Original Article

Seroprevalence of brucellosis among women with miscarriage at Ahmadu Bello University Teaching Hospital, Zaria

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ABSTRACT

Background: Brucellosis in animals has been identified as a common cause of miscarriage. It is the most common zoonotic disease that leads to considerable morbidity in humans. It is rarely diagnosed in hospitals in Nigeria, and debate exists as to whether it is a more common cause of miscarriage in humans compared to other infective agents, especially with the finding of antibrucella activity in human amniotic fluid. Brucellosis in humans is a treatable disease and risk factors for transmission are prevalent in Zaria.

Objective: The objective of this study was to determine the seroprevalence of brucellosis among women with miscarriage.

Materials and Methods: This was a descriptive cross-sectional study involving 121 women aged between 15 and 49 years with miscarriage who presented to Ahmadu Bello University Teaching Hospital (ABUTH), Zaria from August 2014 to May 2015. Information on socio-demographic characteristics, reproductive profile, and risk factors for contracting *Brucella* infection were obtained using a questionnaire. Blood samples were obtained and analysed for *Brucella* IgG and IgM using indirect enzyme-linked immunosorbent assay kits. The data was analysed with SPSS, version 20.0.

Results: The mean age of the participating women was 29.07 years [standard deviation (SD) ±6.74]. The seroprevalence of brucellosis was 19.0%; 17.4% of the women had a recent infection, and 1.7% had a chronic infection. Age, history of previous miscarriage, consumption of milk products and consumption of roasted meat/barbecue had positive relationships with recent *Brucella* infection ($\chi^2 = 9.706$, $P = 0.046$; $\chi^2 = 7.300$, $P = 0.026$; $\chi^2 = 3.169$, $P = 0.049$; $\chi^2 = 3.012$, $P = 0.050$, respectively). Chronic *Brucella* infection had a positive relationship with number of pregnancies ($\chi^2 = 8.036$, $P = 0.018$). Regression analyses showed that age, history of previous miscarriage and history of recent miscarriage in animals reared were positively correlated with *Brucella* seropositivity and miscarriage ($\chi^2 = 13.200$, $P = 0.022$; $\chi^2 = 9.795$, $P = 0.007$; $\chi^2 = 7.890$, $P = 0.005$, respectively).

Conclusion: There is a high prevalence of brucellosis among women with miscarriage in Zaria. The burden of the disease should be appreciated and routinely tested to prevent reoccurrence.

Key words: Brucellosis; miscarriage; prevalence.

Introduction

Brucellosis is a zoonosis transmitted directly or indirectly by exposure to infected animals.[1-6] It is the most common zoonotic disease that leads to considerable morbidity and loss of man-days across the globe and thus perpetuates

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poverty.[4,5] It is endemic in Nigeria causing severe economic losses to livestock farmers and ranchers and is a serious risk to human health.[3,7,8] It is rarely diagnosed in hospitals in Nigeria despite suggestions that the magnitude of infection may be greater than appreciated.[2,3] This may be due to other diseases such as malaria and typhoid fever with similar clinical signs that are endemic and hence often diagnosed.[3] The disease if left untreated can lead to malaise, orchitis, infertility, miscarriage, stillbirth, etc., with its consequent socio-economic implications.[3,8]

Brucellosis is caused by *Brucella* bacteria, which is a gram-negative, facultative, intra-cellular organism.[9,10] There are four types of *Brucella* species, namely *Brucella abortus*, *Brucella melitensis*, *Brucella suis* and *Brucella canis*.[10] The organisms can affect cattle, sheep/goat, swine, and dogs. Humans are susceptible to all species of the organism, and cross-infections in animals have also been reported.[11] Consumption of infected meat, unpasteurised dairy products and occupational contact are the major risks of human infection. Contact with infected materials such as aborted foetus, placentas, urine, manure, carcass and salvaged animals has also been implicated.[12,13] During pregnancy, brucellosis carries the risk of spontaneous miscarriage, intra-uterine transmission to the foetus, foetal demise and pre-term delivery.[5,12-14]

