SURVEY OF BRUCELLOSIS AMONG PEOPLE AT RISK IN LAGOS, NIGERIA

by

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I declare that the above dissertation is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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DEDICATION

I dedicate this thesis to God almighty. He was there for me.

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ABSTRACT

Brucellosis is one of the neglected diseases in Nigeria. In Lagos, the commercial capital of Nigeria with about twenty one million people, a descriptive cross-sectional study was carried out in order to determine the sero-prevalence of brucellosis among people at risk in some selected abattoirs and secondary health care facilities (hospitals) in the state. Mixed sampling method was employed at the abattoir while convenient sampling method was used in sampling the respondents at the hospitals. Sera samples from three hundred and one (*n=301*) abattoir-based workers and traders; and one hundred and twenty one (*n=121)* hospital-based individuals which include people with febrile illnesses and blood donors were tested for brucellosis using Rose Bengal Plate test (RBPT), with indirect ELISA being used as a confirmatory test. Of the 301 abattoir-based workers and traders, 27 (8.97%) were sero-positive to the infection when Rose Bengal Plate test antigen was used. The twenty seven individuals consists of fifteen (15) butchers; four (4) veterinarians; two (2) meat transporters and bone/cow horn dealers each as well as one each of blood meal producer, abattoir engineer, water seller and meat supplier. When blood samples from the sero-positive individuals were subjected to ELISA, 3 (11.1%) were sero-positive to the brucellosis, while one is equivocal. These results confirm that agglutination observed on RBPT might be related to unknown cross-reactions and confirmation with a different test was necessary. None of the hospital-based respondents is sero-positive to the infection. The clinical signs significant for the infection in this study were fever, joint pain, lower backache, regular headache and miscarriage. Brucellosis awareness level among the respondents was very low. Data was analysed using (SPSS) version 20.0 at α0.05 significant level. The significant risk factors for human brucellosis according to this research are consumption of *fura*

(unpasteurized milk) and *wara* (fresh cheese). The study revealed that brucellosis is not only an occupational disease but can also affect people who trade or live in proximity with infected animals.

Key words: Brucellosis, RBPT, ELISA, *B. abortus*, *B. canis*, *B. melitensis*, *B. suis*, people at risk

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LIST OF ABBREVIATIONS AND SYMBOLS

BCV	*Brucella*-containing Vacuole
BPAT	Buffered Antigen Plate Agglutination Test
DNA	Deoxyribonucleic acid
CbetaG	Cyclic β-1, 2-glucans
c-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
ELISA	Enzyme-Linked Immunosorbent Assay
EEA	European Economic Area
EU	European Union
ER	Endoplasmic Reticulum
FAO	Food and Agriculture Organization
FAOSTAT	The Food and Agriculture Organization Corporate Statistical Database
ILRI	International Livestock Research Institute
LAMP	Loop-mediated Isothermal Amplification
LPS	Lipolysaccharides
MLVA	Multiple Locus VNTR Analysis
MRT	Milk Ring Test
NOAA	National Oceanic and Atmospheric Administration
NIMR	Nigerian Institute of Medical Research
OIE	Office International des Épizooties
O.D	Optical Density
PCR	Polymerase Chain Reaction
PCR-EIA	Polymerase Chain Reaction and Enzyme Immunoassay
PCR-RFLP	PCR-Restriction Fragment Length Polymorphism Assay
RBPT	Rose Bengal Plate Test
RBB-RPAT	Rose Bengal Rapid Plate Agglutination Test
SAT	Standard Agglutination Test
T4SS	Type 4 secreting system
TMP/SMZ	Trimethoprim/Sulfamethoxazole
VNTR	Variable-Number Tandem Repeat
WHO	World Health Organization
2-ME	2-Mercaptoethanol

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CHAPTER 1
GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

A Gram-negative bacteria belonging to the genus *Brucella* cause brucellosis. *Brucella* organisms are small (0.5 to 0.7 by 0.6 to 1.5 µm), non-encapsulated, flagellated, facultatively intracellular coccobacilli (Centre for Food Security & Public Health, 2009; Wang, 2016). The genus *Brucella* belongs to the family Brucellaceae within the order of Rhizobiales, in the class of Alphaproteobacteria and phylum of Proteobacteria (Liu, 2015). Other genera of Alphaproteobacteria affecting mammals are *Bartonella, Rickettsia* and *Ehrlichia* (Fitch, 2010). Phylogenetic studies showed that years back prior to domestication of livestock, *Brucella species* had a common ancestor *B. ovis* (Foster *et al.*, 2009; Rossetti *et al.*, 2017).

Brucella genus comprises several species: *Brucella abortus*, *Brucella melitensis, Brucella canis, Brucella suis, Brucella ovis, Brucella neotomae, Brucella ceti, Brucella microti and Brucella inopinata* (Jimenez de Bagues *et al*., 2014). The division of the genus into six classical species (*B. melitensis, B. abortus, B. suis, B. canis, B. ovis* and *B. neotomae*) is still widely used based on historical and clinical reasons (Osterman and Moriyon, 2006). Though *Brucella* species are genetically similar, they have different host preferences (Table 1)

1.2 HISTORY OF BRUCELLOSIS

Hippocrates (Nielsen & Yu, 2010) first described brucellosis in the Mediterranean in 450 BC. The presence of the disease was also confirmed by the microscopic observation of coccoid microorganisms similar to *Brucella* species in carbonized cheese (Capasso 2002; Nielsen & Yu, 2010). A study reported on lytic lesions of the lumbar vertebrae in "southern ape of Africa" (*Australopithecus africanus*) suggestive of brucellosis. The suspected source of infection could be the consumption of infected tissues from wild animals (D'Anastasio *et al.*, 2009; Rossetti *et al.*, 2017).

Human brucellosis is fatal in 1 to 2% of cases. Reports of 72 cases deaths were recorded in the British literature, although in only few of the cases pathological findings were described (Hunt and Bothwell, 1967). Marston suffered from a disease that he described as being different from typhoid fever (Vassalo, 1992). Brucellosis, called "Mediterranean fever" was a debilitating chronic sickness with rheumatism complication that affected many Royal Naval seaman and sailors aboard ships each year (Wyatt, 1999). In 1887, Australian-born British physician, Dr. David Bruce, diagnosed brucellosis, named Malta fever at the time, (Rossetti *et al.*, 2017). Ten years later, Benhard Bang, a Danish Veterinarian isolated *Brucella abortus* while Sir Themistocles Zammit identified unpasteurized milk as the major source of infection for the disease. Overtime, brucellosis has been referred by various names which included Bang fever, Undulating fever, Malta fever, Mediterranean fever, Rock fever, Gibraltar fever, Cyprus fever, Crimean fever, Milk sickness, Goat fever, Satan's fever, Febris melitensis, Febris undulans, Melitensis septicaemia, Jones disease, Brucelliasis, Scottish Delight, Chumble fever and Bruce's septicaemia.

In some parts of Africa, brucellosis is called "Ugonjwa ya maziwa" meaning the disease from milk in Kiswahili, one of the local African languages (Lifecore Ltd, 2014). Worldwide, the last decade has been faced with challenges that include human population growth and environmental changes that have resulted in increased number of people living in close contact with wild and domestic animals. Re-emergence of diseases has been observed and the status of a number of conditions has now become questionable. Metabolic and infectious diseases such as diabetes, HIV and tuberculosis are widely distributed. Any effective recommendation and intervention should be multidisciplinary for solutions to current situation.

1.3 DIAGNOSIS OF HUMAN BRUCELLOSIS

Clinical signs alone are not sufficient for brucellosis diagnosis. Hence, a sensitive, specific, rapid and inexpensive method is required. Early and appropriate diagnosis of this disease is effective in improving public health as well as disease control and

eradication (Molavi *et al.*, 2014). Diagnosis of human brucellosis can be by culture, serological test, biochemical and molecular identification.

1.3.1 Diagnosis of brucellosis by culture

The gold standard for the diagnosis of brucellosis is blood culture. Blood is the most frequently used materials in *Brucella* culture, though the pathogen can be isolated from cerebrospinal fluids, pus, wounds, bone marrow (which gives the highest isolation rate) among others. For the microbiological growth media, serum dextrose broth with corresponding solid phase is recommended. It must be noted however, that *Brucella* grows on most high quality peptone based media used for blood culture. Incubation should be conducted in air supplemented with 5% Carbon dioxide (Poester *et al.*, 2010; Baddour, 2012). The automated lysis centrifugation method is used for the isolation of the pathogens from clinical samples (Baddour, 2012) because of increased sensitivity and reduced time of culture (Durmaz *et al.*, 2003 and Baddour, 2012). This automated culture system replaced the traditional biphasic Ruiz-Castañeda system used for *Brucella spp* isolation from clinical samples (Ruiz-Castañeda, 1954; Ganado & Bannister, 1960; Yagupsky, 1999 and Baddour, 2012). The number of bacteria found in clinical samples may vary widely, as the isolation of *Brucella* is highly dependent on the infection stage (acute versus chronic), antibiotic pre-treatment, the existence of an appropriate clinical specimen and the culturing methods used (Al Dahouk, *et al.*, 2003).

BACTEC 9204 and BacT/Alert are the newer automated blood culture systems used in the isolation of *Brucella* organisms. These methods shorten the time taken for the detection of the pathogens considerably, with *Brucella* being detected by the third day of incubation. The preceding culture system, BACTEC NR 730 could not detect appreciable number of samples, which were positive by conventional blood culture systems. The conventional Castaneda blood culture hardly gives positive result by the fourth day of incubation. Majority of the positives are identified between seven to twenty one days of incubation; with about 2% being positive by the twenty seventh day. For this reason, incubation of blood culture should be carried out for about forty five days before it is discarded as being negative for *Brucella* (Baddour, 2012). It must be noted that

isolation rates are much higher during the initial two weeks of symptomatic disease and in blood cultures taken during the pyrexial phase (Al-Dahouk, 2013)

Colonial morphology, Stamps or modified Ziehl Neelsen staining, slide agglutination with anti-*brucella* serum (smooth or rough), urease, catalase and oxidase tests are the basis for a culture to be identified as belonging to the genus *Brucella.*

1.3.1.1 Identification and typing of *Brucella* organisms after Culture

At incubation temperature of 37°C for 48-72 hours, Poester *et al*. (2010) reported growth of *Brucella* colonies of convex and circular outline, 0.5 to 1.0 mm in diameter. The smooth *Brucella* strains are transparent, yellow, with a semblance of honey droplets, and shiny surface when observed in transmitted light. The rough colonies are opaque with a granular surface. Dissociation of *Brucella* can be detected by the emulsification of a colony in 0.1% w/v aqueous acriflavine. The smooth colonies produce a uniform yellow suspension while the rough strains are seen as granular agglutinates.

Once *Brucella* is identified after culturing, species and biovar classification is important. This should be done in reference or specialized laboratories. These tests are cumbersome and include carbon dioxide requirement, production of hydrogen sulphide (H_2S), dye sensitivity (thionin and basic fuchsin), phage lysis, agglutination with A, M or R specific antisera; and in some cases, it is necessary to use the oxidative metabolic method. For example, Corbel (1984) reported that the three principal *Brucella* species- *Brucella melitensis, Brucella abortus* and *Brucella suis* have been differentiated largely based on their requirement for supplementary carbon dioxide on primary isolation, the production of hydrogen sulphide, ability to grow in the presence of selected dyes, and their reaction in agglutination tests with absorbed antisera. This latter test is time consuming and hazardous to laboratory personnel.

1.3.1.2 Molecular diagnosis of human brucellosis

This method of diagnosis in humans involves the use of polymerase chain reaction (PCR) in *Brucella spp.* identification. Types of polymerase chain reaction include Standard PCR, Real-time PCR as well as Nested and Semi-nested PCR. For Standard PCR, according to Gupte and Kaur (2015), a PCR assay with one pair of primers was developed, which amplifies the target genomic sequence of *Brucella* species. Studies showed that standard PCR is a more sensitive technique than microbiological methods both in the diagnosis of the first episode of infection and early detection of relapses (Wang Y *et al.*, 2014).

For the quantification of nucleic acid in the blood samples of individuals, real-time PCR is used. This is sensitive, specific, rapid and highly reproducible (Wang Y *et al.*, 2014). The nested PCR means that two different pairs of PCR primers are used for a single locus (Gupte and Kaur, 2015). Semi-nested PCR has two different pairs of PCR primers, but the second pair of primers has one primer identical to the first pair (Seah *et al.*, 1995). Nested PCR and Semi-nested PCR assays are now developed for identifying *Brucella* in samples of human blood and then to explore their clinical practice for the diagnosis of human brucellosis (Gupta and Kaur, 2015). Lin *et al.* (2011) reported a nested PCR for the laboratory diagnosis of human brucellosis. Other PCR based assays include a combination of Polymerase Chain Reaction and Enzyme Immunoassay (PCR-EIA) used by Vrioni *et al*.(2004) for the rapid laboratory diagnosis of human brucellosis directly from peripheral blood; and PCR-Restriction Fragment Length Polymorphism Assay (PCR-RFLP) for molecular typing of *Brucella abortus* and *Brucella melitensis* as used by Mirnejad *et al*.(2013)

1.3.1.3 Loop-mediated Isothermal amplification (LAMP) for diagnosing human brucellosis

Loop-mediated isothermal amplification is a single tube technique for the amplification of DNA. The technique involves isothermal nucleic acid amplification technique in which amplification is carried out at a constant temperature; but a thermal cycler is not required (Notomi *et al.*, 2000). This assay is simple in operation, rapid, sensitive and

specific (Ohtsuki *et al.*, 2008). In addition, LAMP is not expensive as its cost per sample is far cheaper than PCR and real-time PCR. Heat block or water bath (which are inexpensive) that provides a constant temperature of 63^{o}C is sufficient for the assay, and the reactivity is directly observed with the naked eye eliminating the need for electrophoretic analysis, unlike PCR (Gupte and Kaur, 2015). Therefore, this assay method is of useful importance in epidemiological studies in countries with limited resources (Soleimani *et al.*, 2013). Loop-mediated isothermal amplification has been used in the brucellosis diagnosis by Ohtsuki *et al,* (2008) and Trangoni *et al.* (2015).

