES) Converge (Reference no 2018 – Oct – 031). The authority to conduct the study was granted by the Ministry of Fisheries and Livestock (Senanga and Mongu districts). Informed written consent was sought from the respondents willing to participate after fully explaining to them the purpose of the study, the importance of the findings, and their right to participate. Confidentiality was maintained and anonymity was ensured.

## Statistical methods

The data obtained from the questionnaire was coded and entered in Microsoft Excel 2010, exported, cleaned, and analyzed using SPSS (version 20, IBM Analytics, Armonk, NY). Descriptive statistics was used to calculate the average serving portions, consumption patterns, and kitchen preparation methods of milk.

Data from the literature search was entered in the Excel version of the SQMRA model developed by Evers and Chardon (2010). The model was run twelve times and came up with results for the exposure assessment following the household exposure pathways shown in Table 2.

**Table 2.** sQMRA input parameters for the serving portion of 99mls under the household risk exposure pathway

INPUT PARAMETERS			
<b>pathogen:</b>			Brucella species
<b>food product:</b>			Milk
<b>population size:</b>			219218
<b>pop. characteristics:</b>			Population of Mongu & Senanga
<b>consumption period:</b>			One year
Number	parameter	Question	value
1	N	portions consumed	2.2E+04
2	M	portion size in grams	99
3	Sr/+	prevalence in retail	8.7%
4	Cr/+	cfu per gram contaminated product	15.0
5	Scc/r	portions causing cross. cont.	45%
6	Fcc	cfu's from portions to environment	30%
7	Fei	cfu's from environment to ingestion	8.2%
8	Sprd/cc	portions prepared done	30%
8	Sprh/cc	portions prepared half-done	50%
8	Sprr/cc	portions prepared raw	20%
9	Fprd	cfu's surviving when prep. done	0%
9	Fprh	cfu's surv. when prep. half-done	13%
9	Fprr	cfu's surviving when prep. raw	100%
10	ID50	ID50 (number of cfu's)	1.0E+02
11	Pill/inf	% people infected who get ill	50%
<b>time stamp:</b>		13/10/2023 21:18	
<b>sQMRA-tool</b>			

