

## ORIGINAL ARTICLES

# Seroprevalence of human Brucellosis and associated risk factors among high-risk occupations in Mbeya Region of Tanzania

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## ABSTRACT

**Background:** Brucellosis is a bacterial zoonotic disease of public health and economic importance. A cross-sectional study was conducted in Mbeya region between November 2015 and January 2016 to investigate the seroprevalence of human brucellosis and identify associated risk factors among individuals in risky occupations.

**Methods:** A total of 425 humans from six occupational categories were serially tested for *Brucella* antibodies using the Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immunosorbent Assay (c-ELISA), for screening and confirmation, respectively. A questionnaire survey was also administered to participants to collect epidemiological data.

**Results:** The overall seroprevalence among the occupationally exposed individuals was 1.41% (95% CI: 0.64-3.12). Seroprevalence was higher among butcher men 5.6% (95% CI: 1.68-5.26), herds men 1.35% (95% CI: 0.18-9.02); and abattoir workers 1.1% (95% CI: 0.26-4.22) although there was no statistical significance. ( $P$  value = .18). Seroprevalence was also higher among men (1.8%) compared to females (0%) ( $P$  value = .19). and also, among those aged < 11 years (2.5%). Individuals who consumed raw milk had a higher seroprevalence (1.56%) compared to those who drunk boiled milk while seropositivity was 0.88% among those who assisted animals during parturition ( $P$  value = .49). Butcher men were at higher risk of exposure compared to other occupational categories. Our findings show the presence of brucellosis in occupationally exposed individuals in Mbeya region.

**Conclusion:** There is need to sensitize the concerned professions in order to reduce the risk of acquiring *Brucella* infections from animals and animal products This also calls for public health awareness about the disease, and implementation of measures to prevent further spread of brucellosis within and outside the study area.

**Key Words:** Brucellosis, Human, Occupation, Seroprevalence, Risk factors

## 1. INTRODUCTION

Brucellosis is a bacterial zoonotic disease of public health and economic importance that affects domestic animals,

wildlife and humans worldwide.<sup>[1]</sup> There are currently twelve known host *Brucella* species, however, this number may change as new species continue to be discovered.<sup>[2]</sup> Zoonotic

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infections are mainly attributed to *B. melitensis*, *B. abortus*, and *B. suis*,<sup>[3]</sup> while *B. canis* has been mainly reported as an occupational hazard among veterinarians and laboratory workers.<sup>[4]</sup> Human brucellosis is a highly debilitating infection that presents as an acute febrile flu-like illness.<sup>[5]</sup> It is characterized by symptoms such as fever, anorexia, fatigue, headaches, depression and weight loss that may easily be confused with malaria or typhoid.<sup>[6]</sup> The source of human infection always resides in domestic or wild reservoirs. It has been observed that most cases of human brucellosis occur in rural areas where people live in close proximity with their livestock and consume raw milk and milk products.<sup>[7,8]</sup> However, information on the brucellosis situation in Sub-Saharan Africa is scarce, particularly in humans. Some studies have reported human brucellosis at seroprevalences ranging from 0.02% to 33.3% in Zambia, Nigeria, Ghana, Cameroon, Sudan and Ethiopia.<sup>[9-15]</sup> In Tanzania, several studies have been done in Katavi, Manyara, Morogoro, Northern Tanzania, Mwanza and Tanga regions which have reported human brucellosis at seroprevalences ranging from 0.6 to 48.4%.<sup>[16-23]</sup> However, there is no previous report on the disease among the high-risk human population in Mbeya region. Therefore, this study was aimed at establishing the seroprevalence and associated risk factors of human brucellosis among occupationally exposed individuals in Mbeya region, Tanzania.