1.3.1.4 Multiple Locus VNTR Analysis (MLVA) for diagnosing human brucellosis

Multiple loci VNTR analysis (MLVA) is a method employed for the genetic analysis of particular microorganisms. It takes advantage of the polymorphism of tandemly repeated DNA sequences. A "VNTR" is a "variable-number tandem repeat". This method is well known in forensic science as it is the basis of DNA fingerprinting in humans (CDC, 2017). The strain and biovar typing of *Brucella* field samples isolated in outbreaks is useful for tracing back the source of infection and may be of help in differentiating between natural outbreak of brucellosis and bioterrorist attack in humans, as *Brucella* is a potential biological warfare agent.

1.3.1.5 Serological tests for diagnosing human brucellosis

Serological diagnosis of brucellosis was first carried out by Wright and Smith in 1897using a simple tube agglutination test (Nielsen and Yu, 2010). Serological tests for diagnosing brucellosis include the use of Rose Bengal Plate Test antigen (RBPT), Standard Agglutination Test (SAT), 2-Mercaptoethanol (2-ME), Enzyme-Linked Immunosorbent Assays (ELISA) for the detection IgG, IgA, and IgM; Buffered Antigen Plate Agglutination test (BPAT), Lateral Flow Assays, Fluorescence Polarization Assays, Immunocapture-agglutination Test (Brucellacapt), Chemiluminescence Assays, Coombs Test, and Complement Fixation Test.

The procedures of the various serological tests, according to Nielsen and Yu (2010), are divided into 2 categories: the conventional tests and primary binding assays. All

conventional tests rely on the antibody performing a secondary function, for instance fixation of complement while in primary binding assays the only function of the antibody is attachment to its antigen. ELISA is an example of primary binding assays for brucellosis with many authors reporting varying specificity and sensitivity for *Brucella* IgG ELISA and IgM ELISA. For example Mohraz *et al*. (2003) reported 100% and 93% for specificity and sensitivity respectively; Vakili *et al*. (2010) 70.6% and 93.7% for specificity and sensitivity; and Dashti *et al*. (2012) 85.38% and 84.09% for specificity and sensitivity. For IgM, Vakili *et al.* (2010) reported 100% and 12.5% for specificity and sensitivity respectively.

Serological tests for the diagnosis of human brucellosis are not standardized internationally, though they seem to be effective. Another demerit of the use of these tests is the detection of antibodies in the sera of *Brucella*-positive individuals after successful therapy. Cross-reaction may also occur (Al Dahouk *et al.*, 2013).

In conclusion, definitive diagnosis of brucellosis is difficult. No single test is perfect. Clinical history coupled with combination of two or more tests reduces diagnostic result errors (Gupte and Kaur, 2015). Most researchers on human brucellosis in Nigeria used only Rose Bengal Plate test for diagnosing brucellosis in their studies. Although the sensitivity of the antigen is high, the probability of false-negative result for this test is high in chronic conditions and complicated cases. The assay also gives false sero-positives because of the cross reaction between *Brucella* species with antibodies to other organisms such as *Yersinia enterocolitica* and *Afipia clevelandensis*. In the light of these, this study will involve the use of Rose Bengal Plate Test antigen and subsequent confirmatory test by the use of Enzyme-Linked Immunosorbent Assay (ELISA) in order to reduce false diagnostic results among the test samples (Poester *et al.*, 2010)

1.4 PREVENTION AND TREATMENT OF BRUCELLOSIS

1.4.1 Prevention in humans

Brucellosis prevention in humans is best achieved by the eradication of the infection in animals, which are the primary hosts (Doerr, 2017). Animals meant for public

consumption should only be slaughtered at designated slaughter slabs and abattoirs so that adequate inspection of meat can be carried out by the veterinarians. In addition, according to the World Health Organization, consumption of unpasteurized milk/milk products should be avoided. Also, avoidance of raw meat consumption, food and personal hygiene, regular hand washing, disinfection; and wearing of protective clothing/protective device for people working with animals or at *Brucella*-vaccine production center/laboratories are very important. Lastly, massive education/public awareness on the infection with community participation is also of utmost importance. Adequate knowledge of the infection by all (including the physicians/health workers) will help in curtailing the spread of the disease in humans (Swai *et al*., 2010)

1.4.2 Prevention in animals

In animals, prevention is the preferred approach. Brucellosis is a listed disease by OIE. Therefore, test and slaughter policy (with necessary compensation of the farmers) instead of treatment is the recommended approach for prevention of brucellosis (OIE, 2018). In addition, vaccination of herd also prevents infection in livestock. Examples of vaccines currently being used for the various animals are live *Brucella abortus* vaccine strain RB51 (which replaced *Brucella abortus* vaccine strain 19), *Brucella abortus* 45/20, live *Brucella melitensis* vaccine strain Rev-1 and live *Brucella suis* vaccine strain 2. Though these vaccines are effective, they have numerous drawbacks such as causing abortion in pregnant dams and pathogenicity in humans (Dorneles *et al.*, 2015). In addition, control of herd/animal movement, surveillance and farm sanitation is also helpful in the control of brucellosis in animals.

1.4.3 Treatment of Brucellosis in humans

The treatment of human brucellosis is dependent on the effective antibiotics administration over an adequate period of time (Corbel, 2006). In addition, therapy of brucellosis must be geared towards prompt control of symptoms as well as prevention of complications and relapses (Wafa, 2017).

Brucellosis is often difficult to treat because therapy takes many weeks; and often, more than one antibiotic is needed for treatment. Oral administrations of 100mg doxycycline twice daily for 6 weeks together with daily, parenteral administration of 1g streptomycin for 2-3 weeks are the World Health Organization recommended two-antibiotic treatment in adults for acute brucellosis. Alternatively, 100mg of doxycycline, given twice daily and 600–900 mg rifampicin can be combined for oral treatment for a least period of 6 weeks (Vassalos *et al.*, 2009). In uncomplicated human brucellosis in children of age eight and above as well as in adults, according to Corbel (2006), 500mg of tetracycline, given every six hours interval for a least period of six weeks is recommended. However, the tetracycline analogue-doxycycline is currently preferred over tetracycline because it causes fewer gastro-intestinal side effects when compared with tetracycline. Doxycycline is given alone in a dose of 100 mg every 12 hours orally and is administered for a period of six weeks. The relapse associated with single administration of tetracycline and doxycycline has led to most authorities recommending the combination of these drugs with an amino-glycoside such as streptomycin and gentamycin for the first two to three weeks of treatment. Secondary alternate therapies include Trimethoprim/sulfamethoxazole (TMP/SMZ, co-trimoxazole), and fluoroquinolones. However, there is high relapse when these drugs are used. Hence, they are combined with the main drugs recommended by the World Health Organization (Corbel, 2006).

In some cases such as spondylitis, cerebral or epidural abscess, endocarditis, splenic abscess and hepatic brucelloma, individualized treatments should be administered to the patients. For example, in spondylitis, quinolones which penetrate and achieve increased concentrations in bone and soft tissue; and which are supposed to shorten the length of treatment are administered. However, they are deemed inferior to other drugs (Skalsky *et al.*, 2008; Pappas *et al.*, 2004; Vassalos *et al.*, 2009). Surgery can also be carried out in brucellosis patients with endocarditis, and hepatic brucelloma. Consensus recommendation on therapy of neurobrucellosis is still being desired; and more information on effective endocarditis treatment is needed (Vassalos *et al.*, 2009).

1.4.4 Treatment of animal brucellosis

Treatment of infected or suspected cases of animal brucellosis using antibiotics is not common and should not be considered in the control of the disease. Although few studies have shown rapid decline in the incidence of the infection when herd or flock were treated (like in the case of animals of special breeding value), however, the procedure is restricted in practice (Corbel, 2006). For example, treatment of *Brucella canis* infection is very difficult and expensive (Illinois Husbandry and Animal Welfare Association, 2015). Hence, the consensus is that vaccination is the best method for brucellosis prevention and control.

Table 1: Host preferences of *Brucella*

Species	Host preference
Brucella abortus	Cattle
Brucella suis	Pig
Brucella melitensis	Sheep, goat
Brucella canis	Dog
Brucella ovis	Sheep
Brucella cetis	Cetaceans
Brucella neotomae	Desert wood rat
Brucella pinnipedialis	Seals
Brucella microti	Common Voles

With the exception of *Brucella microti, Brucella neotomae, Brucella ovis* and *Brucella suis* biovar 5, all other species of *Brucella* cause infection in humans (Lopes *et al.*, 2010). However, zoonotic potential varies among the species (Xavier *et al.*, 2010). This is shown in Table 2 below.

Table 2: Zoonotic potentials of *Brucella*

Species	Zoonotic potential
Brucella melitensis	High
Brucella abortus	Moderate
Brucella suis	Moderate
Brucella canis	Mild
Brucella ovis	Absent
Brucella neotomae	Absent
Brucella ceti	Mild
Brucella pinnipedialis	Mild
Brucella microti	Absent

1.5 LITERATURE REVIEW

1.5.1 Global Report on the most common Zoonotic *Brucella* species

1.5.1.1 *Brucella abortus*

Brucella abortus was discovered in 1897 by Bernhard Lauritz Frederik Bang, the bacterium was then referred to as Bang's bacillus. Later, the pathogen's name changed to *Brucella abortus*. In 1918, Alice Catherine Evans, an American microbiologist demonstrated the zoonotic capability of *Brucella abortus* and its close relationship with the bacteria isolated by Bruce. The bacterium causes abortion and infertility in adult cattle (Dorneles *et al.*, 2015). It also affects other domestic and wild animals namely buffaloes, deer, horses, dogs, camels, sheep, goats and man (Xavier *et al.*, 2010; Dorneles *et al.,* 2015). *Brucella abortus* is the most widespread cause of brucellosis. Infection in man is often sub-clinical and usually less severe than that caused by either *Brucella melitensis* or *Brucella suis* (Corbel, 2006). *Brucella abortus* is classified into seven biovars; namely biovars 1-6 and biovar 9. Within the last two decades, a number of developed countries have eradicated, or significantly reduced, the prevalence of *Brucella abortus* infections (FAO, 2010).

1.5.1.2 *Brucella melitensis*

Brucella melitensis (*B. melitensis*) was discovered and isolated from the spleen of a man that died of 'Malta Fever' in the year 1887 by David Bruce, the British army surgeon. The pathogen was initially named *Micrococcus melitensis* (Galińska & Zagórski, 2013). The mechanism of transmission was determined in 1905 by Themistocles Żammit who found that carrier goats could infect humans with *Micrococcus melitensis* via milk (Wyatt, 2005). It is the most virulent of all the *Brucella* species in humans. A few organisms are sufficient to cause debilitating chronic infection (Fugier *et al.*, 2007). This pathogen is the main cause of ovine and caprine brucellosis. However, susceptibility of sheep breeds varies greatly. Infections caused by *B. melitensis* have also been reported occasionally in cattle, camels and dogs; as well as in wild ruminants such as alpine ibex in Italy and chamois in the French Alps (Centre for Food Security & Public Health, 2009). However, the pathogen rarely infects horses and pigs (Centre for Food Security & Public Health, 2009). This species contains three

biovars- biovar 1, biovar 2 and biovar 3; and is very common in the Mediterranean region. *B. melitensis* biovar 3 was isolated from a Sable antelope in 2002 at Onderstepoort Veterinary Institute, Bacteriology section, South Africa by Prudence Kayoka-Kabongo (Personal communication, January 2018). *Brucella melitensis* infection also occurs in the Central Asia, Middle East, around the Persian Gulf, Africa, some countries of Central America and India. The infection seems not to be endemic in northern part of Europe, Southeast Asia, North America (with the exception of Mexico), Australia, or New Zealand (Centre for Food Security & Public Health, 2009).

1.5.1.3 *Brucella suis*

Brucella suis (*B. suis*) was included in the genus *Brucella* in 1929 (Huddleson, 1929; Cabi, 2017). It comprises five (5) biovars; with pigs being the most common host of biovars 1 and 3 which are distributed all over the world (Díaz, 2013; Cabi, 2017). Biovar 1, which is the most widespread, is found in Australia, many Pacific Islands, the United States and Southern China (Diaz, 2013; Pedersen *et al.*, 2014). Biovar 2 appears to be widely distributed from Scandinavia to the Balkans (Godfroid and Kasbohrer, 2002; Al Dahouk *et al.*, 2005; Cabi, 2017); and is rarely pathogenic for humans, whereas biovars 1 and 3 are highly pathogenic (in both pigs and humans), causing severe disease (OIE Terrestrial Manual, 2009). *Brucella suis* biovar 4 is enzootic in reindeer and caribou in Arctic regions of North America and Europe. This biovar is apparently not pathogenic in swines, but it causes brucellosis in man (Cabi, 2017). *Brucella suis* biovar 5 is associated with murine brucellosis but has been rarely reported since initial description in the former Soviet Union (Cabi, 2017).

Brucella suis continues to occur in domesticated herds in some countries of Central and South America; and Asia. Cases of *B. suis* infections are occasionally reported in some African nations, including Uganda and Cote d'Ivoire (Centre for Food Security & Public Health, Iowa State University, 2009; Bello *et al.*, 2012). There is no report of the pathogen in South Africa.

1.5.1.4 *Brucella canis*

Brucella canis is found primarily in dogs, worldwide. It has been reported in United States (particularly the southern states), Canada, Central and South America (including Mexico), some European countries, China, Tunisia, South Africa, Taiwan, Nigeria, Madagascar, Malaysia, Philippines, Malaysia, India, Korea and Japan (Centre for Food Security & Public Health, 2012). However, the Oceanian countries appear to be free of the pathogen.

Brucella canis was firstly described in United States in 1966 following mass abortions of beagles (Morisset & Spink, 1969). It is a zoonotic pathogen, though symptomatic human infections are rare. However, human infections with *Brucella canis* may be underdiagnosed which could explain its low prevalence or being rarely diagnosed (Centre for Food Security & Public Health, 2012; Krueger *et al.*, 2014). Cases of human *B. canis* infection have been reported in Argentina (Lucero *et al.*, 2009), in Japan (Nomura *et al.*, 2010) and United States (Krueger *et al*., 2014; Dentinger *et al.*, 2015).

1.5.1.5 *Brucella ceti*

Miller *et al.* (1999) reported that *Brucella* species infections and related lesions have been found in marine mammals namely bottlenose dolphins (*Tursiops truncatus*); striped dolphins (*Stenella coeruleoalba*) (Gonzalez *et al.*, 2002), Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Foster *et al.*, 2002), common dolphins (*Delphinus delphi*), harbor porpoises (*Phocoena phocoena*) (Dagleish *et al.*, 2008), and a minke whale (*Balaenoptera acutorostrata*) (Foster *et al.*, 2002).