Maternal infection with intra-uterine, trans-placental transmission has been implicated in 10–25% of second trimester pregnancy losses.[15] This is more closely linked to pregnancy loss in developing countries.[2] Although brucellosis can result in miscarriage in humans, it has been debated whether it is any more frequent than other bacterial infections,[16] especially with the finding that human amniotic fluid has antibrucella activity.[17] Reports from areas where *Brucella melitensis* infection is endemic suggest that there is an increased rate of miscarriage in asymptomatic pregnant women.[18]

Despite the physical and emotional trauma that pregnancy loss causes, studies on the contribution of brucellosis as an infective cause of miscarriage in Nigeria are scarce, even though it is acknowledged that human contact with animal reservoirs and consumption of unpasteurised milk and/or uncooked or under-cooked meat are common practices.[3,17,18] The purpose of this study was to determine the seroprevalence of *Brucella* infection among women who presented with miscarriage at Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria.

Materials and Methods

The study was carried out at ABUTH Zaria, Kaduna state, Nigeria. The study population included women in the reproductive age group between the ages of 15 and 49 years who had a miscarriage and presented to the emergency unit, reproductive health unit or gynaecological clinic/ward of ABUTH, Zaria. For this study, miscarriage was defined as spontaneous termination of pregnancy before 28 weeks of gestation, which is the age of foetal viability in the study area. It was a cross-sectional study, and purposive, non-probability sampling technique was used.

Patients enrolled for the study were consenting women with spontaneous miscarriage (complete, incomplete, inevitable, missed or septic) in whom pregnancy was confirmed with urine/serum pregnancy test strip and/or by pelvic ultrasound scan who presented within 1 week of onset of symptoms.

Non-consenting women, patients with induced/therapeutic abortion and women who had had recent treatment with antibiotics (doxycycline, tetracycline; riampicin; sulfamethoxazole/trimethoprim; ciprofloxacin) within the last 6 weeks prior to presentation were excluded from the study.

Enzyme-linked immunosorbent assay (ELISA) kits manufactured by Diagnostic automation, Incorporated, California, USA was used for the detection of IgG and IgM *Brucella* antibodies. This Diagnostic Automation's *Brucella* IgM and IgG indirect ELISA test kits were manufactured for the detection and quantitative/qualitative determination of specific IgM and IgG antibodies against *Brucella* in serum and plasma. *Brucella* antigen is pre-coated on the surface of the microtiter strips in the kit. The sensitivity and specificity of these test kits in the detection of *Brucella* is 100%. The intra-assay precision was 9.2% for IgM and 8.7% for IgG; the inter-assay precision was 5.0% for IgM and 7.9% for IgG and the shelf life was 12–18 months from the date of manufacture. Biosafety was ensured throughout the process.

Results

The mean age of the participants in this study was 29.07 years [standard deviation (SD) ±6.74] with a range of 17–49 years. Most women (26.6%) were aged between 25 and 29 years. Majority (79.3%) of the women resided in urban areas and 94.2% (114) were married. The Hausa ethnic group was the predominant ethnic group (66.1%); 80.2% were of the Islamic religion; 51.2% were housewives; 37.2% of the participants' husbands were civil servants and 30.6% had acquired tertiary education.

Most of the participants (54.5%) had a history of 1–4 pregnancies prior to the index miscarriage. The mean number of pregnancies was 4.18 pregnancies (SD ± 3.0). Women with no history of prior miscarriage constituted

52.1% of the participants, whereas those who had a history of previous miscarriage constituted 47.9% of the total study population. The mean gestational age at which miscarriage occurred in the women was 5.73 weeks (SD ± 7.5). Among the 47.9% participants with a history of previous miscarriage, majority (53.7%, 29) had pregnancy loss at 12 weeks gestation or more. Out of the 121 participants, 96.7% had no history of preterm birth; 5.8% had a history of fresh stillbirth; 3.3% had history of macerated stillbirth and 74.4% had history of a live birth. The index miscarriage occurred at gestational age of 12 weeks or more in 54.6% (66) of the participants.

