# 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

**Robert Makala**
Manyara Regional Referral Hospital
**Mtebe Venance Majigo** ( mmajigo@gmail.com )
Muhimbili University of Health and Allied Sciences https://orcid.org/0000-0003-3726-2816
**George M. Bwire**
Muhimbili University of Health and Allied Sciences
**Upendo Kibwana**
Muhimbili University of Health and Allied Sciences
**Mariam M. Mirambo**
Catholic University of Health and Allied Sciences
**Agricola Joachim**
Muhimbili University of Health and Allied Sciences

**Research article**

**Keywords:** Infection, Pregnancy, brucellosis, sero-prevalence, Tanzania

**Posted Date:** August 17th, 2019

**DOI:** https://doi.org/10.21203/rs.2.12994/v1



**Version of Record:** A version of this preprint was published on February 18th, 2020. See the published version at https://doi.org/10.1186/s12879-020-4873-7.

# Abstract

Background Brucellosis is a zoonotic disease transmitted to human through contact with infected animals, animal products or consumption of infected dairy products. Infection during pregnancy is of special interest due to associated adverse pregnancy outcome. This study determined the sero-prevalence and factors associated with Brucella infection among pregnant women around human-wildlife-livestock interface area in Ngorongoro Ecosystem, Northern Tanzania. Methods Pregnant women receiving antenatal care were invited to participate in a cross sectional study between April and May 2018. A structured questionnaire was used to collect socio-demographic and obstetric characteristics in addition to behavior and practices related to human brucellosis. Presence of serum immunoglobulin against Brucella was determined using Rose Bengal Plate Test. The positive samples were further assayed for presence of IgG and IgM using Enzyme-Linked Immunosorbent assay. Data were analyzed using Statistical Package for Social Sciences version 20. Results The overall Sero-prevalence of Brucella infection was 10.9%. Of 34 sero-positive individuals, 27(79.4%) had IgG antibodies while 8(23.5%) had IgM antibodies indicating 2.6% (8/313) recent infection. Regular contact with manure (OR 3.59, 95% CI 1.19 – 10.80, p = 0.023), consumption of raw milk (OR 3.58, 95% CI 1.12–11.39, p = 0.031), raw meat (OR 3.07, 95% CI 1.28 – 7.35, p = 0.012) and raw blood (OR 3.16, 95% CI 1.25–7.95, p = 0.015), increased the odds of being infected with Brucella. Majority of social-demographic and obstetric factors were not associated with the occurrence of brucellosis Conclusion Brucellosis is highly prevalent in areas with interactions of humans; livestock and wildlife. The risk of infection increased with regular contact with manure and consumption of raw foodstuff. These findings emphasize the need for interventional strategies to reduce risk of exposure and improve early detection of infection in pregnant women.


# 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, 3] Wild life animals in close proximity with human and domestic animal may act as reservoirs to both. [4] Meat processor, veterinarians, livestock farmers, milkers, abattoir workers and laboratory workers are populations at high risk of getting *Brucella* infection. [5, 6] The major challenge of *Brucella* infection in human is the sharing of clinical presentation to other febrile illness like: - Malaria and typhoid fever. Consequently, under reporting and mismanagement is common in areas with limited laboratory diagnosis. [7, 8]

*Brucella* infection in pregnancy is the major public concerns associated with several detrimental pregnancy outcomes such as spontaneous abortion, preterm delivery, and fetal death [2, 3, 5, 9] The risk of low birth weight has been demonstrated to be higher in pregnant women infected with *Brucella* than non infected9]The burden is mostly seen in poor individuals who regularly live in close contact with their animals.[10]

Previous studies conducted in Tanzania have reported up to 13% prevalence of brucellosis in the area of pastoral and agro pastoral communities [10, 11]. However, there is 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 attracted the need to determine the Sero-prevalence of *Brucella* infection and associated modifiable factors among pregnant women. Information generated from this study may be of help for policy and interventional strategies.

## Methods

# Study design and setting

This was health facility based cross-sectional study conducted between May and June 2018 in Ngorongoro District, Arusha region of Northern Tanzania. The study involved six health facilities providing antenatal services. These included: Wasso designated district hospital, Sakala and Loliondo health centres as well as Muholo, Sale, and Samunge dispensaries.. The District host part of the wildebeest migration, at the same time cattle goat and sheep rearing is a common practice in the district.

