# Seroprevalence of Brucella Infection and Associated Factors among Pregnant Women Receiving Antenatal Care around Human, Wildlife and Livestock Interface in Ngorongoro Ecosystem, Northern Tanzania. A Cross Sectional Study

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# Abstract

Background Brucellosis is a zoonotic disease transmitted to humans through contact with infected animals, animal products or consumption of infected dairy products. Brucella infection during pregnancy is of special interest due to associated adverse pregnancy outcomes. This study determined the seroprevalence and factors associated with Brucella infection among pregnant women around the human-wildlife-livestock interface area in Ngorongoro Ecosystem, Northern Tanzania.

Methods A facility-based cross-sectional study was conducted between May and June 2018 at six health facilities that provide antenatal services. Pregnant women receiving antenatal care were invited to participate. A structured questionnaire was used to collect socio-demographic and obstetric characteristics in addition to behavior and practices related to the occurrence of human brucellosis. The presence of serum immunoglobulin against Brucella was determined using Rose Bengal Plate Test (RBPT). The positive samples were further assayed for the presence of IgG and IgM using Enzyme-Linked Immunosorbent assay. Bivariate analysis was conducted to determine the variables associated with Brucella seropositivity. Multivariable logistic regression analysis was performed to examine the factors independently associations with Brucella seropositivity after adjustment for other explanatory variables.

Results A total of 313 participants were enrolled in the study. The overall seroprevalence of Brucella infection was 10.9 % (34/313) determined by RBPT. Of 34 positive individuals, 27(79.4%) and 8(23.5%) were positive in the ELISA specific for IgG and IgM Brucella antibodies respectively. Preference for raw animal blood (AOR 4.45, 95 % CI 1.5–12.9, p = 0.01), raw meat (AOR 7.59, 95 % CI 1.6 – 35.4, p = 0.01) and fresh milk (AOR 3.76, 95 % CI 1.2–11.5, p = 0.02), increased the odds of being infected with Brucella. Regular contact with manure and contact with the placenta were not statistically associated with Brucella seropositivity after adjustment.

Conclusion This study has found that brucellosis is an important public health problem among pregnant women in areas with interactions of humans; livestock and wildlife. The risk of infection increased with the preference of raw foodstuffs like animal blood, meat, and milk. We emphasize the need for interventional strategies to reduce the risk of exposure


# Background

Brucellosis is one of the neglected zoonotic diseases, acquired through contact with infected animals, consumption of infected dairy products, or inhalation of aerosols. [1, 2] Wildlife animals near human and domestic animal may act as reservoirs to both. [3] Veterinarians, livestock farmers, milkers, abattoir workers and laboratory workers are occupations at high risk of getting *Brucella* infection. [4, 5] Exposure of wildlife animals to *Brucella abortus* in the Ngorongoro ecosystem has reached 24% and 17% for buffalo and wildebeest populations respectively.[6] The prevalence of brucellosis in domestic ruminants

free-range grazing system in Ngorongoro conservation was found to range from 3% to 14.28% in different animals [7]

The community health significance of *Brucella* infection in humans is a severely devastating disease that requires prolonged treatment leaving health problems and disabling results.[8] The major challenge is the similarity of clinical presentation to other febrile illnesses such as malaria and typhoid fever. Consequently, under-reporting and mismanagement may be common in areas with limited laboratory diagnosis. [9, 10] Infection in pregnancy is of major public concerns as it associate with several detrimental pregnancy outcomes like spontaneous abortion, preterm delivery, and fetal death [1, 2, 4, 8] The risk of low birth weight has been demonstrated to be higher in pregnant women infected with *Brucella* than non infected [8]The burden is mostly seen in poor individuals who regularly live in close contact with animals, with poor access to health care service that makes them delay to present to hospital.[11]

Previous studies conducted in Tanzania have reported up to 13% prevalence of brucellosis in the area of pastoral and agro-pastoral communities [11, 12]. However, there are limited published data regarding *Brucella* infections among pregnant women in Tanzania, especially in the area of interactions of humans, livestock and wildlife. This limited information highlights the need to determine the seroprevalence of *Brucella* infection and associated modifiable factors among pregnant women. The information generated from this study may be of help to policy and interventional strategies. Ngorongoro was selected as the study area based on the presence of high interactions among the human-animal-wildlife interface which could play a role in the maintenance of the disease.