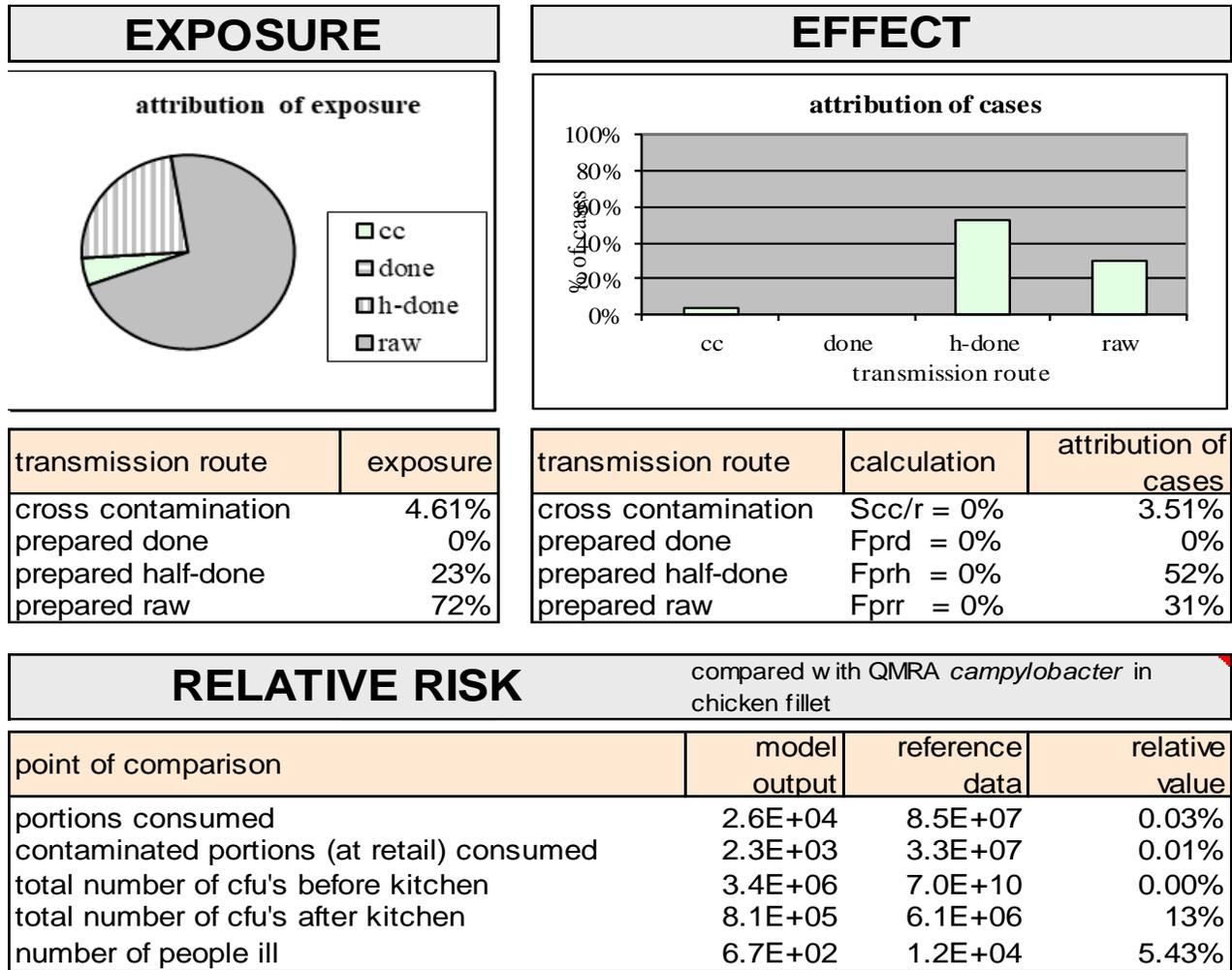


Figure 1. Model output at 99 mls serving portion, 15cfu/ g, and ID 50at 100 cfu (probability for the household risk exposure pathway)

## RESULTS

### Participants

The study included a convenient sample size of 122 small-scale farmers in the two districts which are Mongu and Senanga in Western province. This included 60 from Mongu and 62 from Senanga to obtain representative estimates on serving portions and consumption patterns. The study included cattle farmers who were above 18 years of age excluded those who were below 18 years old and those who did not own cattle were left out of the study.

### Descriptive data and outcome data

#### Consumption data

A portion size of milk (litres) in this study was considered the amount of milk consumed in one meal

or during a single eating occasion. Regardless of the low production of milk, that is, an average of 1.5 litres per cow per day(11), family members consumed most of the milk produced by small-scale farmers, and part of it sold (51.6% of farmers used to sell milk regularly) locally to milk collection centres and nearby markets. The commercial sector of the livestock industry constitutes 20% of the total population and the traditional sector comprises 80% of the total population<sup>26</sup>. The study found out that 80% of the traditional cattle farmers in Western province consumed milk because most of them owned cattle. The survey revealed that, on average, milk was consumed once (breakfast). Therefore, the number of portions consumed by the population was calculated by multiplying the one served by the total population

(219 218), which was 219, 218. Milk derived from clinically healthy animals can also contain *Brucella* which originates from environmental contamination occurring during the collection and storage of milk<sup>3</sup>.

#### *Serving portions and consumption data*

A serving portion was the measured amount of milk, that is, 100 mls of milk. Results from the survey showed that on average, the serving portion of milk per serving at a household level was 100mls (range of 66mls to 100mls). That is, 53.3% of farmers consumed milk daily and the quantity was 100mls, 1.8% (95% CI: 1.6 to 2.0) per person per day. The preparation of milk at the household level was well done 30 % (boiled), half-done accounted for 50% (sour milk) and prepared raw 20%. Sour milk was considered to be half-done because fermentation reduces milk-borne health risks by reducing the number of brucella pathogens but does not eliminate milk borne health risks<sup>28</sup>. Most farmers (39.3%) consumed milk once a day, 36.9% consumed milk twice a day, and 23.8% consumed milk once a week.

#### *Contamination of milk at retail outlets*

Pasteurized milk can also be contaminated during packaging and dispensing at shops and/or restaurants(12). In Mali and Belgium, for example, it was estimated in 2003 and 2019 that up to 30% and 20.5% of milk and dairy products at selling points in Bamako were contaminated with *Brucella*(13). More recently, in 2014, it was estimated that 25.6% of milk in the Adamawa and North Regions of Cameroon was contaminated with *Brucella*. A study carried out in Kenya and Iraq also revealed that 5% to 25% of raw and pasteurized milk sold in rural and urban areas was contaminated with *Brucella* (14).

The study used Salmonella bacteria at 30 % of portions that could contaminate the environment such as hands, and the percentage of coliform forming units (CFUs) on a portion that would contaminate the environment was 45%<sup>12</sup>.

