Serological and Molecular Detection of Dual Brucella and Toxoplasma Gondii Infections and Plausible Risk Factors Among Abattoir Workers and Livestock Sellers in Uyo-Nigeria

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ABSTRACT

Toxoplasmosis and brucellosis are global public health zoonoses, but scarcely is dual infection reported in Nigeria. This study investigated the occurrence of dual brucella/Toxoplasma gondii infection and associated factors in abattoir workers and livestock sellers in Uyo, Nigeria. This was a descriptive cross-sectional study of abattoir workers and livestock sellers in Uyo. Sociodemographic information was collected using a pre-tested interviewer-administered questionnaire. Serological and molecular detection of Toxoplasma *gondii,* ***brucella species, and HIV were conducted using standard methods. Out of 339 participants, 14 (4.1%) and 189 (55.8%) were seropositive for anti-brucella IgG and anti-T. gondii IgG antibodies, respectively. Of these, PCR positives were 9 (64.3%) and 166 (87.8%), respectively. Brucella abortus and Brucella melitensis accounted for two-thirds (66.7%) and one-third (33.3%) of brucella infections as detected by PCR. Nine (5.4%) of the 166 participants with toxoplasmosis were HIV seropositive. Of the 175 participants with the zoonoses (9-brucella and 166-T. gondii), 6 (3.4%) had dual brucella/T. gondii infection, mostly among butchers/meat sellers, while one (16.7%) had ocular complications. Consumption of raw/unpasteurized raw milk and/or drinking of raw egg were significant risk factors associated with dual brucella/T. gondii infection (OR=6.4, 95% CI: 0.74,55.42). Higher frequency of infection was noted in participants with >5 years of work duration. Dual brucella/T. gondii infection among butchers/meat sellers is of serious public health concern. Prolonged occupational exposure and consumption of unpasteurized dairy products were the plausible risk factors. Awareness creation about these zoonoses among butchers, meat sellers, livestock traders and other at-risk populations is paramount.***

Keywords: Dual human brucellosis/toxoplasmosis, Livestock sellers, Abattoir workers.

INTRODUCTION

Brucellosis and toxoplasmosis are important global public health zoonoses and are among the neglected tropical zoonoses in Nigeria. While human toxoplasmosis is associated with mostly congenital malformations and ocular complications,[1] human brucellosis can affect any organ, from internal organ systems such as bones/joints, GIT, liver/biliary, respiratory, genitourinary, cardiovascular, neurological to external - ophthalmic and cutaneous systems.[2] The clinical features of these diseases in humans are non-specific, mimicking other febrile diseases such as malaria and typhoid and often result in missed-diagnoses of the zoonoses.3 Brucellosis and toxoplasmosis are associated with opportunistic infections in immunocompromised patients causing severe diseases, particularly in patients with full-blown AIDS and cancer.[4,5] Human brucellosis is caused by bacteria of the genus, *Brucella.* It is usually transmitted from infected animals to humans via the consumption of non-pasteurized dairy products, uncooked raw meat or by contact through skin, blood, conjunctiva, gastrointestinal or respiratory tracts.[6] Consumption of unpasteurized dairy products and occupational exposure by veterinarians,

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abattoir workers, and animal breeders are the most common risk factors for human brucellosis.[7] The epidemiology of human brucellosis and, to a lesser extent, the severity of the disease, is influenced by the type of organism and its source.[2] Hosts of brucellosis are usually cows, camels, sheep and goats. *B. abortus* species is normally associated with cattle, *B. melitensis* with sheep and goats, *B. suis* with swine. *B. abortus* and/or *B. melitensis* are predominant species associated with human brucellosis and are characterized by inflammation of the genitals and fetal membranes, abortions, sterility and lesions in the lymphatic system and joints.[8,9]

Human toxoplasmosis is caused by *Toxoplasma gondii*, an obligate, intracellular, parasitic protozoan. There are several risk factors associated with human toxoplasmosis, such as cat contact, consumption of raw or undercooked contaminated meat with tissue cysts, ingestion of *T. gondii* oocysts from water, soil, or cat litter, and occupational exposure.[10,11] The main routes of transmission are food and waterborne, though other routes of transmission such as accidental inoculation, blood transfusion, and organ transplant have been reported.[12] The asymptomatic nature of human toxoplasmosis, with its high morbidity and severe complication makes early laboratory detection and proper management of infected persons imperative.