# Methods

## Study design and setting

This was a facility-based cross-sectional study conducted between May and June 2018 in Ngorongoro District, Arusha region of Northern Tanzania. The district plays host to parts of the wildebeest migration at the same time cattle, goat and sheep rearing is a common practice in the. The population of the Ngorongoro District is around 130,000. The major ethnic group in the District is the Masai and Sonjo people.

The Ngorongoro District has 20 public health facilities including 14 dispensaries, four health centres, and two hospitals. Each of the two hospital records between 25- 40 new antenatal clinic attendances per week. The study involved six health facilities providing antenatal services. These included: Wasso designated district hospital, Sakala and Loliondo health centers as well as Muholo, Sale, and Samunge dispensaries.

## Study population, sample size, and sampling procedure

All pregnant women attending the antenatal clinic at selected health facilities were invited to participate in the study. Pregnant women who lived in the study area for more than three months and accepting to participate by signing written informed consent were enrolled. The sample size was estimated using Kish Leslie formula [13], at 95 % confidence interval (CI) considering 7.7% seroprevalence of Brucella infection in Arusha Tanzania [12] and a 3% margin of error. Participants who met eligibility criteria were consecutively enrolled in the study until reaching a representative sample size.

## Data collection

A structured questionnaire was used to collect the required information from each participant. Data for socio-demographic and obstetric characteristics included: age, marital status, education level, occupation, location, gestation age, gravidity, parity and history of spontaneous abortion. Factors related to animal care and animal product consumption, with potential risk for transmission and presence or absence of exposure at the individual level of *Brucella* infection, were also collected. The questionnaire included contact with animals and animal products, participant's involvement in milking, sharing water sources with animals, assisting animals to give birth or drink animal fresh milk.

## Study variables

The dependent variable was *Brucella* serostatus and independent variables were behavior and practices with potential risk for *Brucella* infection. Regular contact with animal manure was defined as exposure to manure at least once in every week in the last three months. Participants were counted to contact the placenta if assisted animals giving birth at least once in the last three months and washing animals at home was counted when performed at least once every week for three months. Preference of foodstuffs like fresh milk, raw animal blood, and raw meat was defined as consumption of the same at least once every week in the last three months.

## Specimen collection

Experienced health personnel normally working at the facilities collected 4 ml of venous blood aseptically using a plain vacutainer system. The collected specimens were labeled with the specific participant's identification number. Serum samples were separated from whole blood by centrifugation at 3,000 rpm for five minutes. The specimens were kept at room temperature for 30 min then at 4 °C up to 24 h before processing

## Laboratory Procedure

## Rose Bengal Plate Test

The *Brucella* serology was first determined by Rose Bengal Plate Test (RBPT) a rapid agglutination test as previously described.[14] The test does not differentiate antibodies against different *Brucella* species like *Brucella abortus and Brucella melitensis.* Briefly, a drop of the test serum (50µl) was taken using a clean micro-pipette and placed onto the test plate beside an equal (50µl) drop of RBPT antigen. These then were mixed well-using applicator stick. The mixture was then rocked manually for 4 minutes before examination. The presence of any visible reaction was considered to be positive[15].

## Enzyme-Linked Immunosorbent Assay

Positive samples were kept at minus 20 $^{O}$C before transportation to the reference laboratory in Dar es Salaam for the detection of Immunoglobulin M and G antibodies. The commercially available test kits of enzyme-linked immunosorbent assay (ELISA), SERION ELISA classic *Brucella* IgG/IgM/IgA (Institut Virion/Serion GmbH) was used to detect IgM and IgG antibodies. The technique was performed according to the instructions from the manufacturer. In brief, 100 ml of diluted serum samples and ready to use control were added to the micro test wells with antigen. The samples were then incubated at 37 $^{O}$C for 60 minutes, after which the first wash was performed. Later, anti-human IgM or IgG conjugated with an enzyme was added and incubated for 30 minutes at 37ċ. All wells were then washed to remove excess conjugate, followed by a new incubation for 30min at 37ċ with the enzyme-substrate. Finally, the reaction was stopped by adding 100 ml of stopping solution. The enzyme reaction with the Substrate yields a colored product. The color intensity is proportional to the amount of specific antibody and can be measured by the photometric method.