## 2. MATERIALS AND METHODS

### 2.1 Study area

The study was carried out in Mbeya Region in the Southern highlands of Tanzania between November 2015 and January 2016 in three selected districts namely; Mbarari, Mbeya and Momba. Geographically, Mbeya region lies about 5,500 feet above sea level and experiences subtropical highland climate with humid summers and dry winters. The temperature ranges between -6°C in the highlands and 29°C on the lowlands, while the average rainfall is 900mm per year. Details of the study area have been described in our earlier publication.<sup>[24]</sup> According to the 2012 national census, the region has an estimated human population of about 2,707, among which 1, 297,738 are males and 1, 409, 672 are females. A majority of the population (1, 809,298) dwell in the rural areas whereas 898, 112 are found in urban areas.<sup>[25]</sup>

### 2.2 Study population

The study population consisted of two groups. One group consisted of individuals from households where cattle had tested positive for *Brucella* from our earlier cattle survey.<sup>[24]</sup> The second group consisted of purposively sampled high-risk individuals that were involved in the cattle value chain: livestock officers, herdsmen, butcher men and abattoir workers.

### 2.3 Study design and sample size calculation

This was a cross-sectional study that was strategically designed in order to determine the seroprevalence of human brucellosis in high-risk individuals. The sample size was pre-determined from our earlier study. A total of 425 humans were purposively sampled in this study which included herdsmen and milkers from the herds earlier screened against brucellosis.<sup>[24]</sup> These included 74 herdsmen and 72 milkers, 184 abattoir workers from all 3 butcheries in the 3 districts, 54 butcher men, 11 livestock officers and 30 other individuals.

The selected study region encompassed a strategic population of individuals whose culture encourages the use of animal products for proteins, thus predisposing them to zoonotic diseases.

### 2.4 Collection of samples and epidemiological data

A health personnel (phlebotomist) was assigned to aseptically collect 5 ml of blood from the participant's brachial vein using a sterile disposable syringe into pre labelled plain vacutainer tubes. The samples were incubated overnight at room temperature and then centrifuged at 3000 xg to get clear serum. All collected samples were assigned identification numbers and stored in a mobile refrigerator until shipment to the University of Zambia, Public Health laboratory where they were stored at -20 degree until serological analysis. A pre-tested structured questionnaire was administered to the participants in order to collect information on demographic data, socioeconomic data, variables on exposure to animals and animal products, consumption of dairy and animal source products.

### 2.5 Laboratory analysis

#### 2.5.1 Rose bengal plate test

All collected sera samples were screened using Rose Bengal Plate Test (RBPT), antigen manufactured by Ubio Biotechnology Systems Pvt Ltd for detection of *Brucella* antibodies according to the test procedure recommended by OIE (1). Briefly, 20 µl of RBPT antigen and 20µl of the test serum were placed alongside on one well of the glass plate and mixed thoroughly. The slide was gently rocked for 4 minutes and thereafter, any visible agglutination was considered as a presumptive positive result.

#### 2.5.2 Competitive enzyme-Linked immunosorbent assay (c-ELISA)

RBPT positive sera were thereafter subjected to competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) as a confirmatory test, adopting a test procedure and interpretation of results as recommended by the manufacturer (Svanova Biotech AB SE-751 Uppsala, Sweden) and as described by

Muma et al.<sup>[26]</sup> According to the ELISA kit manufacturer's instructions, serum was regarded as positive if the PI value was > 30%. Only individuals that tested positive to both RBPT and c-ELISA were regarded as *Brucella* seropositive.

## 2.6 Data management and analysis

Data obtained from the serological tests and a questionnaire survey was stored in an Excel® spreadsheet database before being imported into STATA 13® statistical software for analysis. Categorical variables were summarized as frequency and percentages; continuous variables were summarized as mean or standard deviation (SD). *P*-values of .05 or less were considered statistically significant. A person was considered to be seropositive when tested positive to both RBPT and c-ELISA. The degree of association between each risk factor was assessed using the chi-square test and for all analysis, a *p*-value of ≤ .05 was taken as significant.

## 2.7 Ethical consideration

Ethical approval (reference number NIMR/HQ/R.8a/Vol.1X/2050) was obtained from the Medical Research Committee of the United Republic of Tanzania prior to the study. Individual written consent was obtained from guardians for individuals that were less than 18 years prior to enrollment. Informed consent was obtained from all participants using written and verbal explanation of the study purpose and procedure in the Swahili language.