There exist several reports on the epidemiology of single human brucellosis and toxoplasmosis among different populations and sub-populations in many countries of the world.[13-15] However, reports on dual *brucella*/*Toxoplasma gondii* infections among occupationally exposed populations, particularly abattoir workers and livestock sellers are scarce. Akwa Ibom State-Nigeria has over the years been grappling with the burden of double digits HIV seroprevalence. Advanced HIV infection can lead to immunocompromised conditions thereby predisposing susceptible occupationally exposed individuals like abattoir workers/livestock dealers, to severe consequences of single or dual brucella/ *Toxoplasma gondii* infection. This study aimed to investigate the occurrence of dual brucella/*T. gondii* infection and associated risk factors among butchers and livestock handlers in Uyo, Akwa Ibom State-Nigeria.

MATERIALS AND METHODS

This descriptive cross-sectional community-based study is a continuation of an earlier study on seroprevalence of anti-*Toxoplasma gondii* IgG antibody and risk factors among abattoir workers in Uyo, Southern Nigeria- published elsewhere.[16] The study participants comprised workers in three large abattoirs and livestock markets located in Uyo metropolis and environs. Uyo, Akwa Ibom State capital is located along Latitude 5° 01' 59" N and Longitude 7° 55' 35" E, and is one of the fastest growing cities in Nigeria; economically and population-wise. It is the commercial nerve centre of Akwa Ibom State and can be accessed by inhabitants of the neighbouring Cross River, Rivers and Abia States - for economic and social activities. Subsistence agriculture accounts for 55% of the total workforce of the inhabitants.

A total of 339 consented adult abattoir workers and livestock sellers (cattle, goat, sheep), of all sexes, ethnic groups and educational status, present at the abattoir/markets at the time of visit were included in the study. They were meat sellers (wholesale and retailers of raw meat), butchers (slaughtered and dressed the animals), and livestock farmers/traders (raised livestock for sale). Participants were recruited sequentially as they visited the abattoirs and livestock markets.

The sample size of 339 was calculated using STATCALC in Epi-info version 7.2.1.0 with published *T. gondii* and brucellosis seroprevalence of 26.7%[14] and 24.1%[17] among abattoir workers in Sokoto and Abuja, respectively.

Data collection

All participants who consented to the study were interviewed using a well-structured

interviewer-administered, pretested and validated questionnaire. Socio-demographic information and potential risk factors associated with toxoplasmosis and brucellosis such as consumption of dairy products, contact with live animals, undercooked meat consumption, workplace exposure, source of drinking water and hand washing habit, among others were documented.

Sample collection and processing

Seven millilitres (7ml) of venous blood were collected using sterile vacutainers, shared into plain and EDTA tubes, and transported in a cold box to Immunology Laboratory, University of Uyo Teaching Hospital within 5 hours. Blood sample in plain tubes was centrifuged at 3000 rpm for 10 minutes and serum was separated and stored at -20 ºC until required. Buffy coat from EDTA tubes was separated and stored at 2 – 8 ºC and later transported in ice packs to the Molecular Diagnostic Laboratory, Niger Delta University, Bayelsa State for Polymerase Chain Reaction assay.

Serological testing for anti-b*rucella* IgG and anti-*Toxoplasma gondii* IgG antibodies

Each sample was screened for anti-*Brucella* IgG antibodies using Rose Bengal Plate Test (RBPT) (Chronolab Systems, Barcelona, Spain) and Enzyme-Linked Immunosorbent Assay (ELISA) (Calbiotech Inc., California, USA). Detection of anti-*Toxoplasma gondii* IgG antibodies in serum were conducted using the Enzyme-Linked Immunosorbent Assay kit (Chemux BioScience, Inc, California, USA). Sample testing and interpretation of results were conducted according to the kit manufacturer's instructions.

