Predominance of *Brucella abortus* antibodies among women with spontaneous abortion in the city of Mwanza: unrecognized link or coincidence?

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**Abstract**

**Objective:** This study investigated the association of Brucella seropositivity and spontaneous abortions in human population in the city of Mwanza, Tanzania.

**Results:** A comparative cross sectional study which used 148 sera from women with spontaneous abortion and 250 sera from full-term delivered women was conducted in July 2017. Detection of *Brucella abortus* and *Brucella melitensis* antibodies was done using slide agglutination test. Data were analyzed using STATA version 13 software. The median age of the study participants was 25 (interquartile range 21–30) years. The overall seropositivity of Brucella antibodies was significantly higher among sera from women with spontaneous abortion than full term delivered women; (86/148, 58.1%: 95% CI 50–66 vs. 65/250, 26%: 95% CI 18–33, P < 0.001). Seropositivity of *B. abortus* was significantly higher among sera from women with spontaneous abortion than full-term delivered women (31.8% vs. 10.8%, P < 0.001). Women with abortion had 3.59 odds of being brucella seropositive compared to full term women (OR: 3.59, 95% CI; 2.25–5.74, P < 0.001). Seropositivity of Brucella antibodies is significantly higher among women with spontaneous abortion than full-term delivered women necessitating a need to investigate the relationship between Brucellosis and adverse pregnancy outcomes.

**Keywords:** Brucella, Antibodies, Spontaneous abortion, Mwanza, Tanzania

**Introduction**

Brucellosis is a neglected bacterial zoonotic disease that is common in the Sub Saharan African countries [1, 2]. Brucellosis is of public health concern particularly in the agropastoral communities. It is associated with economic losses and reduced productivity as a result of spontaneous abortions in animals such as goats, sheep and cattle [3]. The disease is widespread in Western Europe, sub-Saharan Africa, Middle East, Central Asia and South America [2, 4–7]. The incidence of human brucellosis in endemic regions ranges from <0.01 to >200 per 100,000 population [8]. In Tanzania, the prevalence of brucellosis in human population has been found to be as high as 46% among livestock keepers [9].

In humans, the clinical presentation of the disease varies from asymptomatic to severe life threatening disease [1, 10]. Brucellosis is well established cause of spontaneous abortion in animals particularly ruminants, however, its role in causing spontaneous abortions in human is unclear [11]. Abortion in animals is mainly due to the presence of high content of erythritol in animal placental tissues which is believed to play an important role in the localization of *Brucella* spp. [12]. In human, it is believed that maternal bacteremia, toxin production, disseminated intravascular coagulation and acute febrile reaction...
contribute to spontaneous abortion and intrauterine fetal death [13–15].

Bone marrow cultures has been considered as the gold standard for the diagnosis of brucellosis [16], nevertheless, harvesting bone marrow for culture is an invasive procedure and unreliable. The serum agglutination test remains to be widely used diagnostic method whereby the titers above 1:160 are considered diagnostic parallel with clinical presentations [17].

Spontaneous abortion is a condition which occurs in about 20% of women before 20 weeks of pregnancy with the majority of them occurring in the first 12 weeks of gestation [18]. The rate of spontaneous abortion in Tanzania is estimated to be approximately 12%, however the causes of these abortions have never been thoroughly studied [19]. Infectious agents such as Toxoplasma gondii, viral infections including rubella, cytomegalovirus, parvovirus B19 and many other agents have been implicated as common causes of spontaneous abortions [20–23]. Brucellosis is considered to be endemic in the sub-Saharan region including Tanzania and it has been associated with other adverse pregnancy outcomes such as stillbirth [24, 25]. However, its role in spontaneous abortion has never been established. This study for the first time in Mwanza attempted to compare seroprevalence of brucella antibodies between women with spontaneous abortion and that of full term delivered women. This information might be useful as a baseline information and may stimulate further studies to establish the pathogenesis and a clear link between brucellosis and abortion in human.

Main text
This was a comparative cross-sectional study, carried out in July 2017 in the city of Mwanza, Tanzania. A total of 148 and 250 archived sera from women with spontaneous abortion and full-term delivery women, respectively were retrieved and analyzed for brucella antibodies (B. abortus and B. melitensis). Samples were collected from four health facilities in the city of Mwanza namely Bugando Medical Centre, Sekou Toure regional hospital, Buzuruga health centre and Nyamagana hospital between 2015 and 2016.

Sample size was estimated using Kish Leslie formula using prevalence of 20% from a previous study in Kenya [26]. The minimum sample size obtained was 245, however a total of 398 sera were available and included in the study. Sera with complete information including storage conditions were serially collected and analyzed. Socio-demographic data and other relevant information were obtained from preexisting records.