Women who reared animals constituted 34.7% (42) of the participants [Table 1]. Goat (14.9%) was the most common type of animal reared. Majority (79.3%) of the participants did not keep pets. The most common type of pet kept by the women was cat (56%). Only 19% (23) cases showed that the animals kept by the participants had recently delivered, and in 5.8% (7) the animals had recently miscarried a pregnancy. Most of the women (81.8%) washed their hands sometimes after having contact with animals. Majority of the participants (51.2%) had history of sustaining accidental cuts on the hand while slaughtering an animal or cutting meat. Only 54.8% of these women reported continuing with animal slaughtering or meat cutting after sustaining the cut.

Consumption of fresh, unboiled (unpasteurised) milk was a common practice found in 62.0% (75) of the participants. Majority of these women (94.2%) also ingested milk products such as yoghurt and cheese. Eating roasted meat/barbecue was also a common practice among 98.3% (119) of the participants. However, most women (81.8%) had never been transfused with blood.

The overall seroprevalence of brucellosis among the participants was 19.0%. The proportion of recent infection was 17.4% and chronic infection was 1.7% among women with miscarriage in the study [Table 2].

A positive relationship between age, history of previous miscarriage, consumption of milk products, consumption of roasted meat/barbecue and recent *Brucella* infection was found in this study ($\chi^2 = 9.706$, $P = 0.046$; $\chi^2 = 7.300$, $P = 0.026$; $\chi^2 = 3.169$, $P = 0.049$; $\chi^2 = 3.012$, $P = 0.050$, respectively) [Tables 3-5]. Chronic *Brucella* seropositivity was also found to have a positive relationship with the number of pregnancies participants had in the past ($\chi^2 = 8.036$, $P = 0.018$) [Table 4]. There was, however, no relationship between the residential area, marital status, ethnic group, religion, educational level, history of pre-term birth/still-birth, gestational age of index miscarriage of the participants and *Brucella* infection [Tables 3 and 4].

Table 1: Factors associated with *Brucella* infection among participants

Factor	Number (N=121)	Percentage
Animal rearing		
Yes	42	34.7
No	79	65.3
Pet keeping		
Yes	25	20.7
No	96	79.3
Type of pet kept		
Dog	11	44
Cat	14	56
Recent animal delivery		
Yes	23	19
No	98	81
Recent miscarriage in animals		
Yes	7	5.8
No	114	94.2
No hand washing after touching animals		
Always	3	2.5
Sometimes	99	81.9
Never	19	15.7
Accidental cut while slaughtering animals/ cutting meat		
Yes	62	51.2
No	59	48.8
Continuation with slaughtering/meat cutting with cut on the hand		
Yes	34	54.8
No	28	45.2
Drinking unpasteurized milk		
Yes	75	62.0
No	46	28.0
Milk products consumption		
Yes	114	94.2
No	7	5.8
Eating locally roasted meat/barbecue		
Yes	119	98.3
No	2	1.7
History of blood transfusion		
Yes	22	18.2
No	99	81.8

Multi-nominal logistic regression analyses showed that age, history of previous miscarriage and history of recent miscarriage in animals reared were positively correlated with *Brucella* seropositivity ($\chi^2 = 13.200$, $P = 0.022$; $\chi^2 = 9.795$, $P = 0.007$; $\chi^2 = 7.890$, $P = 0.005$, respectively) [Table 6].