Detection of Brucella species and *Toxoplasma gondii* by Polymerase chain reaction

i) **DNA extraction and quantification:** The procedure was carried out using the Quick-gDNA™ MiniPrep commercial kit (Zymo Research, California, USA) according to the manufacturer's manual. DNA quantification and purity testing were done using Nanodrop-1000-Spectrophotometer (SN 1844 ND-1000UV/VIS spectrophotometer, USA).

ii) ***Brucella species* DNA amplification**: The genes from three *Brucella* species were amplified using the following forward and reverse primers synthesized by Inqaba Biotechnological Ind. Ltd, Pretoria, South Africa:

- *Brucella abortus*, Forward sequence: 5' -TGCCGATCACTTAAGGGCCTTCAT-3', Reverse sequence: 5' -GACGAACGGAATTTTTCCAATCCC-3' 498 bp;
- *Brucella melitensis*, Forward sequence: 5' -TGCCGATCACTTAAGGGCCTTCAT-3', Reverse sequence: 5' -AAATCGCGTCCTTGCTGGTCTGA-3' 731bp; and
- *Brucella suis*, Forward sequence: 5'-GCGCGGTTTTCTGAAGGTTCAGG-3', Reverse sequence: 5' TGCCGATCACTTAAGGGCCTTCAT- 3' 345bp.

The PCR components had a final volume of 50 µl. The components were Taq Polymerase, dinucleotide triphosphate, magnesium chloride, buffer, primers, Template (extracted DNA) and nuclease-free water. The amplification was performed using a GenAmp

PCR system (Eppendorf, USA) as follows: initial denaturation (3 min at 94 °C) followed by 35 cycles each of denaturation (30 sec at 94 °C), annealing (30 sec at 53 °C, 55 °C and 56 °C for *B. abortus*, *B. melitensis* and *B. suis,* respectively and extension (40 sec at 72°C). A final extension cycle (72°C for 5min) was included. Appropriate positive and negative controls, and ladder markers (100 bp) were included in the assay. The PCR product was resolved on a 1.5% agarose gel containing 3% ethidium bromide (1 µg/mL) at 130V for 25 minutes and visualized on a UV

transilluminator. The presence of obvious bands of 498bp for *B. abortus*, 731 for *B. melitensis* and 345bp for *B. suis* were documented.

iii) *T. gondii* DNA amplification: The conventional PCR assay was performed on all *Toxoplasma gondii* seropositive samples to amplify a fragment of restriction endonuclease sequence. The repetitive 529 bp DNA fragment in *T. gondii* was targeted. This is a highly conserved sequence, specific and sensitive target for the diagnosis of toxoplasmosis.[18] The specific primer pair for amplification of the sequence of *T. gondii* DNA included forward primer sequence: 5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3' and reverse primer sequence: 5'-CGCTGCAGACACAGTGCATCTGGATT-3. The primers were synthesized by Inqaba Biotechnological Ind. Ltd, Pretoria, South Africa. The PCR components were Taq Polymerase, dinucleotide triphosphate, *Magnesium Chloride*, Buffer, primers, Template (extracted DNA) and nuclease-free water reaction. The PCR reaction was at a final volume of 25 µl. PCR conditions were set as follows: Initial denaturation: 95 °C for 3 min, 35 cycles of denaturation 95 °C for 30 s, annealing 55 °C for 30 s, extension 72 °C for 1 min and final extension 72 °C for 7 min. Amplified PCR product was separated on 1.5 % agarose gels, stained with 3 % ethidium bromide (1 µg/mL) and visualized under UV illumination. Positive and negative controls and ladder markers (100 bp) were included in the assay. The presence of obvious bands of 529 bp was considered a significant result for *T. gondii*

HIV antibody testing

HIV testing was conducted using three rapid HIV Enzyme Immunosorbent Assay (EIA) kits – Determine HIV 1/2 (Abott, Japan), Unigold (Trinity Biotech, Ireland) and Stat-Pak HIV 1/2 (Chembio, USA) according to the national serial algorithm. Determine was used for first-line testing followed by Unigold. Statpak was used as tiebreaker. Test procedures and interpretation of results were conducted according to the kit manufacturer's manual.