#### *Kitchen cross contamination*

Milk was consumed raw, boiled and fermented (sour milk). Sour milk was preserved and kept for several

hours. Unhygienic environments (holding containers washed in contaminated water), and storage of milk could expose the milk to contaminants. Contamination of milk was likely to be due to unhygienic handling and poor sanitation. *Brucella abortus* may be transmitted by fomites when containers and other utensils are washed with contaminated water(15).

Therefore, it was less likely that *Brucella* could contaminate milk in a cup or container from the environment. Nevertheless, in situations where there were some spills of milk from the cups into the environment, a likelihood of contaminating the kitchen environment could occur<sup>15</sup> *Brucella* species have been reported to survive in the environment for periods ranging from less than a day to greater than 8 months(16). The percentage of portions that could contaminate the environment such as hands was estimated at 3% and the percentage of coliform forming units (CFUs) on a portion that would contaminate the environment was estimated at 2%<sup>5</sup>. Consequently, even in domestic kitchens, contamination could further involve other equipment, utensils, and other food products. *Brucella* survives well at a temperature reached by refrigerators or freezers; other refrigerated or frozen foods may become contaminated(17).

#### *Kitchen preparation*

The questionnaire survey on milk preparation revealed that 30% of the milk was prepared well (boiled). Consumption of raw milk was also estimated at 68.7% while that of cultured milk was 87.3%<sup>8</sup>, although this pattern could have slightly changed over time. The percentages of microorganisms surviving in a contaminated portion of milk were 0% when milk was well done, 13% when half done, and 100% when raw. Boiling was used to represent milk that was well done because the process achieves higher temperatures than pasteurization and could potentially destroy all zoonotic pathogens(18). Evers and Chardon (2010) also used 0% in well done and 100% when prepared raw in their SQMRA model.

#### *Hazard Identification*

*Brucella abortus* is a common source of human infection and has a high zoonotic potential. Its ability to

successfully survive and replicate within different host cells explains its pathogenicity(19). Human brucellosis caused by *Brucella abortus* also called Bang’s disease is characterized by a prolonged and recurrent undulating fever that can last for many months or even years if not treated<sup>6</sup>. In Zambia, only *Brucella abortus* biovar 2 has been isolated from cattle milk in the Southern province(20).

## Exposure assessment

### Case definition

A consumption period of one year was used to assess the number of people who would get ill from brucellosis in the study, giving the number of people who would get ill per year. The population of the Western province was taken to be 219, 218 in the model as projected by the Central Statistical Office(21). Brucellosis was defined as a “confirmed case” when *B. abortus* was isolated from patients who had common symptoms such as fever, fatigue, anorexia, headache, weakness, profuse sweating, chills, arthralgia, depression, weight loss, hepatomegaly, splenomegaly, and generalized aching(22).

### Infection and illness

The dose (no CFUs) per ml of a portion that would cause infection or disease (brucellosis) in 50% of the total

exposed host population (ID 50) was taken to be a minimum of 10 CFUs/ml and a maximum of 100 colony forming units (CFU) per ml(23). 15% to 30% of *Brucella* survives after ingestion, therefore, in the study it was assumed that 17% of people would get ill after ingesting milk that was contaminated with *Brucella* at the dose mentioned above<sup>15</sup>. The average concentrations of cfu per ml in a contaminated portion were taken to be 100 cfu/ ml as minimum and 10000 cfu/ ml as maximum(24)<sup>1</sup>.

### Risk characterization

A total of 8 simulations (4 for those who consumed 99 ml of milk and 4 for those who consumed 100 ml of milk) were simulated. Each simulation produced a summary of the input parameters (table 2) and the output model results for the risk of developing brucellosis through the consumption of raw milk among traditional cattle farmers (Figure 1) in a household risk pathway. Table 3 (risk characterization) summarises the results of the outputs of the 8 simulations. Of the 8 case scenarios, scenario 3 for those who consumed 99 ml and scenario 3 for those who consumed 100 ml of milk produced the highest results. This simply means that 3200 out of the population of 219,218 people were at risk of exposure to *Brucella* pathogen through the consumption of raw milk.