Discussion

Brucellosis is an endemic zoonotic disease with a world-wide incidence of less than 0.01 to greater than 200 per 100,000

Table 2: Seroprevalence of brucellosis by age group

Age group	Serum IgM				Serum IgG			
	Negative		Positive		Negative		Positive	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
<20 years	5	4.1	0	0.0	5	4.1	0	0.0
20-24 years	16	13.2	9	7.4	25	20.7	0	0.0
25-29 years	26	21.5	6	5.0	32	26.4	0	0.0
30-34 years	25	20.7	4	3.3	28	23.1	1	0.8
35-39 years	21	17.4	0	0.0	20	16.5	1	0.8
>39 years	7	5.8	2	1.7	9	7.4	0	0.0
Total	100	82.6	21	17.4	119	98.3	2	1.7
Overall Prevalence	23 (19.0%)							

Table 3: Statistical relationship of the socio-demographic characteristics of participants with *Brucella* infection

Characteristic	Recent infection (Positive IgM)	χ^2	*P*	Chronic infection (Positive IgG)	χ^2	*P*
Age	21 (17.4%)	9.706	0.046	2 (1.7%)	2.285	0.68
Residential area		0.040	0.841		0.530	0.46
Rural	4 (3.3%)					
Urban	17 (14.40%)			2 (1.7%)		
Marital status		1.316	0.518		0.125	0.93
Single	2 (1.7%)					
Married	19 (15.7%)			2 (1.7%)		
Separated						
Ethnic group		1.370	0.849		7.047	0.13
Hausa	13 (10.7%)			4 (0.8%)		
Fulani	1 (0.8%)					
Yoruba	3 (2.5%)			1 (0.8%)		
Igbo	1 (0.8%)					
Others	3 (2.5%)					
Religion		0.010	0.921		1.164	0.28
Islam	17 (14.0%)			1 (0.8%)		
Christianity	4 (3.3%)			1 (0.8%)		
Occupation		3.367	0.762		10.917	0.09
Artisan						
Business	2 (1.7%)			2 (1.7%)		
Civil service	2 (1.7%)					
Housewife	13 (10.7%)					
Student	4 (3.3%)					
Farming						
Education		0.320	0.988		4.802	0.30
None	1 (0.8%)					
Quranic	6 (5.0%)					
Primary	2 (1.7%)					
Secondary	6 (5.0%)			2 (1.7%)		
Tertiary	6 (5.0%)					

Key: χ^2=Pearson's Chi-square; $P \leq 0.05$ (significant)

population.[6] It is rarely diagnosed in Nigerian hospitals even though the burden of the disease may be greater than appreciated.[3] The prevalence of *Brucella* infection derived in this study (19.0%) was higher than 1.8% prevalence reported in Jordan with a similar study population.[19] The Jordanian study was a hospital-based case-control study that involved 445 women with miscarriage and those without a history of miscarriage. The rose bengal plate test and complement fixation test were used in analysing the blood samples. The study found no statistical significance of *Brucella* seroprevalence among women with miscarriage and those with no history of miscarriage. This study used diagnostic tests that were not 100% sensitive and specific. A prospective, hospital-based study conducted at Saudi Arabia showed *Brucella* infection prevalence of 12.2% with an incidence of miscarriage of 27.27%.[20] In another study from Saudi Arabia,

Table 4: Statistical relationship of the reproductive profile of participants with *Brucella* infection

Profile	Recent infection (Positive IgM)	χ^2	*P*	Chronic infection (Positive IgG)	χ^2	*P*
Previous pregnancy no		1.655	0.437		8.004	0.018
0	2 (1.7%)			1 (0.8%)		
1-4	13 (10.7)					
>4	6 (5.0%)			1 (0.8%)		
History of previous miscarriage		7.300	0.026		0.927	0.629
None	14 (11.6%)			1 (0.8%)		
1	7 (5.8%)			1 (0.8%)		
>1	0 (0.0%)					
History of preterm birth (weeks)		1.095	0.578		0.085	0.957
None	21 (17.4%)			2 (1.7%)		
28-32	0 (0.0%)					
33-37	0 (0.0%)					
History of stillbirth		0.486	0.784		0.163	0.922
None	20 (16.5%)			2 (1.7%)		
1	1 (0.8%)					
>1	0 (0.0%)					
Gestational age of index miscarriage		2.480	0.289		1.004	0.605
<12 weeks	17 (14.0%)			2 (1.7%)		
≥12 weeks	4 (3.3%)			33 (30.6%)		