Serological surveys have shown that cetacean brucellosis may be found all over the world in the oceans. *Brucella ceti* isolates have been included within the same species but there are three different groups based on preferred host, bacteriological properties, and distinct genetic traits: *Brucella ceti* dolphin type, *Brucella ceti* porpoise type, and *Brucella ceti* human type. It seems that *Brucella ceti* porpoise type is more closely related to *Brucella ceti* human isolates and *Brucella pinnipedialis* group, while *Brucella ceti* dolphin type seems ancestral to them. The more likely mode of transmission of

Brucella ceti seems to be through mating, maternal feeding, aborted fetuses, placental tissues, vertical transmission from mother to the fetus or through fish or helminth reservoirs. The *Brucella ceti* dolphin and porpoise types seem to display variable virulence in land animal models and low infectivity for humans.

1.5.1.6 *Brucella pinnipedialis*

Brucella pinnipedialis is the aetiological agent of brucellosis in seals. Seals are pinnipeds. Pinnipeds, also known as fin-footed mammals, are a widely distributed and diverse group of fin-footed marine mammals, which are semi-aquatic comprising the families Odobenidae. Distribution of the diseases in seal is worldwide. The disease can also be transmitted to humans from seals.

1.5.2 ANIMAL BRUCELLOSIS IN AFRICA

Brucellosis is widespread in Africa (Ducrotoy *et al*., 2014; Ducrotoy *et al.*, 2017; Mangen *et al*., 2002; McDermott *et al*., 2013); and is endemic within most African countries (Akakpo & Bornarel, 1987; McDermott and Arimi, 2002; Aworh *et al*., 2013). The condition is often under-reported due to limitations in diagnostic tools and facilities (Ducrotoy *et al.*, 2017).

In Egypt (North Africa), brucellosis is prevalent in many species of farm animals in all parts of the country (Wareth *et al.*, 2014). In a research work covering Kenya and Tanzania, East Africa by Chota *et al.* (2016), it was reported that brucellosis was endemic in both countries when different serological diagnostic techniques were used in ascertaining the prevalence of the infection among 2349 ruminants. Results showed a sero-prevalence of 11.4% in the Southern Highland Zone (*n=799*), 2.4% in the Eastern Zone (*n=169*) and 1% in the Northern Zone (*n= 408*) when Rose Bengal plate test was used together with competitive enzyme linked immune-sorbent assay (c-ELISA) as confirmatory test. Tasiame *et al.* (2016) got a sero-prevalence of 22.9% out of 315 cattle which were subjected to Rose Bengal Plate test in North Tongu district of Ghana in West Africa. Bovine brucellosis has also been reported by Nakoune *et al.* (2004) in Central African Republic (4.9%); Bayemi *et al.* (2009) in Cameroon (8.4%); and

Mekonnen *et al.* (2010) in Ethopia (4.9%). Outbreaks of animal brucellosis in South Africa have been reported between 1993 and 2014 by Mbizeni (2015). All these reports confirm the existence of brucellosis in Africa.

1.5.3 Human brucellosis in Africa

Human Brucellosis is considered endemic in Africa (Tuncel *et al.*, 2008). Pappas *et al.* (2006) reports on human brucellosis divided the continent into two: North Africa and Sub-Saharan Africa. The northern part of Africa has traditionally been considered endemic for brucellosis. However, the disease also exists in Sub-Saharan Africa. There are many reports of brucellosis in various parts of Africa. For example, Tasei *et al.* (1982) reported that the disease is spread throughout Mali. They reported a high prevalence rate of 24.4% in the Sahelian region of Gourma. This report was corroborated by another research by Dao *et al.* (2009) which revealed that of the one hundred and fifty (150) sera samples from individuals in Mopti city, 58% tested positive for *Brucella melitensis* while 49% tested positive for *Brucella abortus*. Besides this report, another serological survey conducted by Fayomi *et al.* (1987) in Republic of Bénin showed that of the 221 sera from exposed workers in slaughtering-houses and breeders tested with Rose Bengal test, Wright sero-agglutination test, indirect immunofluorescence test and counter-immuno electrophoresis , 17.7% were positive to the disease". In Ethiopia, Regassa *et al.* (2009) reported the occurrence of the disease in traditional pastoral communities in people with recurrent unresolved febrile illness. Kassahun *et al.* (2006) had earlier reported a brucellosis prevalence rate of 4.8% among three hundred and thirty six (336) individuals that worked at Addis Ababa abattoirs and different dairy farms in the same country. In Akwapim-south District of the Ghana, Kubuafor *et al.* (2000) reported no incidence of human brucellosis in their research work, although there was 6.6% prevalence of *Brucella abortus* in cattle. Mensah *et al.* (2011) also reported low incidence of human brucellosis among herdsmen and the members of their family in the coastal savanna of Ghana. However, McDermott and Arinmi (2002) reported that human brucellosis might probably be endemic in Ghana and Togo. In Egypt, Hassanain and Ahmed (2012) reported a sero-prevalence of 6.26% out of four hundred and seventy nine (479) animals-contact

persons tested for the infection. Brucellosis is considered a health hazard in Egypt, especially in the rural areas. In Kenya, although there is no adequate record of brucellosis, Muriuku *et al.* (1994) showed that brucellosis occurred throughout the years within a seven-year report of 1986-1992 in Narok District. Rujeni (2007) reported a sero-prevalence rate of 25% in pregnant women in Rwanda.

1.5.4 Animal Brucellosis in Nigeria

The first report of brucellosis in Nigeria was of ten cases of bovine brucellosis (Contagious abortion) in 1927. Reports dating back to 1960s revealed that brucellosis disease in animals and humans is endemic in Nigeria (Adams and McKay, 1966; Alausa and Awoseyi, 1976). Esuruoso, (1979) reported the disease in goats, sheep, cattle, pigs and dogs.

Slaughter surveillance and serological testing of local and exotic breeds of cattle across the nation, particularly in ranches, livestock centers and dairy farms showed prevalence rate ranging between 3.7 - 48.8%. A prevalence of 7.1 - 8.6% was also recorded in unvaccinated nomadic herds, and as high as 26% in cattle concentrated around watering points, particularly among extensive management systems (Rikin, 1988). Cadmus *et al.* (2006) reported serological prevalence rate of between 0.20% and 79.70% in the various parts of the country up to Year 2006. Ocholi *et al.* (2005) reported *Brucella abortus* infection in sheep (which is naturally acquired and sporadic) on a privately owned farm in Toro, Bauchi State, northeast Nigeria. The abortions occurred in a flock of twenty-eight Yankassa breed of sheep, involving five ewes at the third month of pregnancy. Serum and milk samples from the flock were examined for *Brucella* antibodies by the Rose Bengal plate test, serum agglutination test (SAT) and milk ring test (MRT). The SAT gave 14.3% prevalence rate. The five milk samples examined by Milk Ring Test were positive. Out of three of the milk samples and four vaginal swabs of ewes that had aborted, seven isolates of *Brucella abortus* biovar 1 were identified and biotyped. This biovar was also isolated from cattle reared with sheep on the same farm. This is a proof of cross infection of *Brucella* organisms among animals in Nigeria. In addition, Onoja *et al.* (2008) reported a prevalence rate ratio of 1 (0.8%) in ram to 12

(69.2%) in ewes (and overall prevalence rate of 76%) in a flock of seventeen sheep in Zaria, which is located in the north central part of the country.

A sero-epidemiological study of brucellosis in sheep and goat, carried out in Plateau state, in the same north-central geo-political zone of Nigeria, showed that out of a total of 1,347 serum samples (*n = 851* goats and *n= 496* sheep) collected from nine randomly selected Local Government Areas, the prevalence rate was 14.5% in sheep and 16.1% in goats (Bertu *et al.,* 2010). In cattle, Cadmus *et al.* (2009) reported a prevalence rate of 8.6 ± 1.78% out of a total of 479 cattle slaughtered at two major abattoirs in Lagos State, whereas Mohammed *et al.* (2011) reported prevalence rate of 4.04% (23 cattle) and 3.86% (22 cattle) out of a total 570 cattle when Rose Bengal Plate Test and c-ELISA were used respectively and simultaneously in Jigawa State. Jigawa is located in the North-eastern part of Nigeria. A similar study on the sero-prevalence of brucellosis in puerperal cows in Zaria Nigeria by Ate *et al.* (2007) revealed that about 67% of the herds studied were sero-positive for brucellosis, when Rose Bengal plate agglutination test was carried out. This disease prevalence was higher than in post-partum cows.

In birds, the incidences of brucellosis have also been reported in Nigeria. These include detection of *Brucella melitensis* in local chicken in Sokoto, North-western Nigeria by Junaidu *et al.* (2006). Out of one thousand local chicken birds that were subjected to the Rose Bengal Plate test, serum agglutination test (SAT) and competitive ELISA (Compelisa) using both *Brucella abortus* and *Brucella melitensis* antigens, 30 (3.0%), 28 (2.8%) and 26 (2.6%) were positive for *Brucella melitensis* respectively. A similar study by Alaga *et al.* (2012), aimed at detecting agglutinating antibodies to *Brucella* organisms in pigeons, local chicken and Muscovy ducks in Nasarawa State, North Central geo-political zone, revealed that of 1120 birds of both sexes (*n= 355* pigeon, *n=510* local chickens and *n=255* Muscovy ducks), the prevalence rate was 2.8%, 2.3% and 1.9% respectively, when the sera were tested using Rose Bengal Rapid Plate Agglutination Test (RBB-RPAT). Gugong *et al.* (2012) also carried out a study to assess the status of brucellosis in local chickens in four Local Government Areas of Kaduna state, Nigeria.

A total of 150 sera sample were tested, with one (0.67%) being positive for *Brucella* antibodies on the Rose Bengal Plate Test (RBPT). In the south-eastern part of the nation, Onunkwo *et al*. (2011) reported 0.6 % brucellosis prevalence in pigs.

Livestock brucellosis is endemic in Nigeria. Unfortunately, there is no national control programme for the disease in both animals and humans. Nigeria is one of the countries with highest zoonosis burden and widespread mortality/morbidity in the world (ILRI, 2012), with brucellosis being one of the zoonotic diseases.

1.5.5 Human Brucellosis in Nigeria

Nigeria, together with Ethiopia and India, have the highest zoonotic burden in the world, with brucellosis being one of the most important zoonosis in terms of human and livestock impact. Brucellosis is one of the zoonotic diseases often neglected by primary healthcare managers and policy makers in the country (Ehizibolo, 2011). Furthermore, there are few publications on the prevalence of human brucellosis in Nigeria. In addition, major gaps exist in epidemiological data, diagnostics, control, and misconceptions surrounding brucellosis in the country.

Almost 100 years after the first brucellosis control programme was established in Nigeria, there is absence of good-quality information, which is essential for evaluation of zoonotic potential and for establishment of control measures (Ducrotoy *et al.*, 2014). The government appears not to perceive the health risks and implications of brucellosis on livestock/animal productivity. This is alarming, in the light of reported prevalence range of 6-28% among hospital patients; and 28-57% among abattoir workers in the country (Collard 1962; Falade 1974; Falade 1978; Ocholi 1993; Asanda and Agbede, 2001)

The first cases of human brucellosis confirmed by laboratory tests were reported in 1941 and 1962 respectively (Elmes, 1941; Collard, 1962). During this period, under detection was suspected (Collard, 1962). Years later, few laboratories were established to carry out tests on brucellosis but the low specificity of the diagnostic tests available

then could have missed positive cases (Alausa and Osoba, 1975). Alausa (1977) stated that 60% of the human population in Nigeria show serological evidence of *Brucella abortus* infection. Higher antibody titre values were found among occupationally exposed people, than the general population of blood donors.

In addition, the incidence and level of human infection was significantly higher in people living in the northern parts of the country than those living in the western area of the country. This may be due to the frequent physical contact by northern Nigerians with livestock because of the high animal population in the region.

Other historical evidence of human brucellosis in the country as reported by Ducrotoy *et al.* (2014) was in the 1970s when there was widespread epidemic of bovine brucellosis. The Fulani herdsmen, who reared the animals, complained of being unwell and were unable to look after their cattle. Falade (1974) also reported occurrence of *Brucella* agglutination antibodies in the sera of some people from Ibadan and its surrounding districts, located in the south-west region of the country. Other human brucellosis research work in Nigeria include the study of Baba *et al.* (2001) in which patients with pyrexia of unknown origin were tested for the presence of *Brucella abortus* antibodies using RBPT and SAT tests at Maiduguri, Northeast Nigeria. Sero-positive prevalence rate of 5.2% (26) were reported among 500 patients. Cadmus *et al.* (2006) in Ibadan, Southwest Nigeria, got 31.82% (7) sero-positive prevalence rate (using Rose Bengal Test) among butchers; while Ofukwu *et al.* (2007) research work on patients with febrile illnesses at Federal Medical Centre, Makurdi, North Central part of the country over a 12 months period revealed overall prevalence of 7.6% out of sera samples of 1040 individuals screened using Rose Bengal Plate test and SAT test simultaneously. The infection in the sero-positive individuals were caused by *Brucella abortus* (77.2%) and *Brucella melitensis.* (22.8%). In the same geo-political zone of the country, Gusi *et al.* (2010) got a prevalence rate of 5% (9 butchers) and 3.9% (4) when Rose Bengal Plate test and SAT test were used respectively for the sero-diagnosis of brucellosis among 101 butchers at Jos abattoir, Plateau State. However, Olabode *et al.* (2012) study efforts to determine infection with brucellosis among women that process meat to form

'siki', a local delicacy at Ilorin; Kwara State was unsuccessful as the women did not subject themselves to sera samples collection. Relatively, recent research work by Aworh *et al.* (2013) at two abattoirs located in the Federal Capital Territory, Abuja (which is the national capital of the nation) involving 224 abattoir workers led to the observation of a prevalence rate of 24.1% when the workers were screened for *Brucella abortus* and *Brucella melitensis* antibodies. Meanwhile Kudi *et al.* (2015) got a prevalence rate of 32.5% out of 246 sera samples obtained from patients with febrile illness symptoms such as malaria and typhoid between 2012 and 2014 at General Out-Patient unit, Federal Teaching Hospital, Gombe, Northeast Nigeria. Aworh *et al.* (2013) used ELISA and Rose Bengal plate antigen for their research work while Kudi *et al.,* (2015) used Rose Bengal plate antigen alone. In summary, human brucellosis is suggestively endemic in Nigeria.