# Study population, sample size and sampling procedure

All pregnant women attending 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, considering 7.7% seroprevalence of Brucella infection in Arusha Tanzania [11] and 3% margin of error .. Participants who met eligibility criteria were consecutively enrolled in the study until reaching representative sample size.

# Data collection

Structured questionnaire was used to collect 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 abortion. Factors related to animal care and animal product consumption, alleged to influence transmission and persistence of *Brucella* infection were also collected.

# Specimen collection

Experienced health personnel collected 4 mls of venous blood aseptically using plain vacutainer system. The collected specimens were labelled with specific participant's identification number. Serum samples were separated from whole blood by centrifugation at 3,000 rpm for five minutes.

# Laboratory Procedure

Presence of non differentiated *Brucella abortus and/or Brucella melitensis* antibodieswas determined by Rose Bengal Plate Test (RBPT) a rapid agglutination test as previously described [12, 13]. Observation of distinct pink granules was recorded as positive test. Positive samples were kept at minus 20 $^{O}$C before transportation to reference laboratory in Dar es Salaam for detection of Immunoglobulin M and G antibodies. The commercially available test kits of enzyme-linked immunosorbent assay (ELISA) (Institut Virion/Serion GmbH) was used to detect IgM and IgG antibodies as previously described [14].

# Data analysis

Data was analysed using Statistical Package for Social Sciences version 20.0. Descriptive analysis of categorical variables was summarized in form of frequencies and percentages, while continuous variables were summarized as median ± inter-quartile range (IQR). Pearson's Chi-square test was performed to observe the significance of proportion differences of variables. The *p*-value of less than 0.05 was considered to be statistically significant. Bivariate analysis was conducted to determine the factors associated with *Brucella* sero-positivity and odds ratio was obtained at 95% confidence intervals (CI). All variables with $p < 0.2$ at bivariate analysis were considered for inclusion in multivariable logistic regression analysis to establish associations between factors and *Brucella* seropositivity.

## Results

# Characteristics *of participants and sero-prevalence of Brucella infection*

*A total of 313 participants were enrolled in the study, the median age was 25 years, IQR 20–30 years. Majority 299 (95.5%) were Agro-pastoralists, 150 (47.9%) had informal education, 288 (92.0%) were married, and 201(64.2%) had 28 or less weeks of gestation age. Above 50% of participants 161(54.4%) had more than three gravidity, while out 237 with prior pregnancy 35(14.8%) had history of abortion (Table 1).*

*All participants were screened for Brucella antibody using rapid RBPT test, among them 34(10.9%) were sero-positive. Of 34 Sero-positive individuals, 27(79.4%) had IgG antibodies for Brucella while 8(23.5%) had IgM antibodies equivalent to 2.6% (8/313) recent infection. Overall Sero-prevalence was higher in participants above 25 year (11.8%) than 25 years or less (10.0%). There was less sero-prevalence (10.4 %) among Agro-pastoralists than those with formal employment (21.4%). High prevalence were also recorded among participants with education above primary level, not married, gestation age ≤ 28 weeks, and gravida 1 to 2. Of 237 participants with prior pregnancy, sero-prevalence was more among those with history of abortion (17.1%) than without history of abortion (9.9%). The differences observed in demographic and obstetric characteristics were not statistically significant ($p > 0.05$) (Table 1).*

## Table 1: Socio-demographic and obstetric characteristics in relation to Brucella sero-positive

| Variable | Frequency | % participants | Sero-positivity N (%) | p - Value |
|---|---|---|---|---|
| Overall-seroprevalence | 313 | | 34 (10.9) | |
| IgG | 27 | 8.6 | | |
| IgM | 8 | 2.6 | | |
| IgM/IgG | 1 | 0.3 | | |
| **Age group** | | | | |
| ≤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) | |
| **Gravidity** | | | | |
| 1-2 | 152 | 48.6 | 17(11.2) | 0.859 |
| ≥3 | 161 | 54.4 | 17(10.6) | |
| **History of abortion (n=237)** | | | | |
| No | 202 | 85.2 | 20(9.9) | 0.206 |
| Yes | 35 | 14.8 | 6(17.1) | |