Key: χ^2=Pearson's Chi-square; $P \leq 0.05$ (significant)

incidence of miscarriage of 43% was reported,[21] which is similar to 35% incidence found in Kuwait,[22] but higher than 11.6% and 24.14% incidence found in other Middle-east studies in Iran[23] and Turkey,[24] respectively, as well as also the 19.0% found in this study.

Recent *Brucella* infection was found in this study to have a positive relationship with age, with the highest prevalence (5%) found in the age group of 25–29 years, which is similar to studies reported from Iran[25] and Mobarakeh[26] where those aged between 20 and 24 years and 11 and 20 years, respectively, had the highest record of contracting the disease. This study revealed that women in the reproductive age group, aged between 15 and 49 years, who live in endemic areas had nine times increased risk of contracting this zoonotic infection and having a spontaneous miscarriage. However, study from Egypt found no statistical significance between the age of patients, gestational age and spontaneous miscarriage in women with brucellosis.[10] Furthermore, no statistical relationship was also found between participants' occupation, animal rearing and residential location in this study, as was found in an Indian study.[13] Most patients (62.8% and 50.4%) found to be infected in Albania[27] and Mobarakeh[26] resided in rural areas, respectively. Occupation of infected patients was also found to be associated with *Brucella* infection in Iran.[25] Another study conducted among abattoir workers in Abuja showed that occupational exposure greater than 5 years and slaughtering animals while having open wounds were significantly significant with *Brucella* infection.[28] This finding was not corroborated in this study.

A positive relationship between history of previous miscarriage and recent *Brucella* infection was found in this study. This underscores the prevalence of associated factors for disease transmission in Zaria as these women have seven times increased risk of having miscarriage. Pregnant women screened with serum agglutination test and found to have antibody titer greater than 1:160 were found to have two-fold increased risk of having spontaneous miscarriage in Kenya.[29] This finding was similar to the finding reported in Egypt where there was statistically significant miscarriage in women with positive *Brucella* antibody titer; miscarriage was higher in women whose *Brucella* antibody titer was greater than 1:160 compared to those with titers less than 1:160.[10] Pregnant women with brucellosis in Turkey were also found to have miscarriage rate that substantially exceeded the rate among the general population of women in the hospital.[24] The number of pregnancies the participants have had was found to have a positive relationship with chronic *Brucella* infection. The disease has the capacity to cause persistent disease by circumventing the innate and adaptive immunity.[20] Thus, with an increasing number of pregnancies and undiagnosed and/or untreated *Brucella* infection, the women have eight-fold increased risk of miscarriage.

Consumption of local delicacies such as milk products (e.g., cheese and yoghurt) and locally roasted meat/barbecue were found to have a positive relationship with contracting recent *Brucella* infection and increasing the risk of having miscarriage three-fold, respectively. This is similar to the findings found in Tanzania where eating undercooked or raw

Table 5: Statistical significance of risks associated with contracting *Brucella* infection in participants