1.6 BRUCELLOSIS OUTSIDE THE AFRICAN/OTHER CONTINENT

1.6.1 Europe

Brucellosis infection in Europe is mainly in the Mediterranean countries. The Mediterranean European countries refer to Southern European countries such as Greece, Albania, Macedonia, Spain, Portugal, Andorra, Italy, Bulgaria, Serbia and Malta. However, the epidemiology of the disease has been changing over the past decade because of socio-economic, sanitary and political factors; as well as international travel (Tzaneva *et al.,* 2009; Pappas *et al.*, 2006). The disease is endemic in the region. According to Lytras *et al.* (2016), the country with the highest reported incidence of brucellosis in the European Union is Greece. He further stated that human brucellosis cases of 2159 were reported in Greece between November 2013 and December 2015. The mean incidence rate was 1.62 per 100 000 population per year, with *Brucella melitensis* being the most common pathogen in the country (Taleski *et al.*, 2002). It must be noted though, that there are reported cases of *Brucella abortus* infection in the ancient nation too (Giannakopoulos *et al.*, 2006; Stephen Berger, 2017). However, most Islands in the country enjoy a brucellosis eradication status as at June 2009 (Karagiannis, 2009).

In Italy, the incidences of human brucellosis have been on a decline as reported by De Massis *et al.* (2005) and Mancini *et al.* (2014). Brucellosis is still a common notifiable disease in the country, more widespread in southern regions of Italy, especially in Sicily, (including Apulia, Campania, Calabria), in human, sheep and goat populations (De Massis *et al.*, 2005). Rapisarda *et al.* (2005) reported five cases of *Brucella* infection due to occupational exposure in a slaughter-house in South-Eastern Sicily, Italy. The five individuals consisted of four slaughter-house operators and a veterinary surgeon.

Brucellosis is also endemic in Turkey (Yumuk and O'Callaghan, 2012), although the country lies in Eurasia region. In 2014, 354 confirmed cases of brucellosis were reported by 18 European Union and European Economic area (EU/EEA) countries, with an overall rate of 0.1 per 100 000 population. Eleven Member States reported zero cases, while Greece, Spain and Portugal reported the highest numbers of cases (135, 60 and 50, respectively), corresponding to 69.2% of all cases reported in EU and EEA (European Centre for Disease Prevention and Control, 2016).

Many EU and Western Europe countries have been granted brucellosis-free status. Countries with brucellosis-free status include Belgium, Netherland, Sweden, Denmark, UK (excluding Northern Ireland), Finland, Austria, Germany and Luxembourg. Norway and Switzerland are not part of the EU countries, but are also considered as brucellosis-free state (Pappas *et al.*, 2006).

It must be noted however that small number of brucellosis cases are still being reported annually in most of these countries, mainly in travellers to endemic countries or immigrants from endemic areas (Al Dahouk *et al.*, 2005; Pappas *et al.*, 2006; Norman *et al.*, 2016). Also, countries such as France and UK which were previously declared as brucellosis-free countries have experienced re-emergence of the disease (Mailles *et al.*, 2012) and UK (MRCVSonline, 2017).

In conclusion, according to European Centre for Disease Prevention and Control (2016), brucellosis remains a rare disease in the EU/EEA, although it is still found in some countries in the continent, with *Brucella melitensis* and *Brucella abortus* being the common cause of the infection.

1.6.2 Brucellosis in Asia

Brucellosis is endemic in the Middle East, Central and South East Asia. Rubach *et al.* (2013) reported that the Middle East and Central Asia have the highest reported incidence of human brucellosis all over the world. Incidence rates of more than 100 in 100,000 person-year was reported in Saudi Arabia, Jordan and Iraq. In Saudi Arabia, infection of humans by *Brucella melitensis* is common (80%–100%), while infection with *Brucella abortus* is less frequent (Al-Eissa, 1999; El-Koumi *et al.*, 2013). However, infection with other species has not been reported (Al-Eissa, 1999; El-Koumi *et al.*, 2013).

The people are commonly infected through direct contacts with infected animals, inhalation of infectious aerosols (for example workers in abattoirs and microbiological laboratories), consumption/ingestion of cheese, meat or raw milk; and products discharged by animals, which include conception discharge (among farmers, veterinarians and shepherd). (El-Koumi *et al.*, 2013; Young, 2004). For example, Al-Sekait (1993) reported a prevalence rate of 4% among 1200 abattoir workers in the same country. Also in Saudi Arabia, Khan *et al.* (2001) wrote about the incidence of spontaneous abortion and intrauterine death among a retrospective cohort of 92 pregnant women with acute brucellosis. *Brucella melitensis* was the primary cause of the infection, and the prevalence rate was 46%. It was observed that the rates of spontaneous abortion and intrauterine death was substantially higher in women that had acute brucellosis infection than the general population of pregnant women at their institution during corresponding pregnancy trimester. Majority of these women are older than 20 years of age, not primigravid and had not experienced spontaneous abortion prior to the time of the research study.

In Iraq Kurdistan, Jaff (2016) reported that the prevalence of human brucellosis in the three provinces was high. Prevalence rate of 6.36% was reported in Dohuk in 2011(Omar *et al*., 2011); 10.7% in Erbil city (Rasul & Mansoor, 2012). 976 cases were recorded in Sulaimani province in 2013. This record is the highest incidence of brucellosis infection in Kurdistan region and Iraq as a country (Mohammed, 2015; Jaff, 2016). Moreover, human and livestock infections exist in all governorates of the country (Karaawi, 2016); with *Brucella melitensis* and *Brucella abortus* being the common species of *Brucella* found in Iraq. Al-Tawfiq and Abukhamsin (2009), Abu Shaqra (2000) and Yacoub *et al.* (2006) also reported high incidence of human brucellosis in Central Asian countries such as Azerbaijan and Kyrgyzstan. As in the European Mediterranean countries, brucellosis exists in the corresponding Asian nations.

In Israel, most reported cases were due to *Brucella melitensis*, biovar 1 in southern Israel, and biovar 2 in northern Israel (Banai, 2010). Mongolia has one of the highest reported human incidence rates of brucellosis - greater than 60/100 000 people (Roth, 2010). Sero-prevalence rate of 40.8% (1080) out of 2648 individuals were reported in Xianjing China. These individuals were all infected by *Brucella melitensis* biotype 3 (Guoqiu *et al*., 2013).

Quazilbash and Bari (1997) also worked on sero-prevalence of human brucellosis among tuberculosis suspected patients in Islamabad, Pakistan. Of the tested samples, 44 (19.21%) were positive for brucellosis, using SAT. Majority of these people were from the rural areas. Brucellosis frequency among these tuberculosis suspected patients was found to be higher in women (73.91%) in comparison to the men (52.38%). Syria has one of the highest annual incidences of brucellosis worldwide, with alarming cases of 1603/million per year, according to the data from OIE (World Organization for Animal Health, 2004).

Although brucellosis is a common human disease in many parts of Asia, investigation of cases is not commonly practiced in the Asian-pacific countries, despite the seriousness of the reports (Garin-Bastuji *et al.,* 2014).

1.6.3 Brucellosis in South and Central America

Human brucellosis is a disease that is also considered to be endemic in South America. *Brucella melitensis* is prevalent in Peru and West Argentina; and *Brucella abortus* in East Argentina (Trujillo *et al.*, 1994; Samartino, 2002; Nöckler *et al.*, 2009) and other South American countries (Pappas *et al.*, 2006). Mexico is also one of the most important human brucellosis reservoirs (Pappas *et al*., 2006). In Central America, there is existence of animal brucellosis throughout the area (Moreno, 2002), but the human disease is not endemic.

The global map of brucellosis as presented by Pappas *et al.*, (2006) is shown below.

Table 3: Annual cases of brucellosis by countries (Pappas *et al*., 2006)

Country and Reference	Incidence per million of population
Europe	
Albania	63.6
Bosnia and Herzegovina	20.8
Denmark	0.7
France	0.5
Former Yugoslav Republic of Macedonia	148
Georgia	27.6
Germany	0.3
Greece	20.9
Ireland	1.3
Italy	9
Netherlands	0.5
Norway	0.7
Portugal	13.9
Russia	4.1
Serbia and Montenegro	8.4
Spain	15.1
Sweden	0.3

Switzerland	1.5
UK	0.3
Africa	
Algeria	84.3
Cameroon	Endemic, no specific data available
Egypt	2.95
Eritrea	5.48
Ethiopia	Endemic, no specific data available
Mali	2
Namibia	4.9
Tunisia	35.4
Uganda	0.9
North America	
Canada	0.09
USA	0.4
Mexico	28.7
Central and South America	
Argentina	8.4
Chile	0.6
Colombia	1.85
Guatemala	15.7
Panama	10.1
Peru	34.9
Asia	
Afghanistan	3.8
Armenia	31.3
Azerbaijan	52.6
China	8
India	No data available, possibly endemic
Iraq	278.4
Iran	238.6
Israel	9.2
Jordan	23.4

Kazakhstan	115.8
Korea, South	71
Kuwait	33.9
Kyrgyzstan	362.2
Lebanon	49.5
Mongolia	605.9
Oman	35.6
Pakistan	No data available, possibly endemic
Saudi Arabia	214.4
Syria	1603.4
Tajikistan	211.9
Turkey	262.2
Turkmenistan	51.5
United Arab Emirates	41
Uzbekistan	18
Oceania	
Australia	26

1.7 PATHOGENESIS OF BRUCELLOSIS

The phagocytic macrophages, the antigen presenters-dendritic cells (DCs) and trophoblasts (cells that make up the outer layer of a blastocyst, responsible for embryo feeding and which develop into the large part of a placenta) are the major target cells for *Brucella* micro-organisms. *Brucella* micro-organisms are capable of invading epithelial cells of the host; usually through the alimentary canal (Invasion through the digestive tract is associated with epithelial transmigration of bacteria preferentially through M cells). Intra-epithelial phagocytes may also transport *Brucella* from the intestinal lumen to the lamina propria (Figure 1) (Ackermann *et al.*, 1988; Salcedo *et al.*, 2008 and Xavier *et al.*, 2010). *Brucella* organisms can also gain entry through the respiratory tracts and can survive in both phagocytic (Celli, 2006; Xavier *et al.*, 2010) and non-phagocytic host cells by finding ways to avoid the immune system (Xavier *et al.*, 2010;

Wafa, 2017). Therefore, these bacteria can affect almost all organ system of the body. Hence, it is a systemic disease.

Once *Brucella* gains entry into the bloodstream, the organisms become intracellular pathogens, found in circulating granulocytes or polymorphonuclear, leucocytes and macrophages. Many mechanisms are used to suppress or avoid bactericidal responses. The lipopolysaccharides (LPS) coat (smooth in *Brucella melitensis*, *Brucella abortus*, and *Brucella suis*; and rough in *Brucella canis*) is likely to play a major role in the intracellular survival of the micro-organism. This could be due to the inhibition of phagosomal fusion and the oxidative burst process as a result of adenine and guanine monophosphate production.

Inside the agranular (mononuclear phagocytic) cells, *Brucella* micro-organisms are found in *Brucella*-containing vacuole (BCV), which is a special vacuole where it modifies intracellular trafficking and changes the vacuole into a brucellosome, a replicating compartment (Lamontagne *et al.*, 2009; de Figueiredo *et al.*, 2015).

The micro-environment in the BCV has limited nutrients availability as has been shown experimentally (Lamontagne *et al.*, 2009). The organism adapts to this environment soon after invasion (de Figueiredo *et al.*, 2015). The adaptation of the organism include the switching to alternate sources of energy and the altering of the respiration process as a result of low oxygen tension (this is made possible as a result of quantitative reduction of gene expression and protein synthesis involved in anabolic mechanism while amino acid catabolism is being increased). Over time, the brucellae organisms recover gradually the expression of key metabolic process-encoded genes (de Figueiredo *et al.*, 2015). The BCV invaginates with endoplasmic reticulum in epithelial cell lines, macrophages and placental trophoblasts (Anderson and Cheville, 1986; Pizarro-Cerdá *et al.*, 1998; Celli *et al.*, 2003 and Xavier *et al.*, 2010), with chronic infection being established once they are in the compartment.

In conclusion, *Brucella* lacked classical virulence factors in bacteria such as cytolysins, exotoxins, plasmids, fimbriae, capsule, lysogenic phases as well as inducers of apoptosis in host cells among others (Moreno and Moriyón, 2006; Poester *et al.*, 2013); but it is still able to replicate intracellularly. Its virulence are functions of two-component system BvrR/BvrS (TCS BvrRS; necessary for the modulation of the host cell cytoskeleton and regulation of the expression of outer membrane protein); the type IV secretion machinery VirB (T4SS VirB) (O'Callaghan *et al.*, 1999; Hong *et al.*, 2000 and Poester *et al.*, 2013) and Cyclic β-1, 2-glucans (CbetaG) which is produced by the organism for the targeting of its replication niche- endoplasmic reticulum of the host species (Poester *et al.*, 2013).