Statistical analysis

Data were analysed using the STATA statistical software version 12 (special edition) (Stata Corp, Texas, USA). Chi-Square was used to test for association between categorical variables. Significance was set at P value $\leq$ 0.05.

Ethical consideration

Ethical clearance was obtained from the Health Research Ethics Committee of the Akwa Ibom State Ministry of Health. Permission was obtained from the leaders of the respective livestock sellers and abattoir workers' Associations. Written informed consent was obtained from every participant before their data were collected and ethical considerations were duly observed.

RESULTS

Out of the 339 participants, 14 (4.1%) and 189 (55.8%) were seropositive for anti-brucella IgG and anti-*T. gondii* IgG antibodies, respectively. Of the 14 seropositive for anti-brucella IgG, 9 (64.3%) tested PCR positive while 166 (87.8%) of the 189 seropositive for anti-*T. gondii* IgG antibodies were confirmed by PCR. Out of a total of 175 participants that were either brucella or *T. gondii* positive by PCR, 6 (3.4%) had dual brucella/*T. gondii* infection and butchers/meat sellers were the majority (5/175, 2.9%) (Table 1). Among those that tested PCR positive for *T. gondii* infection, 9/166 (5.4%) were HIV seropositive. None of the individuals with either brucella infection or dual brucella/*T. gondii* infection tested HIV seropositive (Table 2). There was no statistically significant difference between ELISA and PCR results for *Toxoplasma gondii* positivity (P>0.05).

Of the 9 *Brucella* species characterized by PCR, *B. abortus* accounted for 6 (66.7%) while *B. melitensis* were 3 (33.3%) (Table 3). Participants with dual *B. abortus/T. gondii* and

B. melitensis/T. gondii infections were 3 (50%) each. None of the participants was infected with *Brucella suis* species. Clinically, 1 (16.7%) of the participants with dual *B. abortus/T. gondii* infection had ocular complications (Table 3).

Table 1: Distribution of participants' occupation according to their serological and molecular test results

Occupation	**Anti-IgG Brucella antibody positive (%)**	**Brucella positive by PCR (%)**	**Anti-IgG T. gondii antibody positive (%)**	***T. gondii* positive by PCR (%)**	**Dual infection by PCR (%)**
Butcher/meat seller	11/174 (6.3)	8/11 (72.7)	112/174 (64.4)	105/112 (93.8)	5/113 (4.4)
Livestock seller	3/168 (1.8)	1/3 (33.3)	77/168 (46.7)	61/77 (79.2)	1/62 (1.6)
Total (%)	14/339 (4.1)	9/14 (64.3)	189/339 (55.8)	166/189 (87.8)	6/175 (3.4)

Table 2: HIV seropositivity among participants with single and dual brucella/*T. gondii* infections

Participants' Infection status	**No. ELISA Positive (%)**	**No. PCR positive (%)**	**No.(%) HIV infected**
Brucella only	14/339 (4.1)	9/14 (64.3)	0
T. gondii only	189/339 (55.8)	166/189 (87.8)	9/166 (5.4)
Dual infection	6/203 (3.0)	6/175 (3.4)	0

Dual brucella/toxoplasma infection mostly occurred among butchers/raw meat sellers, OR: 4.9 (95% CI 0.56 - 41.98), but with no significant risk (P>0.05). However, the risk of dual brucella/toxoplasma infection was significantly higher among those with the habit of raw/unpasteurized milk and/or raw egg consumption than those without (5, 3.3% vs 1,0.5%), respectively; OR: 6.4(0.74-55.42), P < 0.05. Five participants (2.7%) whose work duration was 5 years and above were more frequently reported with dual infections, compared to less than 5 years' work duration (1, 0.7%), but with no significant association with dual infection; OR: 0.2 (95% CI 0.02 – 2.06) (P>0.05) (Table 4).