## Data analysis

Descriptive analysis of categorical variables was summarized as frequencies and proportions and continuous variables as median ± inter-quartile range (IQR). Group differences were examined using Pearson's Chi-square test. Bivariate analysis was conducted to determine the variables associated with *Brucella* seropositivity and crude odds ratio (cOR) with 95% confidence intervals (CI). Multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment. Associations in the multivariable logistic models were presented as adjusted odds ratios (AOR) with 95% confidence interval (CI). The crude association of each independent variable was determined, and then variables were entered into a multivariable logistic model. Interactions between independent variables were examined, and the Wald test was used to test the associations of the variables and interactions. The Hosmer-Lemeshow test was used to examine the overall fitness of the model. Statistical Package for Social Sciences version 20 was used for all data analyses. The level of significance was specified at 0.05.

## Results

## Characteristics of participants and seropositivity of *Brucella* infection

A total of 313 participants were enrolled in the study, the median age was 25 years, interquartile range 20–30 years. The majority 299 (95.5%) were Agro-pastoralists, 150 (47.9%) had no formal education, 288 (92.0%) were married, and 201(64.2%) had ≤ 28 weeks of gestation. Out of 237 with prior pregnancies, 35(14.8%) reported a history of spontaneous abortion (Table 1).

All participants were screened for antibodies against y *Brucella* using a rapid RBPT test. Out of 313 participants, 34(10.9% [7.9–14.8]) were seropositive. Of 34 Seropositive individuals, 27(79.4%) and 8(23.5%) were positive in the ELISA specific for IgG and IgM antibodies respectively. Based on the detection of IgM antibodies, 2.6% (8/313) of participants were deemed to have had recent *Brucella* infection. The seropositivity observed for demographic (age, occupation, education, marital status) and obstetric characteristics (gestation age, number of pregnancies, history of spontaneous abortion) were not significantly deferent ($p > 0.05$) (Table 1)

Table 1 Descriptive characteristic of participants and *Brucella* seropositivity by using rapid RBPT test

| Variable | Frequency | % of participants | Seropositivity N (%) | *p*-Value* |
|---|---|---|---|---|
| **Overall-seropositivity** | 313 | | 34 (10.9) | |
| **Age group (years)** | | | | |
| ≤25 | 160 | 51.1 | 16(10.0) | 0.616 |
| >25 | 153 | 48.9 | 18(11.8) | |
| **Occupation** | | | | |
| Agro-pastoralist | 299 | 95.5 | 31(10.4) | 0.194 |
| Formal employment | 14 | 4.5 | 3(21.4) | |
| **Level of education** | | | | |
| Informal | 150 | 47.9 | 11( 7.3) | 0.054 |
| Primary | 98 | 31.3 | 11(11.2) | |
| Secondary and above | 65 | 20.8 | 12 (18.5) | |
| **Marital status** | | | | |
| Single | 25 | 8.0 | 4 (16.0) | 0.389 |
| Married | 288 | 92.0 | 30 (10.4) | |
| **Gestation age (weeks)** | | | | |
| ≤ 28 | 201 | 64.2 | 24 (11. 9) | 0.412 |
| >28 | 112 | 35.8 | 10 (8.9) | |
| **Previous pregnancy** | | | | |
| 0 | 76 | 24.4 | 8(10.5) | |
| 1 | 76 | 24.3 | 9(11.8) | |
| 2 | 58 | 18.5 | 3 (5.2) | 0.421 |
| 3+ | 103 | 32.9 | 14(13.6) | |
| **Spontaneous abortion (n=237)** | | | | |
| No | 202 | 85.2 | 20(9.9) | 0.206 |
| Yes | 35 | 14.8 | 6(17.1) | |

* *P* value according to Pearson Chi-Square test

## Behavior and practice associated with *Brucella* infection

Several factors with the potential risk of brucellosis among humans were assessed and reported in Table 2. Participants who reported to have been exposed to the assessed potential risk factors; had more seropositive cases of *Brucella* except for those reported washing animals at home. At bivariate analysis, regular contact with animal manure increased the probability of *Brucella* seropositivity (cOR 2.7, 95%CI 1.12–6.33). Contact with animal placenta through the assist of parturition had higher odds of being seropositivity (cOR 3.1, 95%CI 1.18–8.37). Preference for fresh milk, raw meat, and raw animal blood, were significantly associated with seropositivity to *Brucella* ($p < 0.05$). The odds of being seropositive among those prefer fresh milk, raw meat and raw animal blood ranged from 2.1 to 3.1 (Table 2). Washing animal at home (p = 0.4) and Sharing water source with the animal (p = 0.82) were not significantly associated with seropositivity to *Brucella*