## Behaviour and practice associated with Brucella infection

*Several factors assumed to increase possibility of Brucella infection were assessed as shown in table 2. Off all participants, 61.3%, 67.1% and 64.2% reported regular contact with animal manure, assist animal during birth, and wash animal at home respectively. Regarding food consummation behavior, 73.2% reported drinking raw milk, 24.3% eating uncooked meat, while 55.6% consumed raw blood. Slightly more than 50% participants reported sharing water source with animal. At bivariate analysis, regular contact with animal manure, contact with animal placenta through assist of parturition, consumption of raw milk, uncooked meats, and raw animal blood, were significantly associated with the occurrence of brucellosis ($p < 0.05$). The odds of being Sero-positive among these factors ranged from 2.1 to 3.1 times (Table 2).*

## 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 | | |
| Assist animal giving birth | | | | | |
| 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 of raw 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 of uncooked meat | | | | | |
| Yes | 76 (24.3) | 13(17.1) | 2.1 | (1.01-4.48) | 0.044 |
| No | 237 (75.7) | 21(8.9) | 1 | | |
| Raw blood consumption | | | | | |
| Yes | 174 (55.6) | 26 (14.9) | 2.9 | (1.26-6.57) | 0.009 |
| No | 139 (44.4) | 8 (5.8) | 1 | | |
| Share 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*

*Table 3 shows the result of multivariate analysis by multivariable regression model performed to measure the relationship between Brucella sero-positivity and independent variables. All variables that showed p-values < 0.2 in the univariate analysis were included. Regular contact with manure (OR 3.59, 95 % CI 1.19—10.80, p = 0.023), consumption of raw milk (OR 3.58, 95 % CI 1.12–11.39, p = 0.031), preference of raw meat (OR 3.07, 95 % CI 1.28—7.35, p = 0.012) and consumption of raw blood (OR 3.16, 95 % CI 1.25–7.95, p = 0.015), increased the odds of being infected with Brucella (Table 3).*

# Table 3: Multivariable analysis of factors for occurrence of Brucella seropositive

| variable | AOR | 95% CI | p value |
|---|---|---|---|
| **Occupation** | | | |
| Agro-pastoralist | 1 | | |
| Formal employment | 2.04 | 0.34 – 12.09 | 0.433 |
| **Level of education** | | | |
| Informal | 1 | | |
| Primary | 3.46 | 1.30 – 9.18 | 0.013 |
| Secondary and above | 7.83 | 2.66 – 23.04 | 0.000 |
| **Regular contact with manure** | | | |
| Yes | 3.59 | 1.19 – 10.80 | 0.023 |
| No | 1 | | |
| **Assist animal giving birth** | | | |
| Yes | 3.13 | 0.96 – 10.22 | 0.059 |
| No | 1 | | |
| **Preference of raw milk** | | | |
| Yes | 3.58 | 1.12 -11.39 | 0.031 |
| No | 1 | | |
| **Preference of uncooked meat** | | | |
| Yes | 3.07 | 1.28 – 7.35 | 0.012 |
| No | 1 | | |
| **Raw blood consumption** | | | |
| Yes | 3.16 | 1.25 – 7.95 | 0.015 |
| No | 1 | | |

*Key: AOR = Adjusted odds ratio, CI = Confidence interval*

## Discussion

*The current study has demonstrated higher (10.9%) seroprevalence of Brucella infection in pregnant women compared to previously report in general population of the same geographical location [11, 15]. In addition, the study has revealed nearly 3% of pregnant women with immunologic evidence of recent Brucella infection. The seroprevalence found among pregnant women suggests that Brucella infection is a public health problem in Ngorongoro District. Elsewhere, diverse level of brucellosis in different population has been reported. The prevalence of 17% was reported in agro-pastoral communities in Uganda [16]; 25% among women with abortion in Rwanda [3]; 5.8% among pregnant women in Pakistan [5] and 11.25% among pregnant women in Nepal [17]. These reported findings signify the endemicity of brucellosis in various countries worldwide.*

*The community where this the present study were conducted comprised around 95% agro-pastoralists, in which, women do most of the work associated with care and harvest of livestock products. Activities commonly done by women include milking, cleaning livestock houses, house repair using cattle dung and handling newly borne calves, which predispose them to brucellosis [6]. Reports suggesting brucellosis as endemic disease in Tanzania, [10, 18] and the proximity of population with livestock-wildlife interface subject then into risk of Brucella infection. The high Sero-prevalence of Brucella infection in pregnant women showed by this study may be attributed to different behaviour and practice towards livestock handling and preparation of food of animal origin in the community. The Current study has identified four independent factors associated with Brucella infection including: contact with manure, consumption of raw milk, meat and blood.*