Table 3: Distribution of *Brucella* species and *T. gondii* genomic makers according to participant's occupation and ocular lesion

Participant Identification No.	Occupation Type	DNA Genomic marker				Dual Infection by PCR	Ocular lesion
		B. abortus (band at 498bp)	B. melitensis (band at 731bp)	B. suis (band at 345bp)	T. gondii (band at 529bp)		
114	Butcher/meat seller	-	+	-	+	+	-
165	Livestock seller	+	-	-	+	+	+*
236	Butcher	-	+	-	+	+	-
238	Butcher	-	+	-	+	+	-
271	Butcher/meat seller	+	-	-	+	+	-
291	Butcher/meat seller	+	-	-	+	+	-
B11	Butcher/meat seller	+	-	-	-	-	-
B16	Butcher/meat seller	+	-	-	-	-	-
B54	Butcher/meat seller	+	-	-	-	-	-
Total		6	3	0	6	6	1*

***Ocular lesion present, 1 (16.7%)**

Table 4: Plausible risk factors among participants with dual brucella and *Toxoplasma gondii* infection

Characteristics	No. Participant	No .(%) Dual Infection	X^2; P value	Odds Ratio (95% CI)
Occupation				
Butchers/Meat sellers	174	5 (2.9)	2.5; 0.11	4.9 (0.56 – 42.0)
Livestock sellers	165	1 (0.6)		
Work duration				
< 5 years	153	1 (0.7)	2.0; 0.16	0.2 (0.02 – 2.06)
> 5years	186	5 (2.7)		
Drink raw unpasteurized milk/raw egg				
Yes	151	5 (3.3)	3.7; 0.05	6.4 (0.74 – 55.42)
No	188	1 (0.5)		

DISCUSSION

Toxoplasmosis and brucellosis are anthropozoonoses of public health significance, particularly among occupationally exposed persons. The diagnosis of these zoonotic diseases is challenging, especially in resource-poor settings. In this study, the seroprevalence of anti-brucella IgG and anti-*T. gondii* IgG antibodies among livestock handlers and abattoir workers were 4.1% and 55.8%, respectively, while 64.3% and 87.8% of the serologically positive samples were PCR positive. There was a relatively high concurrent sero-detection rate and PCR positivity for the two infections with no significant difference (p>0.05). This finding could be attributable to the constant exposure of these persons to these infections during their routine activities at the abattoir and farms. Consequent upon the continuous exposure, is a high IgG antibody yield as detected by serological method and correspondingly, confirmed by PCR. Albeit, these cases may not necessarily portray active infection but rather an indication of humoral immune response of host exposure to the pathogens.[19] This buttresses the point raised by Robert-Gangneux and Dardé,[20] and corroborated by Onosakponome *et al.,*[21] about the likelihood of using standard serological methods to adequately detect latent *T. gondii* infection in exposed persons, where advanced methods are unavailable. The use of standard serological methods for the detection of anti-brucella and anti-*Toxoplasma gondii* antibodies has remained the only available and cheap option in developing countries despite their low sensitivity, possibly due to low antibody production rates, especially in the early stages of these diseases, unlike molecular methods that detect organismic DNA.[22]

The seroprevalence of *T. gondii* infection among the study participants has earlier been documented.[16] For brucella infection, *B. abortus* was the predominant species detected in this study, while similar studies in Maiduguri, Northeast Nigeria[23] and Iran[24] reported *B. melitensis* as the major species. Human infection with *B. abortus* has been associated with less serious sequelae compared to *B. melitensis*[25] but in other studies, both species have been reported to cause life-threatening health complications.[26] In this study, only one-third of those who tested positive for brucellosis by PCR, had *B. melitensis* infection contrary to the report among livestock handlers in Maiduguri where infection by *B. melitensis* was the majority.

In sub-Saharan Africa, especially in areas with high endemicity of infectious diseases, there is a likelihood of having co-existing diseases which could pose diagnostic and management challenges.[27] In this study, the prevalence of dual brucella/*T. gondii* infection was 3.4%, and butchers/meat sellers were the majority. Abattoir workers including butchers, meat sellers, handlers of livestock, dressers of carcasses and disposers of condemned organs have been reported with acquiring zoonoses.[28,29] Documentary evidence of dual *T. gondii*/brucella infection among abattoir workers, livestock sellers, and other at-risk populations is scarce. A similar study in Trinidad did not report co-infection status but documented acute toxoplasmosis as most prevalent compared to *B. abortus* infection in the study population.[13] A recent report of dual brucella/*T. gondii* infection was recorded among pregnant women (0.65%) in Mogadishu, Somalia.[30] In the present study, 50% of participants with dual infection had either a combination of *B. abortus/T. gondii* or *B. melitensis/T. gondii*. The clinical implications of dual brucellosis/toxoplasmosis indicate double jeopardy posing serious public health concerns.