## 3. RESULTS

The overall seroprevalence for human brucellosis in Mbeya was 1.41% (95% CI: 0.64-3.12) (see Table 1). Mbeya district had the highest seroprevalence (1.88%) while Momba district did not record any seropositive individuals (see Table 1). There was no statistically significant association between district and seropositivity (*P* value = .85).

None of the female participants were seropositive (0%) compared to men (1.8%). The highest seroprevalence was recorded among butcher men (5.6%) followed by herdsmen (1.35%) and abattoir worker (1.1%) as shown (see Table 2). Furthermore, seroprevalence was higher among individuals that were aged less than 11 years (2.5%) compared to those aged between 11 and 20 years (1.02%). Results showed that 75.3% of respondents consumed raw milk while 27% assisted their animals during parturition (see Table 2). A higher seroprevalence (1.56%) was recorded among individuals who consumed unboiled milk compared to those who assisted in parturition (0.88%) (see Table 2). Results from univariate analysis show that there was no statistical association between the hypothesized risk factors and *Brucella* seropositivity (see Table 2).

**Table 1.** Overall Seroprevalence of human brucellosis by district in Mbeya region

District	No	Seropositive (%)	95% CI	<i>P</i> value
Mbarari	220	1.36	0.44-4.12	.85
Momba	45	0.00	0.00	
Mbeya	160	1.88	0.06-5.68	
Overall	425	1.41	0.64-3.12	

## 4. DISCUSSION

### 4.1 Seroprevalence of human brucellosis

The aim of this study was to estimate the seroprevalence of human brucellosis and identify associated risk factors among high-risk occupations in Mbeya region of Tanzania. The overall seroprevalence found among butcher men, abattoir workers and herdsmen (1.41%) is different from the rates reported in other parts of Tanzania namely; 5.52% among high-risk groups in Tanga region,<sup>[23]</sup> 1.5% and 14.1% among agro-pastoral herders in Katavi and Mwanza regions respectively<sup>[19,20]</sup> and 48.4% among abattoir workers and meat vendors in Mwanza region.<sup>[21]</sup> The difference can be due to variations in study population, geographical locations and the use of Serum Agglutination Test (SAT) and Microscopic Agglutination Test (MAT) in the previous studies which had specificity and sensitivity of 95.7% and 55.3% respectively. Similar studies in Cameroon, Ethiopia and Nigeria among abattoir workers found seroprevalences of 5.6%, 4.7% and 33.4% respectively,<sup>[10,12,18]</sup> while another one in Ghana among cattle handlers and slaughterhouse workers found seroprevalences of 10.1% and 1.8% respectively.<sup>[11,15]</sup> In contrast, similar studies in South Sudan and Uganda found seroprevalences of 33.3% and 4.4% among cattle herders and livestock farmers respectively.<sup>[14,27]</sup> These findings were higher than those from our study in all occupational groups except for the study among slaughterhouse workers in Ghana.<sup>[15]</sup> Brucellosis has also been reported in butcher and slaughterhouse workers in Iran at 7.9% seroprevalence.<sup>[28]</sup> Our results show that brucellosis is present in high-risk populations in Mbeya region, even though the rate is lower than that reported in other population groups in other regions of Tanzania. The differences could possibly be due to small sample sizes, the different study populations and different diagnostic tests used in the other studies.