*Regular contact with animal manure had increased odds of being sero-positive for Brucella infection. The finding of our study is in agreement with other studies that reported direct contact with livestock excreta being potential sources of Brucella infection [11, 19]. It is documented that Brucella spp from infected animals are found in animal excreta which serve as sources of humans infections [20]. Our study did not find contact with placenta being independent factor for Brucella infection contrary to what has been reported in other studies [21–24]. On the other hand, our finding is in line with findings reported in Uganda [16].*

*Eating habits may expose an individual to Brucella infection if the consumed products from infected livestock are not properly prepared. [11, 16, 25, 26] Substantial number of participants in the current study reported regular habit of drinking raw milk (73.2%), eating uncooked meat (24.3%) and consumption of raw animal blood (55.6%). The habits of consumption of raw foodstuff were among the factors independently constituting risk for Brucella infection among pregnant women in Ngorongoro District. This could be explained by fact that the studied community regularly consume raw milk and include Sonjo tribe which traditionally consume wild animal. Our findings are in agreement with 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 [6, 11]. Other studies in Africa reported the same predictors for transmission of brucellosis, but tend to vary widely depending on customs and taboos of referred community [19, 27]*

*It was interesting to note that having formal education increased the odd of Brucella infection in pregnant women. Further analysis of study participants revealed significant differences of education level with regular contact with manure. Only 18.7% with informal education were in regular contact with manure against 54.1 % and 60.5 % with primary and secondary level of education respectively. The possible reason for the differences observed is that those with education are likely to create modern structure for keeping animal. Due to the need of regular cleaning of the modern structure, increase the likelihood of contact with manure.*

*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 some possible factors associated with occurrence of brucellosis. Reporting error for some measures were reduced by asking participants to recall only recent events. Despite limitations encountered, this study has demonstrated some important factors associated with transmission to human in Ngorongoro ecosystem. Our findings serve as considerable baseline data for prevention and control of the disease and associated adverse effect in pregnancy.*

## *Conclusions*

*Brucella infection among pregnant women around human, wildlife and livestock interface in Ngorongoro ecosystem is remarkable. Regular contact with animal manure and consumption of raw foodstuff are the main factors associated with brucellosis in the study area. We recommend interventional strategies to*

*improve animal excreta handling practices; discourage the habit of raw foodstuff consumption and screening pregnant women for brucellosis as necessary measures for prevention of disease and associated adverse effects in pregnancy.*

## List Of Abbreviations

*ELISA: Enzyme-linked immunosorbent assay, IgG: Immunoglobulin G, IgM: Immunoglobulin M, MUHAS: 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 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 analysed 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 manuscript.*


# Acknowledgements

*We are grateful to all pregnant women who participated in the study. We thank laboratory personnel at Wasso hospital and Central Veterinary Laboratory for technical support. Our sincere gratitude also goes to health workers at Wasso hospital for cooperation and assistance in recruitment and specimen collection.*


## References


*1.Gwida M, Al Dahouk S, Melzer F, Rosler U, Neubauer H, Tomaso H: Brucellosis - regionally emerging zoonotic disease? Croat Med J 2010, 51(4):289–295.*

*2.Kurdoglu M, Adali E, Kurdoglu Z, Karahocagil MK, Kolusari A, Yildizhan R, Kucukaydin Z, Sahin HG, Kamaci M, Akdeniz H: Brucellosis in pregnancy: a 6-year clinical analysis. Arch Gynecol Obstet 2009, 281(2):201–206.*

*3.Rujeni N, Mbanzamihigo L: Prevalence of Brucellosis among Women Presenting with Abortion/Stillbirth in Huye, Rwanda. J Trop Med 2014, 2014:740479.*

*4.Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, Whatmore AM, Cloeckaert A, Blasco JM, Moriyon I et al: Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Prev Vet Med, 102(2):118–131.*

*5.Ali S, Akhter S, Neubauer H, Scherag A, Kesselmeier M, Melzer F, Khan I, El-Adawy H, Azam A, Qadeer S et al: Brucellosis in pregnant women from Pakistan: an observational study. BMC Infect Dis 2016, 16:468.*

*6.Swai ES, Schoonman L: Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. Zoonoses Public Health 2009, 56(4):183–187.*