Detection of brucella antibodies was done using slide agglutination test (SAT) as per manufacturer's instructions (Euromedi equip LTD UK). Reagents and test samples were brought at room temperature for 20 min. Eurocell antigen suspensions was shaken and mixed well before dispensing. The Eurocell-A and Eurocell-M reagents contain ready for use standardized, attenuated Brucella antigen with specific reactivity towards antibodies to B. abortus (Eurocell-A), and B. melitensis (Eurocell-M). The test has 95% sensitivity and specificity of 100%.

Data were extracted from existing database in excel sheet, cleaned and coded then transferred into STATA version 13 for analysis. Percentage/fraction was used to summarize categorical variables while median (IQR) was used for continuous variables. Chi square test was used to show association between Brucella antibodies and associated factors followed by logistic regression analysis to establish adjusted odd ratio. All factors with P value less than 0.2 on univariate analysis were subjected on multivariate logistic regression analysis. A P value of <0.05 at 95% confidence interval was considered as statistically significant.

The median age of the study participants was 25 (IQR 21–30) years. Regarding marital status, 126 (85%) and 215 (86%) of women with spontaneous abortion and full term delivered women were married, respectively. Regarding gravidity; the median number of pregnancies was 3 [IQR 1–4] for women with spontaneous abortion while for full term delivered women was 2 [IQR 1–3] pregnancies. Regarding HIV status, 39/85 (54.1%) and 39/85 (45.9%) had unknown HIV status among women with spontaneous abortion and full term delivered women, respectively (Table 1).

The overall seropositivity of Brucella specific antibodies among sera from women with spontaneous abortion was 86/148 (58.1%, 95% CI 50–66) while that of full term delivered women was found to be 65/250 (26%, 95% CI 18–33), P < 0.001. Seropositivity of B. abortus was significantly higher among sera from women with spontaneous abortion than from full term delivered women [47/148 (31.8%) vs. (10.8%), P < 0.001]. Further analysis showed that there is no significant difference in seropositivity of B. melitensis among sera from women with spontaneous abortion and those from full term delivered women [19/250 (7.6%) vs. 10/148 (6.8%), P = 0.381]. Mixed Brucella seropositivity (B. abortus and B. melitensis) was found to be significantly higher among sera from women with spontaneous abortion than full term delivered women [29/148 (19.5%) vs. 19/250 (7.6%), P = 0.001].

Regarding factors associated with Brucella seropositivity among the entire population studied, only type of population was significantly associated with seropositivity. It was found that women with abortion had 3.59
odds of being brucella seropositive compared to full term women (OR: 3.59, 95% CI; 2.25–5.74, P < 0.001) (Table 2).

This is the first study in the city of Mwanza attempting to compare the Brucella seropositivity between women with spontaneous abortion and those with full term delivery. Overall seropositivity of brucella antibodies among sera from women with spontaneous abortion was found to be significantly higher than that of full term delivered women. The reported seropositivity from this study is higher than the previous reports in Iran, Nigeria and Kenya which documented the seropositivity of 13.6%, 19% and 27% among women with spontaneous abortion, respectively [14, 17, 27]. In addition, the reported seropositivity of Brucella spp. among women with normal deliveries in the current study was also found to be higher than what has been reported in Iran and Iraq which reported the prevalence of 6.2% and 15.2%, respectively [28, 29]. Variations in seropositivity in these studies can be explained by the differences in the diagnostic tests used [30]. The current study used Eurocell slide agglutination test with specificity of 100% and 95% sensitivity.

In the current study, seropositivity of Brucella abortus was significantly higher among sera from women with spontaneous abortion than that of sera from full term delivered women. This observation is different from a previous study in the United Kingdom [31]. Regarding B. melitensis there was no significant differences in seropositivity among the studied groups. The observed seropositivity is comparable to a previous report in the United Kingdom which reported seropositivity of 17.4% [31]. Seropositivity of mixed infection (B. abortus and B. melitensis) was found to be higher among sera from women with spontaneous abortion than among full term delivered women which is similar to a previous report [32]. In the current study on the multivariate analysis, we observed women with abortion to have 3.59 odds of being Brucella seropositive compared to full term women, however history of miscarriage was not found to be associated with brucella seropositivity. This could be explained by the timing of miscarriage and ability of the test to detect brucella antibodies from a distant past infection. Further studies to explore on the risk factors among these populations are highly recommended in Mwanza.

In conclusion the seropositivity of B. abortus antibodies is terrifyingly high among women with spontaneous abortion which call for the need for further investigations on the role of brucellosis in relation to adverse pregnancy outcomes particularly in resource limited countries where the disease is endemic.