Table 2: Factors assessed by bivariate analysis for *Brucella* seropositive

| Variable | Frequency (%) | Sero-positive N (%) | cOR | 95%CI | *p*-value |
|---|---|---|---|---|---|
| **Regular contact with manure** | | | | | |
| Yes | 192 (61.3) | 27 (14.0) | 2.7 | (1.12-6.33) | 0.022 |
| No | 121 (38.7) | 7 (5.8) | 1 | | |
| **Contact with animal placenta** | | | | | |
| Yes | 210 (67.1) | 29 (13.8) | 3.1 | (1.18-8.37) | 0.017 |
| No | 103 (32.9) | 5 (4.9) | 1 | | |
| **Washing animal at home** | | | | | |
| Yes | 201 (64.2) | 20 (10.0) | 0.8 | (0.37-1.59) | 0.487 |
| No | 112 (35.8) | 14 (12.5) | 1 | | |
| **Preference for fresh milk** | | | | | |
| Yes | 229 (73.2) | 30 (13.1) | 3.0 | (1.03-8.83) | 0.036 |
| No | 84 (26.8) | 4 (4.8) | 1 | | |
| **Preference for raw meat** | | | | | |
| Yes | 76 (24.3) | 13(17.1) | 2.1 | (1.01-4.48) | 0.044 |
| No | 237 (75.7) | 21(8.9) | 1 | | |
| **Preference for raw animal blood** | | | | | |
| Yes | 174 (55.6) | 26 (14.9) | 2.9 | (1.26-6.57) | 0.009 |
| No | 139 (44.4) | 8 (5.8) | 1 | | |
| **Sharing water source with animal** | | | | | |
| Yes | 160 (51.1) | 18 (11.3) | 1.1 | (0.53-2.21) | 0.822 |
| No | 153 (48.9) | 16 (10.5) | 1 | | |

Key: cOR = crude odds ratio, CI = Confidence interval, p-value according to Pearson Chi-Square test

Table 3 shows the result of multivariate analysis by a multivariable regression model performed to measure the relationship between *Brucella* seropositivity and independent variables. All variables that showed significant association *p*-values < 0.05 in the bivariate analysis were included. Two interactions between independent variables were also examined. The multivariate logistic regression analysis revealed that preference for raw animal blood, preference for raw meat, and preference for fresh milk remained a risk factor for *Brucella* seropositivity (Table 3).

Besides, we observed negative interactions between regular contact with manure and contact with the placenta as well as preference for raw meat and preference for raw animal blood. The Hosmer-Lemeshow test result was $p = 0.94$, which indicated the fitness of the overall model. There was no statistically significant association found for regular contact with manure (AOR 3.06, 95 % CI 0.5—20.6, $p = 0.25$) and contact with placenta (AOR 2.26, 95% CI 0.5–11.1, $P = 0.313$) after adjustment for other factors

Table 3: Multivariable analysis of variable associations with the RBPT seropositivity of participants

| Variable | B±SE | Wald test | *P* | AOR ( 95% CI) |
|---|---|---|---|---|
| Regular Contact with Manure | 1.12±0.97 | 1.321 | 0.250 | 3.06 (0.5-20.6) |
| Contact with Placenta | 0.82±0.81 | 1.019 | 0.313 | 2.26 (0.5-11.1) |
| Preference for raw animal blood | 1.49±0.58 | 6.621 | 0.010 | 4.45 (1.5-13.9) |
| Preference for raw meat | 2.03±0.78 | 6.668 | 0.010 | 7.59 (1.6-35.4) |
| Preference for fresh milk | 1.32±0.57 | 5.402 | 0.020 | 3.76 (1.2-11.5) |
| Contact Manure by Contact Placenta | -0.38±1.13 | 0.113 | 0.737 | 0.68 (0.1-6.3) |
| Preference for blood by Preference for uncooked meat | -1.52±0.91 | 2.800 | 0.094 | 0.22 (0.04-1.3) |
| Constant | -5.64±0.95 | 35.104 | 0.000 | .004 |

Key: AOR = Adjusted odds ratio, CI = Confidence interval, *P*-value according to logistic regression models

# Discussion

The current study has demonstrated a higher (10.9%, [7.9–14.8]) seropositivity of *Brucella* infection in pregnant women compared to the previous reports in the general population of the same geographical location [12, 16]. Besides, the study has revealed nearly 3% of pregnant women with immunologic evidence of recent *Brucella* infection based on IgM ELISA positivity. The level of seropositivity found among pregnant women in the Ngorongoro District suggests that *Brucella* infection is a public health problem. Our finding on the level of seropositivity of *Brucella* infection is higher compared to a previous report from Pakistan (5.8%) among pregnant women [4] and comparable to report from Nepal (11.25%)

among pregnant women [17]. Besides, our study finding is lower compared to a report from Uganda (17%) in agro-pastoral communities [18] and 25% among women with abortion in Rwanda [2].