**Table 3:** Summary of the outputs of 8 simulations under the household risk exposure pathway

Scenario	Portion (ml)	Cfu/ml	ID 50	Model output (No. of people ill)	Qualitative risk	Probability
1	99	15	100	561	Medium	2.6x 10 <sup>3</sup>
2	99	15	1000	172	Low	7.8x10 <sup>4</sup>
3	99	1000	100	794	High	3.6x10 <sup>3</sup>
4	99	100	100	770	High	3.5x10 <sup>3</sup>
1	100	15	1000	174	Low	7.9x10 <sup>4</sup>
2	100	50	100	733	High	3.3x10 <sup>3</sup>
3	100	100	50	790	High	3.6x10 <sup>3</sup>
4	100	100	100	771	High	3.5x10 <sup>3</sup>

### Uncertainty

Information gaps on consumption patterns and serving portions of milk were identified through a questionnaire survey which was done so that data can be collected from the real areas of the study, instead of using information from other areas. The pathogen numbers were followed throughout the milk chain which in this case starts from the milking areas (kraal) and ends up with the number of people getting ill (farm to folk). The relative risk was compared with the reference point in the model to prevent overestimation of results. To follow the model very well, milk was measured in grams. “Well done” on preparation was taken to be boiled milk, and “half-done” was taken to be fermented (sour) milk while raw milk was taken raw (not boiled). The observation is that what determines the risk is the preparation and consumption of milk.

## DISCUSSION

The present study was carried out to assess the risk of traditional cattle farmers being exposed to *Brucella* pathogens through the consumption of milk in the Western province of Zambia. A total number of 122 respondents were interviewed to determine the consumption patterns and serving portions. The target population was traditional cattle farmers in the Mongu and Senanga districts of Western Province. These farmers consumed milk that came from their animals and sometimes bought milk from the neighborhood if they had not produced any from their animals.

The survey on consumption patterns revealed that 38.5% of farmers consumed milk once a day, 36.9% consumed milk twice a day, and 23.8% consumed milk once a week, while 0.8% consumed milk once a month. The serving portions of milk were 0.099 litres (99mls)/person/ day. That is, 53.3% of farmers consumed milk on a daily basis and the quantity was 99mls, 1.8 (95% CI: 1.6 to 2.0) per person per day, the results are similar to a study carried out in Kenya(25). This contributed to the low serving portion of milk in different households. Milking was done by herdsman and they were not washing their hands during milking, hence leading to high levels of milk contamination. However, milk can be contaminated with *Brucella* due to poor handling, improper cleaning of equipment, and contact surfaces(26). Since most of the farmers used

plastic containers (95%), there was a possibility of milk contamination because it is very difficult to remove all milk residues from the milk contact surfaces of the milking equipment(27). Soap was also not used, and the water used to rinse and wash the milking equipment was from wetlands.

Most of the milk produced was consumed raw with only 21% of farmers who used to boil the milk before consumption. Consumption of sour milk as half-done also contributes to developing brucellosis. Consumption of sour milk as half-done also contributes to developing brucellosis. This led to the high risk of exposure by farmers to *brucella* species, because the pathogen remains active in raw milk. Some of the farmers boiled their milk as mentioned above, hence reducing the risk of exposure to *brucella* species by farmers. This process helps farmers to be free from brucellosis.

## CONCLUSION

Consumption of raw milk was high among the traditional cattle farmers (78%). This suggests a commensurate risk of exposure to *Brucella* spp. and hence infection with brucellosis as a result of consumption. Milk produced on farms with poor hygiene practices may be contaminated with *Brucella*. The hand contact of farmers or the dislodging of dust and dirty particles by the milker also adds some microbes to milk causing a high risk of contamination. Improperly cleaned milking equipment was also one of the sources of milk contamination. Boiling of milk should be encouraged in rural areas among traditional cattle farmers where most households reported consuming raw milk and fermented milk that had been naturally fermented without prior heat treatment. Such fermented milk could expose traditional cattle farmers to *Brucella* pathogens. Boiling of raw milk at 72° C and held for at least for 16 seconds achieves higher temperatures and duration than pasteurization and could destroy all zoonotic pathogens(28). Traditional cattle farmers need to be educated on the risks of drinking raw milk and on the need to ensure that milk is adequately heated before drinking. They also need to be educated on consuming fermented milk that was prepared from unheated milk. Furthermore, farmers

should also be educated on the hygienic handling of milk as contamination of milk is found at all levels of the milk value chain. More studies are needed on the survival of *Brucella abortus* in naturally fermented milk and the consumption patterns of milk at the national level. Studies should also be carried out on the contamination levels and cross-contamination of milk and naturally fermented milk.

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## CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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## AUTHORS' CONTRIBUTIONS

M.M, C.M. and J.M. conceptualized the study. M.M., C.M., J.M., and D.S, M.M, designed the methodology and performed Statistical analyses. M.M, C.M., J.M., D.S., M.M, performed the formal analysis. M.M., C.M., J.M., D.S, M.M., I.N.L., reviewed and edited the manuscript. M.M., C.M., M.M., prepared the tables and performed the visualization. C.M., J.M., D.S., supervised the study. All the authors have read and agreed to the published version of the manuscript.

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