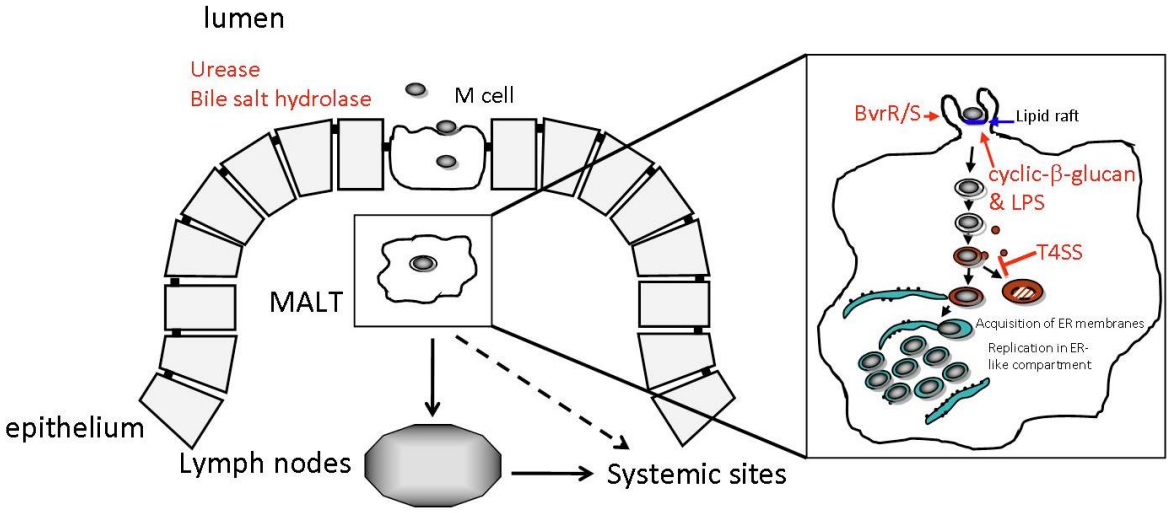

Figure 1: Schematic representation of *Brucella* invasion through the digestive tract. Entry is through M cells and subsequently the bacteria are taken up by macrophages of the mucosa associated lymphoid tissue (MALT). This macrophage transports the bacteria to the lymph nodes and on to systemic sites. Blown up macrophage shows trafficking within the macrophage from entry via lipid rafts, through the endosomal pathway to the ER-like compartment in which *Brucella* replicates (Starr *et al.*, 2008). In red letters are *Brucella* virulence factors that are involved in establishing (Xavier *et al.*, 2010)

1.8 PATHOGENESIS AND CLINICAL SIGNS OF BRUCELLOSIS

1.8.1 Pathogenesis and clinical signs of bovine brucellosis

Brucella abortus is the main cause of brucellosis in cattle. It also affects deer, camels, sheep, goat, horses, dogs, buffaloes and man (Xavier *et al.*, 2010). The presence of the pathogen in affected host is noticed at the time of calving or in aborted cows. Clinical

signs of the infection in cattle include retained placenta after birth, post-partum metritis, mastitis, orchitis in the bull, arthritis, weak calves, inflammation of the epididymis, still birth; and male infertility.

Spontaneous abortion in cows and other animals due to *Brucella* infections is because of lack of anti-brucella activities in the amniotic fluid. In addition, the presence of erythritol (a four carbon sugar that is preferentially utilized by the pathogen; which promotes placental and foetal infections) in the placentas of cows (cattle), dams (goats) and sow (pigs) cause the accumulation of *Brucella* organisms in the organ, thereby leading to the foetal abortion. This was confirmed by prior research by Xavier *et al.* (2010), in which it was reported that *Brucella abortus* induced abortion is associated with necro-hemorrhagic placentitis and fetal lesions, particularly fibrinous pleuritis, pericarditis, and interstitial pneumonia.

Transmission of bovine brucellosis to healthy flock mainly occurs when there is exposure to aborted foetus, contaminated foetal membranes or uterine secretions from infected cows. Infected bulls do not usually play major role in the spread the disease.

1.8.2 Pathogenesis and clinical signs of porcine brucellosis

Brucellosis affects both domestic and wild pigs. The infection is caused by *Brucella suis*. The clinical signs of the disease in sows are abortion in various stages of gestation, irregular oestrus, infertility, metritis, and birth of weak piglets with a high neonatal mortality rate. In boars, orchitis, epididymitis, paralysis, abscesses in various organs, arthritis, osteomyelitis, spondilytis and sterility are the symptoms of the disease (Cabi, 2017)

It must be noted that swollen joints and tendon sheaths, which is followed by lameness and incoordination, can occur in both boars and sows (Lopes *et al.*, 2010). The infection occasionally may be restricted to sexual glands and may not result in impaired fertility (Vandeplassche *et al.*, 1967); while in some cases, fertility of the pigs is permanently

impaired, particularly in boars (Cabi, 2017). However, because porcine brucellosis does not often manifest clinical signs, diagnosis of the infection in pigs is usually difficult.

1.8.3 Pathogenesis and clinical signs of caprine brucellosis

The infection is primarily caused by *Brucella melitensis*. However, goats, which are the main hosts, are also susceptible to *Brucella abortus* infection (Wareth *et al.*, 2015 and Rossetti *et al.*, 2017) and *Brucella suis* (Alton, 1990). The main signs of brucellosis in goats are similar to that of other livestock. These include orchitis, epididymitis, sporadic fever, nervousness, mastitis, stiff/swollen joints and abortion at around the fourth month of pregnancy in the herd. Reduced milk yield, retained placenta, production of weak kids as well as still births are also symptoms of the disease in the animals. Mode of transmission of *Brucella* infection in goat is through vaginal discharges from infected animals, aborted foetus, amniotic fluids and placenta. Most animals become infected by ingestion or through the mucous membranes of the oropharynx, upper respiratory tract and conjunctiva. *Brucella* can also be transmitted through broken skin (Centre for Food Security & Public Health, 2009). The mucous membranes of the male or female genital tract are also other potential routes of infection. In addition to other route of infection, *Brucella melitensis* can also be transmitted by stable fly. To establish infection in the host, genes that encode urease are necessary (these genes are also important for the establishment of infection caused by *Brucella suis* and *Brucella abortus*).

1.8.4 Pathogenesis and clinical signs of canine Brucellosis

Brucella canis bacterium primarily affects the reproductive organs of male and female dogs. Brucellosis can infect dogs of any breed and any age; though it is most common in mature dogs. The infection causes infertility, late abortions (49th-59th day of gestation) and vaginal discharges in female dogs. In male dogs, it can cause inflammation of the testes or scrotum; as well as infertility. This disease can also lead to still birth or weak puppies at birth. Other symptoms of the infection in dogs include swollen lymph nodes, difficulty in walking, back pain, vaginal discharge and lethargy.

The Centre for Food Security & Public Health (2012) reported the symptoms of the disease in dogs to include abortions, stillbirths, and epididymitis, orchitis and sperm abnormalities in dogs. Castrated dogs do not show reproductive signs, but they occasionally develop conditions such as discospondylitis and ocular infection. There is also the occurrence of premature aging in infected dogs.

Although *Brucella canis* infection is also found in other canids such as foxes, wolves, coyotes, dingoes and jackals, it is rarely reported. In addition, infection with other *Brucella* species such as *Brucella suis* and *Brucella abortus* have been reported in wolves, foxes and coyotes (Davis *et al.*, 1979; Urbigkit, 2010; Azevedo *et al.*, 2009).

1.8.5 Clinical Signs of Human Brucellosis

Because of the wide spectrum of the clinical signs of human brucellosis, it has been grouped alongside tuberculosis and syphilis (Andriopoulos *et al.*, 2007; Mantur *et al.*, 2006; Giannakopoulos *et al.*, 2006). However, according to Celebi *et al.* (2007), brucellosis may be asymptomatic. Symptoms of brucellosis in humans are generally non-specific, as most of the signs are obvious in 50% or slightly lower percentage of patients (Pappas, 2008). The onset of brucellosis may be either acute or insidious. The latter mode of presentation causes more difficulties in diagnosis.

The diverse and sometimes deceptive way the disease manifests (i.e. can be localized, acute, sub-acute or chronic infection) may lead to missing or delaying the diagnosis if the attending clinician has a low index of suspicion. Symptoms may appear suddenly over 1-2 days or gradually over 7 days or more. Fever (which is classical undulant; with other patterns being manifested at other times) is commonly observed in 90-95% of the patients, and the infection is a differential in pyrexia of unknown origin.

Other symptoms and the rate of occurrences in patients as reported by Willacy (2010) are malaise (80-95% of patients), tiredness and arthralgias (in 20-40%), chills, sweating (in 40-90%) and myalgias (in 40-70%). Athuri *et al.* (2011) observed the following symptoms: acute undulating fever (>90% of all cases), headache, arthralgia (>50%),

night sweats, fatigue and anorexia. Complications, which may arise thereafter, are epididymoorchitis, spondylitis, abscess formation in the liver, endocarditis and neurobrucellosis. Kyebambe *et al.* (2012) report cervical spondylitic myeloradiculopathy as a symptom in a Ugandan teenager.

The World Health Organization describes the infection as one with acute or insidious onset; continued intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Formation of abscess is a rare complication. Most deaths due to brucellosis are caused by neurobrucellosis and endocarditis (Al-Dahouk and Nockler, 2014)

In summary, many authors (Bashir *et al.*, 1985; Al-Orainey *et al.*, 1987; Rajapakse *et al.*, 1987; Al-Kasab *et al.*, 1988; Al-Aska 1989; Akhtar and Ali, 1989; Halim *et al.*, 1993; and Al-Eissa 1999) reported fever with drenching sweats, loss of weight, nausea, vomiting, anorexia, lethargy, colitis, hepatosplenomegaly, spontaneous peritonitis, lymphadenopathy, sacroiliitis, ileitis, spondylitis, bursitis, osteomyelitis, mild anaemia, leukopenia, pancytopenia, thrombocytopenia and gastro-intestinal complications are clinical symptoms of human brucellosis

1.9 PROBLEM STATEMENT

The population of livestock consumed in Lagos Nigeria is very high, which is more than 40,000 ruminant animals per month excluding pigs and poultry. Nigeria has a large livestock population of about 104 million (FAOSTAT, 2010) which are mainly in the hand of nomadic farmers. These animals harbour *Brucella species*, and yet, investigation of brucellosis is not carried out routinely on these animals. As a result, this place the population of Lagos at risk of *Brucella* infection from livestock. Febrile illness and generalized aches and pains are major symptoms of human brucellosis. These similar signs are frequently observed in other diseases. Unfortunately, due to non-prioritization of brucellosis as a high public health issue by primary health care givers, brucellosis is hardly diagnosed in the health facilities despite the high-risk of exposure in the country. In addition, the nomadic farmers and the butchers who are involved in the handling of

potentially infected animals and their products on a daily basis have not been studied extensively to determine the extent of the problem. Therefore, there is paucity of data on the magnitude of the disease in terms of prevalence and incidence, which justifies the current study.

1.9.1 Hypotheses

(i) The prevalence of brucellosis is low among people at risk.

(ii) The high febrile illness observed among people that visited hospitals for medical reasons are not due to brucellosis.

(iii) Individuals, being in constant contact with animal products such as milk, meat and blood are likely not to be infected by *Brucella* organisms.

(iv) Age and sex do not determine the prevalence of brucellosis in a given population.

(v) Individuals at risk to brucellosis do not know about the disease.

1.9.2 Aims and objectives of the study

The aim of this study is to determine the sero-prevalence of brucellosis among people that are at risk in Lagos Nigeria.

The specific objectives of the study are:

i. To determine the sero-prevalence of brucellosis among veterinarians, slaughterers, butchers abattoir cleaners/blood packers, meat transporters and laboratory workers in some selected abattoirs and hospitals in Lagos Nigeria.

ii. To determine the sero-prevalence of brucellosis among people with febrile diseases that visit some selected hospitals in Lagos Nigeria

iii. To identify the risk factors that predisposes individuals to brucellosis in Lagos Nigeria.

iv. To evaluate the sex and the age bracket of the populace with the highest sero-prevalence of the disease.

v. To determine the awareness level of brucellosis and treatment of the infection among individuals at risk in the selected abattoirs and hospitals.

1.9.3 Significance of the study

The study will help to ascertain the prevalence of human brucellosis among some randomly selected populace in Lagos Nigeria, so that the government and appropriate regulatory bodies for public and animal health can take necessary actions of prevention.

CHAPTER 2

MATERIALS AND METHODS

2.1 STUDY AREA

The study location is Lagos State, the commercial capital of Nigeria, with a population of about 21 million people (Campbell, 2012). The city, with its adjoining conurbation, is the largest in Nigeria, as well as on the African continent (Lagos State Government, 2017). It is located in a coastal area, and is about 400m above sea level. It is at 6.45306 [latitude in decimal degrees] and 3.39583 [longitude in decimal degrees]. Based on Köppen climate classification, the city experiences tropical savannah climate (*Aw*). The wet season is between April and October (with average annual rainfall of 1693mm); while the dry season is between November and March. The average temperature is 27^0C. Geographically, Lagos State is classified into two, namely 'Island and 'Mainland'. Administratively, Lagos consists of twenty local government areas and thirty-seven local council development areas. In addition, the whole state is divided into three constitutionally recognized senatorial districts. These senatorial districts are Lagos Central, Lagos East and Lagos West districts (City Newsline.com.ng, 2013).

Furthermore, according to Healthcare Facilities Monitoring and Accreditation Agency (HEFAMAA), an agency of the government that is involved in the registration of health care facilities in the state, there are twenty-six government-owned general hospitals (about nineteen in Lagos metropolis), two hundred and fifty six primary health care centers, estimated one hundred and sixty trado-medical centres as well as two thousand eight hundred and eighty six accredited private hospitals/clinics/laboratories/ophthalmic/dental/diagnostic centers in Lagos State. In addition, there are two university-teaching hospitals in the state. People in need of treatment for various disease conditions including febrile illnesses visit these medical facilities. Also, many abattoirs are found in the city where thousands of livestock animals such as cattle, sheep, goat and pigs are slaughtered daily for public consumption.

Based on the senatorial districts/zoning of the state, the research was carried out in randomly picked representative hospitals and abattoirs in the three zones. These consist of four abattoirs and three secondary health care providers.

Figure 2: Map of Lagos State, Nigeria (Source: Nigeria Galleria)

Figure 3: Senatorial districts of Lagos State (Source: Umar Yussuf. https://umar-yusuf.blogspot.com/2017/10/map-of-nigeria-senatorial-districts-by.html)

2.2 STUDY DESIGN

It is a descriptive cross-sectional study involving some randomly selected populace at risk to brucellosis in Lagos Nigeria.

2.3 STUDY POPULATION

The study population include veterinarians, slaughterers, butchers, abattoir cleaners, blood packers, abattoir traders and service providers, laboratory workers, people with febrile illnesses and blood donors at the hospitals. The later three groups of participants were found in the hospitals while the initial strata of participants were abattoir-based respondents.

2.4 SAMPLING AND SAMPLING SIZE DETERMINATION

A mixed sampling method was used in population sampling of the abattoir workers while convenient sampling method was the approach at the health care centers. The mixed sampling method adopted at the abattoir involves stratified sampling method in which the respondents were picked from the different strata or groups present at the abattoir; and convenience sampling method for individuals that were easily accessible for participation in the research. The sample size was calculated using Raosoft online software for sample size calculations at 95% confidence interval with a marginal error of 5%. The prevalence rate of 24.1% was used in the calculation based on the research of Aworh *et al.* (2013).