The community where the present study was conducted comprised around 95% agro-pastoralists. In most of the agro-pastoralist communities, women do most of the work associated with care and harvest of livestock products. They actively engage in barn cleaning, herding small ruminants, milking and preparing manure dung.[19] The report indicating activities commonly done by women predispose them to brucellosis [5]; brucellosis being an endemic disease in humans and animals in Tanzania, [11, 20] and the proximity of studied population with the livestock-wildlife interface, can explain the level of seroprevalence found in the studied population.

The higher seropositivity of *Brucella* infection among pregnant women showed by this study could be attributed in part due to the preference for raw foodstuffs like fresh milk, raw meat, and raw animal blood. Eating habits may expose an individual to *Brucella* infection if the consumed products from infected livestock are not properly prepared. [12, 18, 21, 22] A substantial number of participants in the current study reported a preference for fresh milk (73.2%), preference for raw meat (24.3%) and preference for raw animal blood (55.6%). The habits of consumption of raw foodstuff were the risk factors independently associated with *Brucella* infection among pregnant women in Ngorongoro District. Our findings are in agreement with the previous study conducted in Tanzania which reported food preferences and eating behavior to play major roles in *Brucella* infection in pastoral and agro-pastoral communities [5, 12]. Other studies in Africa also reported the similar predictors for transmission of brucellosis, although the main predictors vary depending on customs and taboos of referred community [23, 24]

Regular contact with animal manure and contact with the placenta had increased odds of being seropositive for *Brucella* infection in the bivariate analysis model. However, the association with *Brucella* seropositivity was not observed in the multivariable logistic regression model. Some studies reported direct contact with livestock excreta as a potential route of exposure to *Brucella* infection [12, 23]. Similarly, contact with animal placenta has been reported associated with brucellosis.[25–28] It is also documented that *Brucella* spp from infected animals are found in animal excreta which serve as sources of humans infections [29]. Lack of independent association of brucellosis with exposure to manure and placenta in our study could be explained by negative interaction found between these variables. The majority of participants 54% reported both regular contact with manure and contact with the placenta.

The study relied heavily upon self-reported information which is open to information bias, clustering of events and failure to recall. Participants could have missed out on some possible factors associated with the occurrence of brucellosis. Reporting error for some measures was reduced by asking participants to recall only events in the last three months. Despite limitations encountered, this study has demonstrated some important factors associated with transmission to humans in the Ngorongoro ecosystem. Our findings serve as considerable baseline data for prevention and control of the disease and associated adverse effect in pregnancy.

## Conclusions

This study has found that brucellosis is an important public health problem among pregnant women in the area with interactions of humans; livestock and wildlife. The risk of infection increased with the preference of raw foodstuffs like animal blood, meat, and milk. These findings emphasize the need for interventional strategies to reduce the risk of exposure and improve early detection of infection in pregnant women.

## List Of Abbreviations

*ELISA:* Enzyme-linked immunosorbent assay, *IgG:* Immunoglobulin G, I*gM:* Immunoglobulin M, *MUHAS:* the Muhimbili University of Health and Allied Sciences, *RBPT:* Rose Bengal plate test.

## Declarations

## Ethics approval and consent to participate

The ethical approval was obtained from the Senate Research and Publication Committee, the Institutional Review Board of Muhimbili University of Health and Allied Sciences (MUHAS). Permission to conduct the study was obtained from the District Director and hospital authorities where the study was conducted. Written informed consent was obtained from all patients before being enrolled in the study.

## Consent for publication

Not applicable

## Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

## Competing interests

The authors declare that they have no competing interests.

## Funding

The funding for data collection and laboratory works were obtained from the Ministry of Health, Community Development, Gender, Elderly and Children. The funder had no role in the design of the study, collection, analysis, and interpretation of data and in writing the manuscript.

## Authors' contributions

RM and MVM were involved in conception and design of the study; RM participated in data collection, laboratory work and drafting the manuscript; MVM had overall coordination of the study; RM, MVM, and GMB contributed to the analysis and interpretation of data; MVM, UK, GMB, MMM, and AJ participated in write up and critically revising the manuscript. All authors read and approved the final version of the manuscript.

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