Table 1 Sociodemographic characteristics of the study participants

| Characteristics   | Abortion       | Full term     |
|------------------|----------------|---------------|
|                  | Number/frequency | Percentage (%) | Number/frequency | Percentage (%) |
| Pregnancy status | 148            | 37.19%        | 250            | 62.81%        |
| Age              | 148            | 26.4 [25.4–27.3] | 250            | 25.2 [24.5–26.0] |
| Gravidity        | 148            | 3 [1–4]       | 250            | 2 [1–3]       |
| Marital status   |                |               |                |               |
| Married          | 126            | 85.1%         | 215            | 86%           |
| Single           | 22             | 14.9%         | 35             | 14%           |
| Education        |                |               |                |               |
| Primary          | 82             | 32.3%         | 172            | 67.7%         |
| Secondary        | 48             | 44.0%         | 61             | 56.0%         |
| Tertiary         | 18             | 51.4%         | 17             | 48.6%         |
| Occupation       |                |               |                |               |
| Business         | 27             | 18.24%        | 88             | 35.2%         |
| Employed         | 23             | 15.54%        | 21             | 8.4%          |
| House wife       | 61             | 41.22%        | 74             | 29.6%         |
| Peasant          | 37             | 25.0%         | 67             | 26.8%         |
| HIV status       |                |               |                |               |
| Positive         | 1              | 7.7%          | 12             | 92.7%         |
| Negative         | 101            | 33.7%         | 199            | 66.3%         |
| Unknown          | 46             | 54.1%         | 39             | 45.9%         |
Limitations
Due to the design of the study correlation between the timing of miscarriage and seropositivity was not determined and many known factors of Brucellosis could not be assessed. In addition, detection of acute Brucella infection using molecular techniques was not done.

Authors’ contributions
MMM, FM, EC and SEM participated in the design of the study. MMM, AV and FM participated in the retrieval of the specimens and relevant information. AV and MMM performed serological tests. SEM and EBM analysed and interpreted the data. MMM wrote the first draft of the manuscript. SEM did a critical review of the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data
All data were included.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The protocol for carrying out the study and used archived sera was approved by the Joint Catholic University of Health and Allied Sciences/Bugando Medical Centre (CUHAS/BMC) research ethics and review committee (CREC) with the ethical clearance number CREC/353/2017.

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Table 2 Factors associated with seropositivity of Brucella antibodies among 398 participants

|                     | Univariate | Multivariate |
|---------------------|------------|--------------|
|                     | Positive   | Negative     | P value | OR  | 95% CI | P value |
| Age                 | 26 [22–30] years | 24 [20–30] years | 0.109   | 0.98 | 0.92–1.04 | 0.566  |
| Gravidity           | 2 [1–4]    | 2 [1–3]      | 0.044   | 1.10 | 0.89–1.35 | 0.356  |
| Education           |            |              |         |     |         |        |
| Primary             | 85 (33.5%) | 169 (66.5%)  | 1       |     |         |        |
| Secondary           | 52 (47.7%) | 57 (52.3%)   | 1.45    | 0.57–3.65 | 0.425  |
| Tertiary            | 14 (40.0%) | 21 (60.0%)   | 0.036   | 0.79 | 0.30–2.08 | 0.639  |
| Occupation          |            |              |         |     |         |        |
| Peasants            | 37 (32.2%) | 69 (66.4%)   | 1       |     |         |        |
| H/wife              | 56 (41.5%) | 79 (58.5%)   | 0.68    | 0.28–1.60 | 0.374  |
| Business            | 37 (32.2%) | 78 (67.8)    | 0.74    | 0.31–1.77 | 0.503  |
| Employed            | 23 (52.3%) | 21 (47.7%)   | 0.072   | 0.66 | 0.28–1.56 | 0.349  |
| M/status            |            |              |         |     |         |        |
| Married             | 136 (39.9%)| 205 (60.1%)  | 1       |     |         |        |
| Single              | 15 (26.3%) | 42 (73.7%)   | 0.051   | 2.13 | 0.99–4.57 | 0.05   |
| HIV status          |            |              |         |     |         |        |
| Negative            | 115 (38.3%)| 185 (61.7%)  | 1       |     |         |        |
| Unknown             | 35 (41.2%) | 50 (58.8%)   | 0.94    | 0.54–1.62 | 0.818  |
| Positive            | 1 (7.7%)   | 12 (92.3%)   | 0.066   | 0.15 | 0.02–1.27 | 0.083  |
| Population          |            |              |         |     |         |        |
| Full term           | 65 (26.0%) | 185 (74.0%)  | 1       |     |         |        |
| Abortion            | 86 (58.1%) | 62 (41.9%)   | <0.001  | 3.59 | 2.25–5.74 | <0.001 |
| History of abortion |            |              |         |     |         |        |
| No                  | 135 (38.1%)| 219 (61.9%)  |         |     |         |        |
| Yes                 | 16 (36.4%) | 28 (63.6%)   | 0.819   |     |         |        |