The target sample size was five hundred (*n=500*). The breakdown of the sample size (and strata) is as follows: butchers (*n=78*) out of an estimated total population of 45,000 then slaughterers (*n=53*) out of an estimated population of 147; veterinarians (*n=37*) out of an estimated population of 62; cleaners (*n=58*) out of estimated population of 210; blood packers (*n=69*) out of estimated population of 595, laboratory workers (*n=75*) out of estimated populations of 1587; *Eko* meat van drivers *(n=51*) out of estimated populations of 125 and people with febrile illnesses (*n=90*). However, because of the lack of cooperation and withdrawal of some individuals from the targeted study

populations, other unstated populace which were discovered at the abattoirs were included in the research.

Sera samples were collected from three hundred and sixty two respondents (*n=362*) at the abattoirs. Sixty one (*n=61*) out of these were discarded because of the inability of the serum to separate from whole blood and heamolysis caused by improper storage/improper transportation from the site of bleeding to the laboratory where the serological tests were performed. Therefore, only three hundred and one (*n=301*) sera from abattoir were subjected to Rose Bengal Plate Test.

The hospital-based participants were grouped as professionals, intermediate, manual skilled and unskilled respondents in terms of their levels of education and occupation. Classified under “professionals” among the hospital-based respondents are accountants, doctors, risk managers, bankers and nutritionists among others; while customer care officers, secretaries, interior designers etc. are classified as intermediate workers. The manual-skilled workers include photographers, drivers and security officers. Roadside mechanics, barbers and farmers among the respondents are classified as unskilled workers (appendix J).

Of the 149 hospital-based participants, only 121 were subjected to Rose Bengal Plate test, while the remaining ones (*n= 28*) were discarded due to unsuitability for use in the research i.e. blood sample were haemolysed. Sixty one (*n=61*) respondents out of 121 patients were at the hospital for the treatment of febrile illnesses such as malaria, typhoid while the remainders (*n=60*) were blood donors for either their pregnant wives or as general blood donors.

In all, five hundred and eleven respondents participated in the research; with three hundred and sixty two from the abattoirs and one hundred and forty nine from the hospitals.

2.5 ETHICAL APPROVAL

Ethical approvals for the research were obtained from the Institutional Review Board, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos Nigeria (appendix H); and Health Studies Higher Degree Committee of the College of Human Sciences, University of South Africa (REC-012714-039). The consent of the participants was sought before the commencement of the research.

Approvals for the use of health and abattoir facilities were given by the Lagos Health Service Commission and Veterinary Division, Lagos State Ministry of Agriculture respectively (appendices D and F). However, the demand for another ethical scrutiny in one of the hospitals despite adequate approval by two ethical boards and the government also 'slowed' down the pace of the study. One of the management staff of the hospital later resolved this.

2.6 DATA COLLECTION

2.6.1 Information session

The Heads of the various groups of the people at risk were met and the purpose of the research/study was explained to them. These heads thereafter spoke to their respective team members to participate in the research.

Figure 4: One of the information sessions at Oko-Oba abattoir and Lairage, Agege in Lagos, Nigeria

2.6.2 Questionnaire administration

The respondents were invited to fill the questionnaire after signing the consent/assent form (appendix C & I). However, most of the people at the abattoir were illiterates. Hence, the content of the questionnaire was explained to them in two of the local languages, Hausa and Yoruba (with sometimes, the assistance of an interpreter) by the researcher while they provide the corresponding answer. The questions include socio-demographic information, risk factors for brucellosis, clinical signs, awareness level and any treatment received for suspected symptoms (appendix B).

2.6.3 Blood sample collection

Qualified medical personnel using sterile syringe and needle (Medtrue enterprise co., China product) collected 5 ml of blood aseptically from the respondents (Figures 5 and 6). The blood was then transferred into sterile bottles (without anti coagulants), which were kept in cool container with ice so as to keep it at a temperature of ~4^0C . The blood samples were then taken immediately to the immunology Laboratory at the Nigerian Institute of Medical Research, Yaba Lagos for the serological tests. Sera were extracted immediately and tested for the presence of *Brucella* antibodies using Rose Bengal Plate Test antigen. The sera of sero-positive individuals to Rose Bengal Plate Test antigen were then stored at -20^0C until when they were processed on ELISA as a confirmatory test. The sera for the serological tests were extracted by allowing the effect of gravity in separating sera from whole blood and centrifuging at a rate of 10,000 rpm for 5 minutes.

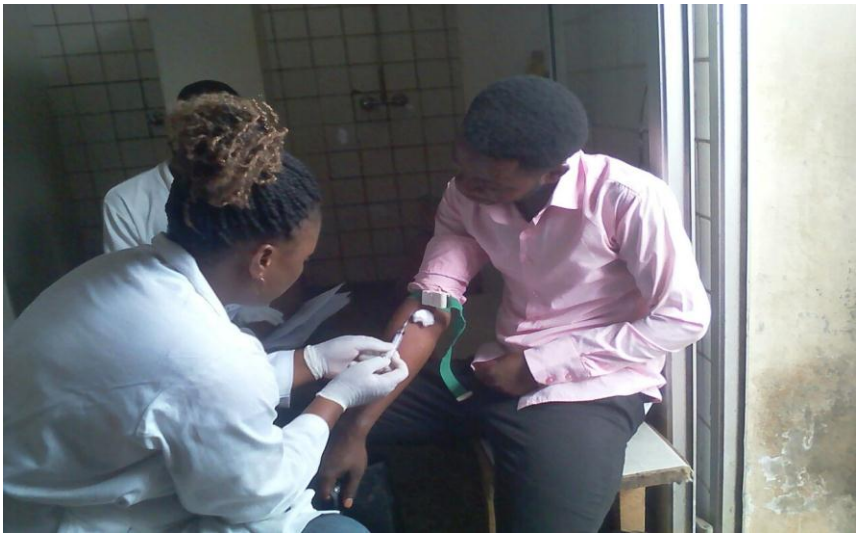

Figure 5: Blood Sample Collection

2.6.4 Serological Tests

2.6.4.1 Rose Bengal plate test

The Rose Bengal reagent used for the study contain *Brucella abortus* biovar 1 (Weybridge 99 strain) inactivated by heat and phenol, coloured with Rose Bengal stain and diluted in an acidified buffer. The reagent was obtained from ID.Vet Innovative Diagnostics, France. Equal volume (30 µl) of Rose Bengal antigen and serum where mixed, using a mixer on a white tile. After 4 minutes under slight agitation, the presence of specific antibodies is demonstrated by the visible agglutinates. In the absence of specific antibodies, the mixture was homogenous.

Figure 6: Rose Bengal plate antigen test at the Nigerian institute of medical research, Lagos Nigeria

Sera that reacted positively to Rose Bengal Plate antigen test were then subjected to ELISA test, which serves as confirmatory test.

2.6.4.2 Indirect ELISA assay

The ELISA test was carried out based on the manufacturer's (Vircell S.L, Spain) instruction. This can be accessed on (http://en.vircell.com/products/brucella-elisa/). The product insert is attached as appendix G. It was validated, with the positive, negative and cut off controls being run (appendix A). The antibody index was calculated from the optical density value obtained, using the formula:

Antibody index= (sample O.D / cut off serum mean O.D.) x 10

The calculated mean optical density (O.D) is 1.7485.

The results interpretation corresponded to the table 4 below:

Table 4: Indirect ELISA Assay result table

Antibody Index	Interpretation
< 9	Negative
9-11	Equivocal
>11	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation; samples with indexes below 9 are considered negative meaning they did not contain IgG specific antibodies against *Brucella*; samples with indexes above 11 are considered positive, meaning IgG specific antibodies against *Brucella* are present.

2.7 DATA MANAGEMENT AND ANALYSIS

Descriptive statistics were used to summarize socio-demographic characteristics of the respondents while patterns of infection, distribution of the clinical signs of brucellosis and level/treatment of the disease among respondents were summarized with proportions and percentages. Bivariate analysis such as Chi-sqaure test was used to investigate the association between location of patients' and selected variables. All analysis was carried out using Statistical Packages for Social Sciences (SPSS) version 20.0 at $\alpha_{0.05}$ significant level.

CHAPTER 3

RESULTS

3.1 SOCIO-DEMOGRAPHY OF RESPONDENTS

Five hundred and eleven respondents (*n=511*) participated in the research study. However, data of only three hundred and twelve (*n=312*) abattoir-based respondents and one hundred and forty nine individuals (*n=149*) hospital participants were analysed. Of the aforementioned, sera of three hundred and one (*n=301*); and one hundred and twenty one (*n=121*) respectively were subjected to serological tests due to reasons explained in the previous chapter.

The socio-demographic characteristics of the respondents (in alignment with objective iv of the study) are presented as follows:

Table 5: Socio–demographic characteristics of abattoir respondents

Variable	Frequency	Percentage
Age of workers (years)		
<18	1	0.3
18 – 24	13	4.2
25 – 34	47	15.1
35 – 44	139	44.6
45 – 59	72	23.1
>=60	22	7.1
Nil	18	5.8
Total	**312**	**100**
Gender of workers		
Male	203	65.1
Female	109	34.9
Total	**312**	**100**

Occupation of workers

Food vendor	13	4.2
Trader	29	9.3
Butcher	118	37.8
Butcher (ponmo)	31	9.9
Nurse	2	0.6
Vets	21	6.7
Cleaner	4	1.3
Slaughterer	3	1
Herdsmen	1	0.3
De–boner	4	1.3
Animal waste packer	1	0.3
Administrative manager	2	0.6
Slab manager	1	0.3
Market coordinator	2	0.6
Student	1	0.3
Meat transporter	37	11.9
Ticketer	1	0.3
Tiler	1	0.3
Herbal/Alcohol seller	2	0.6
Abattoir contractor	1	0.3
Task force	7	2.2
Water seller	1	0.3
Blood meal	1	0.3
Carcass clearance	1	0.3
Meat seller	7	2.2
Civil servant	2	0.6
Skin processor	3	1
Others	12	3.8
Nil	3	1
Total	**312**	**100**

Table 6: Socio–demographic characteristics of hospital-based people at risk to brucellosis

Variable	Frequency	Percentage
Age of respondents (years)		
<18	3	2
18 – 24	19	12.8
25 – 34	60	40.3
35 – 44	34	22.8
45 – 59	14	9.4
>=60	14	9.4
Nil	5	3.4
Total	**149**	**100**
Gender of respondents		
Male	96	64.4
Female	52	34.9
Nil	1	0.7
Total	**149**	**100**
Occupation of respondents		
Professional	25	16.8
Intermediate	16	10.7
Manual skilled	12	8.1
Unskilled	96	64.4
Total	**149**	**100**
Location of respondents		
Lagos General Hosp, Odan	48	32.2
Isolo General Hosp, Lagos	53	35.6
Ikorodu General Hosp, Lagos	48	32.2
Total	**149**	**100**

3.2 GROUPS OF RESEARCH PARTICIPANTS AND THEIR POPULATIONS

The various groups of individuals from which blood were collected are summarized in table 7 and 8 below. This aligns with objectives i and ii of the research study.

Table 7: The various groups of participants at the abattoir

People at Risk to Brucellosis	**Number of Individuals per group**
Main Butchers	167
Ponmo 'butchers' (process skin to *ponmo*, a widely consume 'skin product' in Nigeria)	35
Specialized Slaughterers	3
De-Boning Individuals	3
Veterinarians	31
Animal Sellers/Dealers	8
Blood Meal Makers/Packers	1
Eko Meat Van Drivers/Meat Transporters/Manual Meat Transporter (*Alabo are three*)	38
Cow Horn Dealers	3
Animal Carcass Disposer	1
Food Vendors/traders	37
Abattoir Managers/Taskforce Members/Slab Manager/Clerical officer/Ticketer etc. (Ensures orderliness in the abattoir)	15
Local Herbal drug seller	2
Lotto Coordinator	1
Vets office Assistant	1
Abattoir Engineering Staff	1
Stationery Printer	1
Cleaners/ingesta packers	4
Gall Stone Merchants	2
Tiller	1
Herdsmen	1
Nurse	2
Students	1
Not stated	3
Total number of individuals sampled	**362**

Table 8: Category of participants according to occupations at the hospital (appendix J)

Occupation of Respondents	Number of Participants
Professionals	25
Intermediate	16
Manual skilled	12
Unskilled	96
Total	**149**

3.3 RESULTS OF ROSE BENGAL PLATE TEST

Sera from three hundred and one (*n=301*) abattoir-based individuals and one hundred and twenty one (*n=121*) hospital-based respondents were subjected to Rose Bengal Plate antigen test (RBPT). Twenty seven people (8.97%) were sero-positive to the infection. A bar chart showing the occupations of the sero-positive respondents is presented below:

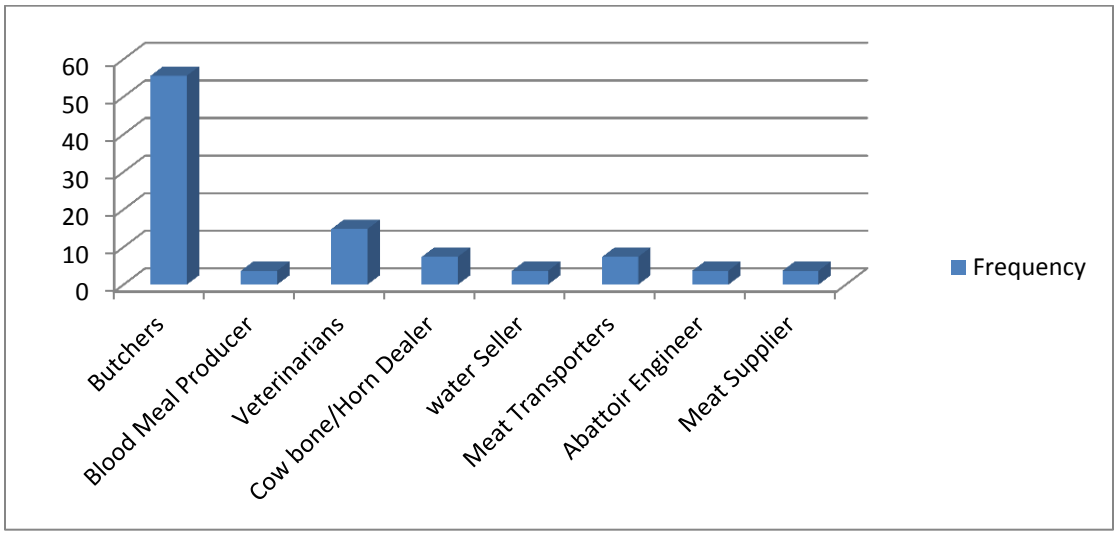

Figure 7: Bar Chart showing the Occupations of the Sero-Positive Individuals on RBPT