### 4.2 Risk factors associated with *Brucella* seropositivity

The prevalence of human brucellosis in occupationally exposed individuals in the Mbeya region of Tanzania varied with the occupational category, age, sex and milk consumption behaviour. However, there was no statistically significant association between the hypothesized risk factors and *Brucella* seropositivity. Butcher men had a higher risk of

exposure to brucellosis than shepherds and abattoir workers, which agrees with findings from Tanga region.<sup>[23]</sup> This could be attributed to that fact butcher men have a higher and constant exposure to slaughtered animal parts i.e. blood, tissues, fluids, with inadequate or poor use of protective wear. The risk of injury (knife-cuts) is also very high during this period as compared to the other categories, which increases the risk of exposure to the *Brucella* pathogen). *Brucella* seropositivity was higher in males (1.41%) than females (0%), similar to other findings<sup>[10, 15, 21, 23, 27]</sup> but contrary to another.<sup>[18]</sup> The high seroprevalence in males can be explained by the fact that most activities in the cattle value chain are carried out by males compared to females. The high disease prevalence among butcher men could be because they tend to spend longer periods handling animal carcasses usually without protective wear and are more likely to be injured when cutting meat and get infected. Hence, they are at a relatively higher risk of infection compared to other groups. Seroprevalence was higher in individuals aged 11 years and below (2.5%). This is in contrast to<sup>[21]</sup> but in agreement with<sup>[15, 29]</sup> in Uganda and Ethiopia. This shows the traditional role that young male individuals play in livestock management where young men start herding livestock at a very young age. They spend more time in close contact with animals

during their daily livestock activities, engage in consumption of unpasteurized milk directly from the teats of cows and assist during deliveries. This increases their risk of exposure to brucellosis. In our study, 75.3% of people consumed raw milk which was higher than the 12.8% reported by<sup>[29]</sup> in Uganda. This can be explained by the fact that over 70% of milk sales in Tanzania is produced by pastoral farmers who do not believe or know that milk could be a potential source of infection to humans; and are not ready to subject their milk to heat treatment.<sup>[30]</sup> Interestingly, seroprevalence was higher among individuals who did not assist their animals during parturition (1.88%) than those who did (0.88%) ( $P = 1.00$ ). This could probably indicate that milk is a significant vehicle for transmission of *Brucella* infection and individuals with a history of consumption of raw milk were more likely to be infected.<sup>[29]</sup> These study findings indicate that butchers and slaughterhouse workers in different areas face different risk levels towards zoonotic infectious diseases attributed to variation in infection rates among animals, differences in human lifestyle and use of PPE. It is possible that hygienic practices, regular medical check-ups and measures put in place by the local authorities can strongly influence the risk of brucella infections.

**Table 2.** Univariate analysis of seroprevalence of human brucellosis by different variables

Variable	Category	Total n = 425	Seropositivity (%)	Odds ratio (95% CI)	P value
Gender	Male	334	1.8	0.81-3.95	.198
	Female	91	0		
Occupation	Herdsmen	74	1.35	0.18-9.02	.180
	Livestock officers	11	0	0.00	
	Butcher men	54	5.6	1.68-5.26	
	Abattoir workers	184	1.1	0.26-4.22	
	Milker	72	0	0.00	
Age	< 11yrs	155	2.5	0.94-6.54	.396
	11-20 yrs	196	1.02	0.25-4.01	
	21-30 yrs	70	0	-	
	30 yrs	0	0	-	
Consumption of unboiled milk	Yes	320	1.56	0.65-3.71	.54
	No	105	0.95	0.13-6.54	
Assist in parturition	Yes	113	0.88	0.12-6.09	1.0
	No	312	1.6	0.66-3.81	

### 5. CONCLUSION

This was the first serological study of human brucellosis in occupationally exposed individuals, specifically abattoir

workers, butcher men and shepherds in Mbeya region. Seroprevalence was higher among butcher men, individuals below 11 years and among those who consumed raw milk. Occupa-

tionally exposed individuals and the public need to be sensitized on brucellosis and the zoonotic dangers of consuming raw milk. There is also need for public health measures to prevent spread of the disease within and outside the study area.

## STUDY LIMITATIONS

One of the limitations in this study was that some herds men from certain cattle herds whose animals were screened earlier could not participate in the study due to the migratory nature of agro-pastoralists in search of water and pasture.

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## CONFLICTS OF INTEREST DISCLOSURE

The authors declare that they have no competing interests.

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