Risk factor	Recent infection (Positive IgM)	χ^2	*P*	Chronic infection (Positive IgG)	χ^2	*P*
Animal rearing		0.423	0.516		1.081	0.29
Yes	6 (5.0%)					
No	15 (12.4%)			2 (1.7%)		
Pet keeping		0.630	0.427		1.068	0.30
Yes	3 (5.0%)			1 (0.8%)		
No	18 (14.9%)			1 (0.8%)		
Recent animal delivery		0.368	0.544		0.477	0.49
Yes	3 (2.5)					
No	18 (14.9)			2 (1.7%)		
Recent miscarriage in animals		3.369	0.066		0.125	0.72
Yes	3 (2.5%)			2 (1.7%)		
No	18 (14.9%)					
No hand washing after touching animals		1.199	0.549		0.452	0.79
Always	1 (0.8%)					
Sometimes	18 (14.9%)			2 (1.7%)		
Never	2 (1.7%)					
Accidental cut while slaughtering animal/cutting meat		0.133	0.715		2.137	0.14
Yes	10 (8.3%)			0 (0.0%)		
No	11 (9.1%)			2 (1.7%)		
Drinking of unpasteurized milk		0.004	0.319		1.247	0.26
Yes	11 (9.1%)			2 (1.7%)		
No	10 (8.3%)					
Milk products consumption		3.169	0.049		0.125	0.72
Yes	18 (14.9%)			2 (1.7%)		
No	3 (2.5%)					
Eating roasted meat/barbecue		3.012	0.050		0.034	0.85
Yes	21 (17.4%)			2 (1.7%)		
No						
History of blood transfusion		0.259	0.611		0.452	0.50
Yes	3 (2.5%)			3 (2.5%)		
No	18 (14.9%)			18 (14.9%)		

Key: χ^2=Pearson's Chi-square; $P \leq 0.05$ (significant)

Table 6: Multinominal logistic regression model of parameters correlating with *Brucella* seropositivity

Variable	χ^2	*P*
Age	13.200	0.022
Residence	0.301	0.584
History of previous Miscarriage	9.795	0.007
Animal rearing	1.003	0.3317
Pet keeping	0.014	0.905
History of recent miscarriage in animals	7.890	0.005
Consumption of unpasteurized milk	0.251	0.617
Consumption of milk products	2.782	0.095

Key: χ^2=Pearson's Chi-square; $P \leq 0.05$ (significant)

meat, seeping raw blood, contact with cattle manure, milking cattle, contact with placenta during assisted parturition and home slaughter of animals were found to be the main risk factors for direct transmission of brucellosis by direct contact.[30] Consumption of unpasteurised milk was not statistically significant to cause *Brucella* infection in this study in contrast to the findings in Tanzania,[30] Albania,[27] and Mobarakeh.[26] A study of brucellosis in low- and middle-income countries revealed that ingestion of unpasteurised dairy products and exposure through direct contact with infected animal fluids or tissues, especially the placenta from aborted animals, were the main risk factors for transmission of the infection.[31]

The multi-nominal logistic regression model showed that age, history of previous miscarriage and history of recent miscarriage in animals reared had a positive correlation with contracting *Brucella* infection and miscarriage, increasing the risk thirteen-fold, nine-fold, and seven-fold, respectively. It will, therefore, be necessary to elucidate these factors in women with miscarriage. An assessment of demographic factors for brucellosis-infected patients in Iran revealed that 73% of the study population had direct contact with the disease carrying animal while conducting miscarriage on the pregnant cow.[25] This finding is similar to those of this study, however, the finding of assisting an animal during the birth process was not statistically significant in this study.[32]

This study has highlighted the significance of brucellosis as a common cause of spontaneous miscarriage in Zaria. Indeed, the burden of the disease (19.0%) and the prevalent risk factors for contracting the disease such as consumption of locally prepared barbecue/roasted meat and/or milk products such as cheese and yoghurt and history of recent miscarriage in animals reared in women aged between 15 and 49 years should be better appreciated. The high seropositivity detected in participants also highlights the need for a standardised screening/detection tool. The indirect ELISA kits which have 100% sensitivity and specificity used in this study may have helped in diagnosing this disease and detecting those with recent or chronic infection.

Recommendations from this study are routine screening of women with miscarriage for *Brucella* infection. Public enlightenment to raise awareness of the population regarding the prevalent risk factors for contracting *Brucella* infection, adoption of safe practices such as consumption of well-prepared barbecue/roasted meat and or milk products such as yoghurt and cheese including washing of hands with soap and water after contact with animals who have a recent miscarriage and/or the product of conception.

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Conflicts of interest
There are no conflicts of interest.

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