From the chart above, 55.6 % (*n=15*) of the sero-positive respondents were butchers; 14.8% (*n=4*) were veterinarians, 7.4% each (*n=2*) were meat transporters and cow bone/horn dealers while the remaining respondent groups were 3.7% each. Of the twenty seven individuals, 66.7% (*n=18*) were males while the remaining 33.3% (*n=9*) were females. This is shown in the bar chart below:

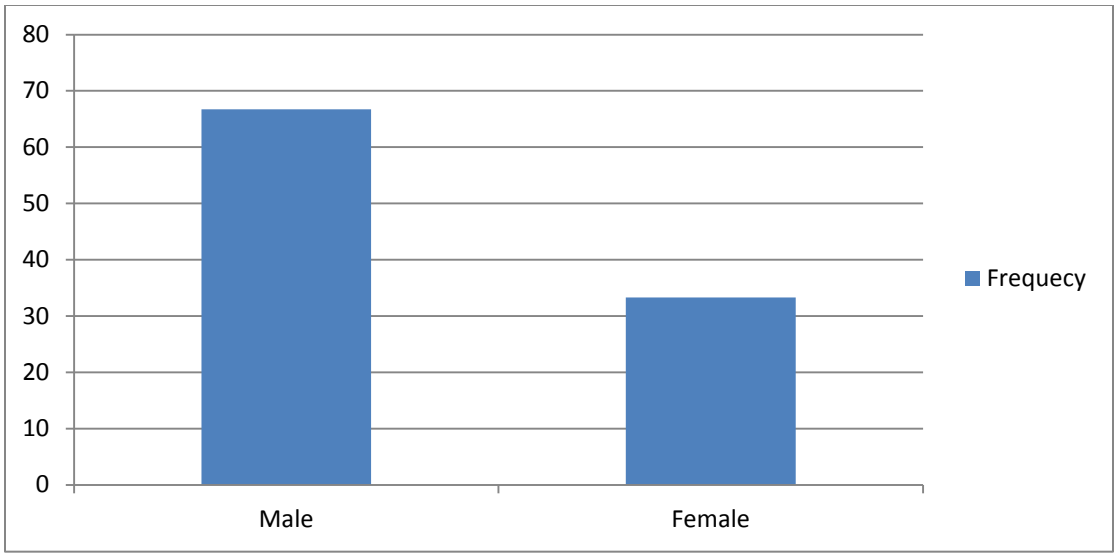

Figure 8: Gender of sero-positive individuals on RBPT

3.4 RESULTS OF INDIRECT ELISA TEST

Sera from the twenty-seven sero-positive individuals to brucellosis on Rose Bengal plate antigen were subjected to *Brucella* indirect ELISA assay as a confirmatory test. Three (*n=3*) were confirmed by the assay, while one was equivocal. This means that 11.1% were confirmed positive by the ELISA test (Appendices E & K).

Table 9: Results of the ELISA Test

Results	Frequency % (n)
Sero-positives	11.1 (3)
Equivocal	3.7 (1)
Sero-negatives	85.2 (23)

3.5 DISTRIBUTION OF BRUCELLOSIS-RELATED CLINICAL SIGNS AMONG THE RESPONDENTS

Some clinical signs associated with brucellosis were observed among some of the respondents. These clinical signs include fever, orchitis, abortion, joint pain, lower backache, regular headache and weakness. The distributions of the clinical signs are shown in Tables 10 and 11

Table 10: Distribution of clinical signs associated with people at risk to brucellosis at the abattoirs

Variable	Frequency	Percentage
Fever		
Yes	109	34.9
No	201	64.4
Nil	2	0.6
Total	**312**	**100**
Joint pain		
Yes	184	59
No	126	40.4
Nil	2	0.6
Total	**312**	**100**
Lower backache		
Yes	197	63.1
No	113	36.2
Nil	2	0.6
Total	**312**	**100**
Regular headache		
Yes	130	41.7
No	180	57.7
Nil	2	0.6
Total	**312**	**100**
Weakness		
Yes	111	35.6
No	198	63.5
Nil	3	1
Total	**312**	**100**
Orchitis		
Yes	20	6.4
No	196	62.8
Nil	96	30.8
Total	**312**	**100**
Miscarriage		
Yes	57	18.3
No	249	79.8
Nil	6	1.9

Total	**312**	**100**

Nil means 'not stated'.

Table 11: Distribution of clinical signs associated with hospital-based respondents

Variable	Frequency	Percentage
Fever		
Yes	17	11.4
No	130	87.2
Nil	2	1.3
Total	**149**	**100**
Joint pain		
Yes	27	18.1
No	114	76.5
Nil	8	5.4
Total	**149**	**100**
Lower backache		
Yes	27	18.1
No	114	76.5
Nil	8	5.4
Total	**149**	**100**
Regular headache		
Yes	25	16.8
No	116	77.9
Nil	8	5.4
Total	**149**	**100**
Weakness		
Yes	40	26.8
No	101	67.8
Nil	8	5.4
Total	**149**	**100**
Orchitis		
Yes	11	7.4
No	85	57
Nil	53	35.6
Total	**149**	**100**
Miscarriage		

Yes	9	6
No	126	84.6
Nil	14	9.4
Total	**149**	**100**

Nil means 'not stated'

Table 12: Distribution of clinical signs associated with sero-positive individuals at the abattoirs

Variable	Frequency	Percentage
Fever		
Yes	11	40.7
No	16	59.2
Nil	0	0
Total	**27**	**100**
Joint pain		
Yes	18	66.7
No	9	33.3
Nil	0	0
Total	**27**	**100**
Lower backache		
Yes	17	63.0
No	10	37.0
Nil	0	0
Total	**27**	**100**
Regular headache		
Yes	11	40.7
No	16	59.3
Nil	0	0
Total	**27**	**100**
Weakness		
Yes	13	48.2
No	14	51.8
Nil	0	0
Total	**27**	**100**
Orchitis		
Yes	0	0
No	18	66.7

NA	9	33.3
Total	**27**	**100**
Miscarriage		
Yes	08	29.6
No	19	70.4
Nil	0	0
Total	**27**	**100**

NA-Not applicable

3.6 RISK FACTORS FOR BRUCELLOSIS

The analyses of the risk factors that pre-dispose individuals to brucellosis are presented in tables 13 and 14 below, in alignment with objective iii of this study:

Table 13: Distribution of possible source of *Brucella* infection to abattoir respondents

Variable	Frequency	Percentage
Location of workers		
Itire slaughter slab	63	20.2
Kwil abattoir	29	9.3
Ikorodu slaughter slab	46	14.7
Oko-oba Agege abattoir	174	55.8
Total	**312**	**100**
***Fura* (unpasteurized milk) consumption**		
Yes	189	60.6
No	120	38.5
Nil	3	1
Total	**312**	**100**
***Wara* (fresh cheese) consumption**		
Yes	176	56.4
No	133	42.6
Nil	3	1
Total	**312**	**100**

***Ponmo* (animal skin) consumption**		
Yes	285	91.3
No	24	7.7
Nil	3	1
Total	**312**	**100**
Meat		
Yes	298	95.5
No	12	3.8
Nil	2	0.6
Total	**312**	**100**
***Suya* (spicy skewered beef) consumption**		
Yes	216	69.2
No	96	30.8
Total	**312**	**100**
Animal rearing		
Yes	89	28.5
No	223	71.5
Total	**312**	**100**

Nil means 'not stated'

Table 14: Distribution of possible source of *Brucella* infection to hospital-based respondents

Variable	Frequency	Percentage
***Fura* (unpasteurized Milk) consumption**		
Yes	103	69.1
No	41	27.5
Nil	5	
Total	**149**	**100**
***Wara* (Fresh Cheese) consumption**		
Yes	21	14.1
No	123	82.6
Nil	5	
Total	**149**	**100**

***Ponmo* (Animal skin) consumption**		
Yes	75	50.3
No	69	46.3
Nil	5	3.4
Total	**149**	**100**
Meat		
Yes	106	71.1
No	38	25.5
Nil	5	3.4
Total	**149**	**100**
***Suya* (spicy skewered beef) consumption**		
Yes	97	65.1
No	51	34.2
Nil	1	0.7
Total	**149**	**100**
Animal rearing		
Yes	25	16.8
No	122	81.9
Nil	2	1.3
Total	**149**	**100**

Nil means 'not stated'

Table 15: Distribution of brucellosis awareness level and treatment against brucellosis among people at risk at the abattoirs

Variable	Frequency	Percentage
Brucellosis awareness		
Yes	55	17.6
No	255	81.7
Nil	2	0.6
Total	**312**	**100**
Brucellosis treatment		
Yes	2	0.6
No	308	98.7

Nil	2	0.6
Total	**312**	**100**

Nil means 'not stated'

Table 16: Distribution of brucellosis awareness level and treatment against brucellosis among hospital based patients

Variable	Frequency	Percentage
Brucellosis awareness		
Yes	17	11.4
No	129	86.6
Nil	3	2
Total	**149**	**100**
Brucellosis treatment		
No	143	96
Nil	6	4
Total	**149**	**100**

Nil means 'not stated'

Table 17: Results of bivariate analysis of Infectious status and gender at the abattoirs

Variable	Gender		χ2	P-value
	Male	Female		
Infection status (unpasteurized milk**)**			4.763	0.027**
Positive	162 (79.8)	27 (55.9)		
Negative	41 (20.2)	79 (44.1)		
Total	**203 (100.0)**	**106 (100.0)**		
Infection status (fresh cheese**)**			4.638	0.031**
Positive	137 (67.5)	39 (35.8)		
Negative	66 (32.5)	67 (64.2)		
Total	**203 (100.0)**	**106 (100.0)**		

** significant p-value

Table 17 showed the test association between infection status and selected variables at the abattoir. There were significant associations between unpasteurized milk (p=0.027) and fresh cheese (p=0.031) and gender.at abattoir.

Table 18: Results of bivariate analysis of infectious status and gender at General Hospital

Variable	Gender		χ2	P-value
	Male	Female		
Infection status (unpasteurized Milk**)**			2.136	0.105
Positive	63 (65.6)	41 (85.4)		
Negative	33 (34.4)	7 (14.6)		
Total	**96 (100.0)**	**48 (100.0)**		
Infection status (fresh cheese**)**			2.774	0.093
Positive	13 (13.5)	8 (16.7)		
Negative	83 (86.5)	40 (83.3)		
Total	**96 (100.0)**	**48 (100.0)**		

** significant p-value

Table 18 showed the test association between infection status and selected variables at the abattoir. There were no significant associations between unpasteurized milk (p=0.105) and fresh cheese (p=0.093) and gender at the General Hospitals

Table 19: Bivariate analysis association of suggestive symptoms of brucellosis with location of the participants

Variable	Location		χ2	P–value
	Abattoir	General Hospital		
Fever				
Yes	109(86.5)	17(13.5)	27.803	*P*<0.001**
No	201(60.7)	130(39.3)		

Total	**310(67.8)**	**147(32.2)**		
Joint pain				
Yes	184(87.2)	27(12.8)		
No	126(52.5)	114(47.5)	62.928	*P*<0.001**
Total	**310(68.7)**	**141(31.3)**		
Lower back ache				
Yes	197(87.9)	27(12.1)		
No	113(49.8)	114(50.2)	76.426	*P*<0.001**
Total	**310(68.7)**	**141(31.3)**		
Regular headache				
Yes	130(83.9)	25(16.1)		
No	180(60.8)	116(39.2)	25.174	*P*<0.001**
Total	**310(68.7)**	**141(31.3)**		
Weakness				
Yes	111(73.5)	40(26.5)		
No	198(66.2)	101(33.8)	2.478	0.115
Total	**309(68.7)**	**141(31.3)**		
Orchitis				
Yes	20(64.5)	11(35.5)		
No	196(69.8)	85(30.2)	0.359	0.549
Total	**216(69.2)**	**96(30.8)**		
Miscarriage				
Yes	57(86.4)	9(13.6)	10.53	0.001**

No	249(66.4)	126(33.6)
Total	**306(69.4)**	**135(30.6)**

Table 20: Bivariate analysis of risk factors with brucellosis status of the participants

Variable	Brucellosis Status		χ^2	P–value
	Positive	Negative		
Age of participants (years)				
<18	0(0.0)	1(100.0)		
18 – 24	0(0.0)	13(100.0)		
25 – 34	5(10.6)	42(89.4)	7.235	0.204
35 – 44	11(7.9)	128(92.1)		
45 – 59	11(15.3)	61(84.7)		
>=60	0(0.0)	22(100.0)		
Total	**27(9.2)**	**265 (90.8)**		
Gender of participants				
Male	18(8.9)	185(91.1)	0.033	0.855
Female	9(7.3)	101(92.7)		
Total	**27(8.7)**	**285(91.3)**		
Occupation of participants				
Professional	3(12.0)	22(88.0)		
Intermediate	3(25.0)	9(75.0)	4.994	0.172
Manual skilled	0(0.0)	3(100.0)		
Unskilled	21(7.7)	251(92.3)		
Total	**27(8.7)**	**285(91.3)**		
***Fura* (Unpasteurized Milk) consumption**				
Yes			7.186	0.007**
No	23(12.2)	166(87.8)		
Total	4(3.3)	116(96.7)		

	27(8.7)	**282(91.3)**		
***Wara* (Fresh Cheese) consumption**				
Yes			5.231	0.022**
No	21(11.9)	155(88.1)		
Total	6(4.5)	127(95.5)		
	27(8.7)	**282(91.3)**		
***Ponmo* (Animal skin) consumption**				
Yes			0.682	0.409
No	26(9.1)	259(90.9)		
Total	1(4.2)	23(95.8)		
	27(8.7)	**282(91.3)**		
Animal rearing				
Yes	6(6.7)	83(93.3)	0.576	0.448
No	21(9.4)	202(91.6)		
Total	**27(8.7)**	**285(91.6)**		

CHAPTER 4

DISCUSSION

The results of the research revealed that brucellosis is mainly related to occupational disease (objectives i & ii). Twenty-seven (*n*=27) sero-positive individuals were from the abattoirs; and none found at the hospitals. This finding correlates with findings of many research works on human brucellosis (Mukhtar, 2008, CDC, 2015; WHO 2018). On Rose Bengal test, the "butchers" category showed the highest number of sero-positive individuals (15 out of 27 or 55.6% of the total *Brucella*-seropositive persons). This result was in agreement with the research work of Aworh *et al.* (2013) at Abuja, Nigeria; Cadmus *et al.* (2006) at Ibadan Nigeria; Swai & Schoonman (2009) at Tanzania; El Kholy *et al.* (2009) at Egypt; and Amegashie *et al.* (2017) at Ghana. The second highest sero-positive group of people to brucellosis was "the veterinarians", accounting for 14.8% of the twenty-seven positives. This result contradicts the earlier research report of Awad, (1998) and Aworh *et al.* (2013) in which the veterinarians were not sero-positive to the infection, attributed to their short exposure to animals during meat inspection, use of personal protective equipment as well as their knowledge of the zoonotic disease. However, Patil *et al*. (2013) and Sambrani *et al*. (2016) reported incidence of brucellosis among veterinarians in their research work. The infection of the veterinarians in this study might probably be as a result of inadequate protection of the veterinarians which may be sequel to non-provision of protective materials by the government

Furthermore, two meat transporters were both sero-positive to RBPT and ELISA tests. These transporters move meat within the abattoir using cart and motorcycle. The level of exposure of these men to raw meat could be responsible for their infection by the bacteria. The other group of meat transporters, *Eko* Meat Van drivers were not sero-positive to the infection. The later group of meat transporters move meat from the abattoir to different part of the state in enclosed vans, with level of exposure to physical contacts with meat relatively minimal when compared with the other group of meat transporter.