Another duality recorded in this study was 5.0% *T. gondii/*HIV coinfection among the participants. The implication of toxoplasmosis as an opportunistic infection in immunocompromised HIV-infected persons has long been documented.[4] Higher prevalence of 23.6% -30.0% have been reported among traders and artisans in Port Harcourt, South-south Nigeria.[21] Traders and artisans are

blanket occupational groups who may engage in several other activities that expose them to zoonotic infections unlike butchers and livestock sellers who are relatively more restrictive occupational groups.

Ophthalmologic probing of the participants in this study showed that 1 (16.7%) of them with dual brucella/*T. gondii* infection had unilateral ocular lesions on the right eye and in the posterior pole. Both zoonoses have been implicated in ocular complications, though ocular brucellosis is rare in endemic areas.[31,32.]

None of the participants with dual brucella/*T. gondii* or brucella infection had a concurrent HIV infection, except for those with *T. gondii* infection alone. In an earlier study, the authors of this article reported a significant association between HIV infection and ocular toxoplasmosis in the same population.[32] As documented by Welker *et al*.,[33] immunocompromised conditions like HIV/AIDS are likely to alter the clinical course of *T. gondii* infection, thereby posing the risk of re-activating latent toxoplasma infection, leading to ocular and neurological complications.[34,35] This finding is instructive because Akwa Ibom State is currently burdened with the highest HIV prevalence of 5.5% in Nigeria, far above the national median of 1.4%.[36]

Consumption of raw/unpasteurized milk and/or raw egg constituted the most significant plausible risk factor for dual brucella/*T. gondii* infection as revealed in this study. In earlier reported studies in southwestern Uganda,[37] southern Thailand,[38] and Iran,[24] ingestion of raw unpasteurized dairy foods was one of the commonest modes of transmission of human brucellosis. However, a systematic review and meta-analysis of *T. gondii* infection and food consumption conducted by Belluco *et al*.[39] reported consumption of raw eggs and unpasteurized milk to be non-significant risk factors. Hence, milk-borne toxoplasmosis may not be as common as brucellosis, but the importance of consuming pasteurized dairy products to prevent infection with these zoonoses cannot be overemphasized.

In this study, the participants who had been in the business for 5 years and above, were more frequently reported with dual brucella/*T. gondii* infections than those with less than a 5-year work duration. It had earlier been documented that brucella and *Toxoplasma gondii* can survive for long periods in the environment, meat tissue and dairy products.[40] Hence, prolonged occupational exposure to these pathogens, particularly to abattoir workers, constitutes a great risk not only to the workers but also to their family members.[28]

There were some challenges encountered during this study, such as difficulty in reaching the highly mobile livestock sellers who tested seropositive for the zoonoses, to collect follow-up blood samples for PCR testing. Majority of them were from the northern part of Nigeria doing business in the south where this study was conducted. Consequently, a few of them were lost to follow-up.

CONCLUSION

The occurrence of dual brucella/*T. gondii* infection among abattoir workers and livestock handlers in Uyo, particularly butchers/meat sellers, is of immense public health concern and thus requires public health interventions. Prolonged occupational exposure and consumption of unpasteurized dairy products were the plausible risk factors for zoonotic infections. To the best of our knowledge, this is the first documented evidence of dual brucella/*Toxoplasma gondii* zoonoses among animal handlers in South-south Nigeria and should instigate a larger population study to ascertain the extent of the problem in the general population considering the medical importance of the zoonoses involved. There is a need for intense awareness campaigns and prevention programmes for butchers, meat sellers, livestock traders and other at-risk

populations, to eliminate the hazard and complications of these zoonoses.

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Conflict of interest:

All the authors declare that there was no conflict of interest.

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