*7.Bosilkovski M, Dimzova M, Grozdanovski K: Natural history of brucellosis in an endemic region in different time periods. Acta Clin Croat 2009, 48(1):41–46.*

*8.Memish ZA, Balkhy HH: Brucellosis and international travel. J Travel Med 2004, 11(1):49–55.*

*9.Vilchez G, Espinoza M, D'Onadio G, Saona P, Gotuzzo E: Brucellosis in pregnancy: clinical aspects and obstetric outcomes. Int J Infect Dis 2015, 38:95–100.*

*10.Kunda J, Fitzpatrick J, Kazwala R, French NP, Shirima G, Macmillan A, Kambarage D, Bronsvoort M, Cleaveland S: Health-seeking behaviour of human brucellosis cases in rural Tanzania. BMC Public Health 2007, 7:315.*

*11.John K, Fitzpatrick J, French N, Kazwala R, Kambarage D, Mfinanga GS, MacMillan A, Cleaveland S: Quantifying risk factors for human brucellosis in rural northern Tanzania. PLoS One, 5(4):e9968.*

*12.Colmenero JD, Reguera JM, Cabrera FP, Hernandez S, Porras J, Manchado P, Miranda MT: [Combined use of rose Bengal and indirect immunofluorescence in the diagnosis of brucellosis]. Enferm Infecc Microbiol Clin 1989, 7(6):316–320.*

*13.Ruiz-Mesa JD, Sanchez-Gonzalez J, Reguera JM, Martin L, Lopez-Palmero S, Colmenero JD: Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clin Microbiol Infect 2005, 11(3):221–225.*

*14.Fadeel MA, Hoffmaster AR, Shi J, Pimentel G, Stoddard RA: Comparison of four commercial IgM and IgG ELISA kits for diagnosing brucellosis. J Med Microbiol 2011, 60(Pt 12):1767–1773.*

*15.Shirima GM, Kunda JS: Prevalence of brucellosis in the human, livestock and wildlife interface areas of Serengeti National Park, Tanzania. Onderstepoort J Vet Res, 83(1):a1032.*

*16.Tumwine G, Matovu E, Kabasa JD, Owiny DO, Majalija S: Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. BMC Public Health, 15:900.*

*17.Thapa S MM: Sero Prevalence of Brucellosis in Pregnant Women Visiting Gynaecology Department of Kathmandu Model Hospital, Kathmandu, Nepal. National Journal of Health Sciences 2018, 9(3):16–19.*

*18.Assenga JA, Matemba LE, Muller SK, Malakalinga JJ, Kazwala RR: Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC Vet Res, 11:189.*

*19.Genene R DM, Yamuah L, Hiwot T, Teshome G, Asfawesen G, Abraham A, Abdoel TH, Smits HL.: Human brucellosis in traditional pastoral communities in Ethiopia. International Journal of Tropical Medicine 2009, 4(2):59–64.*

*20.Young EJ: Human brucellosis. Rev Infect Dis 1983, 5(5):821–842.*

*21.Bikas C, Jelastopulu E, Leotsinidis M, Kondakis X: Epidemiology of human brucellosis in a rural area of north-western Peloponnese in Greece. Eur J Epidemiol 2003, 18(3):267–274.*

*22.Cooper CW: Risk factors in transmission of brucellosis from animals to humans in Saudi Arabia. Trans R Soc Trop Med Hyg 1992, 86(2):206–209.*

*23.Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J: Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev Vet Med 2003, 61(4):279–293.*

*24.Lim HS, Min YS, Lee HS: [Investigation of a series of brucellosis cases in Gyeongsangbuk-do during 2003–2004]. J Prev Med Public Health 2005, 38(4):482–488.*

*25.Hambolu D, Freeman J, Taddese HB: Predictors of bovine TB risk behaviour amongst meat handlers in Nigeria: a cross-sectional study guided by the health belief model. PLoS One, 8(2):e56091.*

*26.Pappas G, Akritidis N, Bosilkovski M, Tsianos E: Brucellosis. N Engl J Med 2005, 352(22):2325–2336.*

*27.Adesokan HK, Alabi PI, Ogundipe MA: Prevalence and predictors of risk factors for Brucellosis transmission by meat handlers and traditional healers' risk practices in Ibadan, Nigeria. J Prev Med Hyg, 57(3):E164-E171.*