Moreover, one of the sero-positive respondents who deals with cow bones confirm the possibility of the transmission of *Brucella* organisms through bones as found in the reports of Scian *et al.* (2012) and Giambartolomei *et al.* (2017). Another sero-positive respondent was a cow horn dealer. The mechanism of infection through animal horn has not been reported but the man might have been infected through the horns placed in contaminated slurry and soils of the abattoir.

The fact that one of the sero-positive individuals is a trader who sells water to the abattoir workers means that non-occupational people who visits the abattoir to buy meat or for any other purpose and those who live in houses very close to the abattoir are also at risk to contracting the infection.

In addition, it must be noted that no sero-positive individuals were found at two (Ikorodu Slaughter Slab and Kwil Abattoir and Farms) out of the four abattoirs visited in the research. Slaughtering and other animal-related activities in these two abattoirs are neater and better carried out when compared with the other two abattoirs. This shows that level of cleanliness and proper waste disposal in an abattoir has influences on transmission of *Brucella* pathogen from animals to humans as was observed in the report of Corbel (2006); WHO (2006) and Wang *et al.* (2015). They reported that *Brucella* could survive in dust, manure, water, manure slurry, aborted fetuses, soil, meat and dairy products for considerable periods, and human/animal infections originate from direct or indirect contact with infected animals or *Brucella*-contaminated environments. However, before the conclusion of this study, the Lagos State Government was already carrying out some reforms at the biggest abattoir in the state (Oko Oba Abattoir & Lairage, Agege), where majority of the sero-positive respondents were found.

The level of brucellosis awareness and treatment for brucellosis among the respondents are also very low (tables 15 and 16). Considering the chronic course of brucellosis as well as its consequences, awareness campaigns should be conducted to inform people

at risk in an endemic region like Nigeria (objective v). Currently, there is no regulation for implementation of an effective control programme of the disease. In addition, significant attention for the treatment of possible brucellosis infection among the populace is not catered for. This perception is based on the few respondents who turned to traditional medicine for brucellosis treatment.

The clinical signs suggestive of human brucellosis in this study were fever, joint pain, lower backache, regular headache and miscarriage. However, orchitis does not have a significant association with the location. The prevalence rate of 6.4% and 7.4% among the abattoir-based respondents and hospital-based respondents respectively is still a source of concern (table 19).

The main risk factors significant for human brucellosis according to this research and as shown in table 20 are consumption of *fura* (unpasteurized milk) and *wara* (fresh cheese). This is in agreement with many research reports worldwide which states that milk and milk products consumption are a major source of human brucellosis (Giannakopoulos *et al*., 2006; Mensah *et al*., 2011; Guoqiu *et al*., 2013; Khazaei *et al*., 2016; Wafa, 2017).

CHAPTER 5

SUMMARY AND CONCLUSION

Brucellosis is present in Lagos State Nigeria. The prevalence rate of 8.97% is a significant infection rate, considering the possibility of higher infection rate due to the operation of illegal abattoirs, slaughter slabs and lack of regulated control measures in the state. In addition, the fact that none of the hospital respondents is sero-positive to brucellosis further shows people that are not working/trading in places where animals are slaughtered are not at much risk to brucellosis as people that work/trade in slaughter houses. However, consumption of unpasteurized milk/milk products predisposes all the populace irrespective of the place of occupation to *Brucella* infection as reported by El-Koumi *et al*. (2013).

In term of gender, males are the dominant gender in the abattoir based occupation/trading, with females accounting for just 34.9% (out of 312 respondents) of the abattoir based respondents. The number of sero-positive males to females is in ratio 2:1. However, the number of females found at the various abattoir shows that there is increasing feminine participation in abattoir activities. There is therefore the need for increased brucellosis awareness among the populace since high feminine brucellosis infection may lead to a decline in the population over time. For the hospital-based respondents, the percentage of women that participated is surprisingly 34.9% (out of 149 participants) too. This shows that women in Lagos make effort in knowing their health status; therefore, educating a woman is educating a nation.

Individuals in age bracket 35-44 are mostly involved in abattoir work and trading, according to the result of this research. 44.4% of the sero-positive individuals fall in this age group, being the highest among them. However, age is not significant in relation to brucellosis infection. Also, brucellosis awareness level and treatment is very low among the respondents.

The prevalence rate of orchitis among abattoir-based respondents (6.4%) and hospital-based (7.4%) in this study is a source of concern. Hence, effort should be made to find out the cause of this inflammation in the state.

In the light of all the aforementioned, I hereby recommend that there should be increased surveillance of brucellosis in collaboration with the surrounding states/country (Ogun State Nigeria and Republic of Benin). Also, there is a need for the government to employ more veterinarians so as to improve meat inspection service in the state. In addition, there should be massive public enlightenment on brucellosis infection. Lastly, units for One Health (which comprises the medical and veterinary personnel) should be established by the government/relevant stakeholders so as to enhance control and treatment of brucellosis and other zoonotic infection.

5.1 LIMITATIONS OF THE STUDY

The challenges encountered in the course of the research were enormous. These challenges include:

(i) Unwillingness of many of the people at risk especially in the abattoir to participate in the research. Time and funds were wasted in trying to convince the people to participate in the research. Hence, the populations of the target abattoir-based

professionals were altered in order to encourage participation of more individuals in the research.

(ii) The progressive, rapid decline of Nigerian Naira in comparison to other major currencies (about 300% increase in exchange rate) between the time the research budget was made and when the research was carried out made the antigens, kits and logistics to be extremely expensive. This major constraint retarded the progress of the research.

5.2 FUTURE PERSPECTIVE

The possibility of *Brucella pinnipedialis* and *Brucella ceti* infection may be high in the state because Lagos is a coastal city, with many of the residents being exposed to the coastal water regularly. Hence, research work on prevalence rate of the above-mentioned pathogens should be carried out.

If possible, isolation and identification of *Brucella spp*. should be conducted in those areas where serological positive individuals have been identified.

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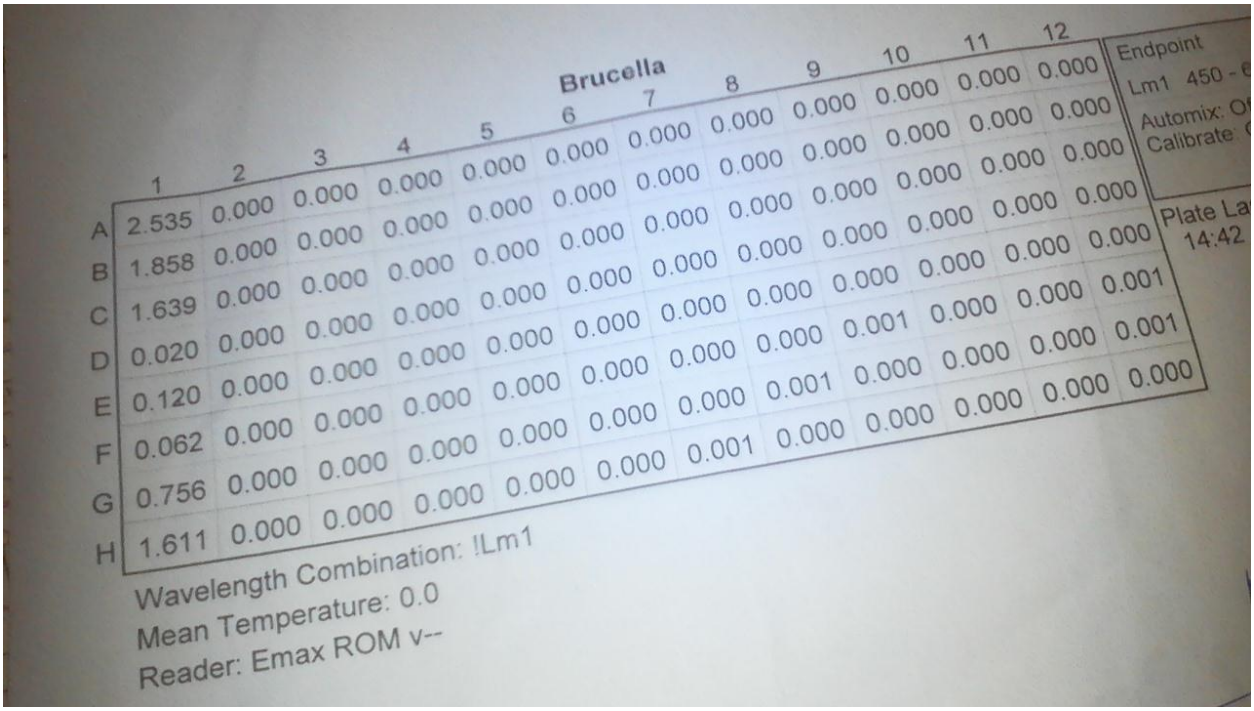
APPENDICES

Appendix A: Print out of ELISA result sheet



Brucella

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.535	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.858	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.639	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001
G	0.756	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.001
H	1.611	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000

Endpoint
Lm1 450 - 6
Automix: O
Calibrate: C
Plate La
14:42

Wavelength Combination: !Lm1
Mean Temperature: 0.0
Reader: Emax ROM v--

Appendix B: Questionnaire used during survey

Survey of brucellosis among people at risk in Lagos, Nigeria

(An MSc Research project of a student of University of South Africa)

(a) Title: (Prof/Mr. /Mrs. /Miss/Dr.)…… (b) Gender: ……. (c) Age:

(d)Address:……..……

(e) Occupation: ……

(f) Which of these do you consume? Milk ☐ Cheese ☐ Cow skin ☐ Meat ☐ (*You can tick as many as you consume raw*)

(g) Do you eat *suya (spicy skewered meat) Yes* ☐ No ☐. If yes, how many times per month? ……..

(h) Do you keep/rear animals ……If yes, which animals?

…….

(i) Did any of your animals mentioned in (h) above experience stillbirth or abortion?

Yes ☐ No ☐ I don't know ☐

If yes, which one ……

(j) Do you experience fever every day? Yes ☐ No ☐

(k) Which of these symptoms have you been experiencing for several weeks?

Joint pain ☐ lower backache ☐ regular headache ☐ weakness ☐ orchitis ☐ none ☐ (*You can tick more than one item*)

(l) Did you experience miscarriage more than twice Yes ☐ No ☐. If yes, how many times? ……

(m) Have you heard of the disease called brucellosis? Yes ☐ No ☐

(n) Have you been treated for brucellosis before? Yes ☐ No ☐ If yes, which drug(s) were administered to you ……

Thanks for participating in this research. Your information will be kept confidential.

Appendix C: Consent form

CONSENT FORM

Project Title: Survey of brucellosis among people at risk in Lagos, Nigeria

Principal investigator: Akinroyeje Kehinde Adeyemi (MSc Student, University of South Africa)

I am conducting a survey in order to establish if people have been exposed to a possible brucellosis infection (a disease that can affect people that work with animal farms, abattoirs and those that handle animals and their products) in Lagos Nigeria. If you are willing to be part of this study, I will be grateful if you allow the medical personnel to collect blood samples that will be tested in the laboratory to check for this disease. You will also fill a questionnaire that will provide us with the information that will assist us during the information sessions and our feedback to you at the end of this research work. Collection of the blood and filling of the questionnaire will take not more than an hour of your time.

The medical personnel will collect about 5 ml of blood. You may feel a little discomfort when the syringe needle is used but that feeling will disappear immediately. A new syringe will be used for each individual.

This research will be of great benefit to you and the country at large. It will help you know more about brucellosis, which is a disease that causes fever and also affects the reproductive system. This disease is preventable and treatable. If you know your status, you will be able to observe the relevant precautions.

I promise to keep all the information from the questionnaire as well as the result of your blood test confidential. However, if the government want to re-use the data generated from this research, it will be able to access them.

Participation in this research is voluntary, you can withdraw at anytime and you will not be penalized. If you are a minor, consent from your parents will be required.

If you have decided to participate in this study, please write your name and append your signature

I ……want to be part of this research study.

…… ……

Signature Date

If you have any question concerning this study, you may contact Dr. Adesina Adeiga at Nigerian Institute of Medical Research (NIMR), Yaba, and Lagos Nigeria. Tel: 08023026544 or 08097631483; email: aadeiga@yahoo.com. You may also contact Dr. Prudence Kayoka-Kabongo at UNISA on Telephone no +27827750754; email:kabonpnk@unisa.ac.za.

If you have any question about your right or if there is any adverse consequence as a result of your participation in this study, please contact the Chairman, Institutional Review Board (IRB), Nigerian Institute of Medical Research, Yaba Lagos Nigeria or Chair of Department, Department of Agriculture and Animal Health, University of South Africa, South Africa.

WITNESSES

1. ..... 2.……

Signature and date

Appendix D: Approval letter from the Lagos State Health Service Commission

LAGOS STATE GOVERNMENT

LSHSC/2222/VOL. XVIII/314

19th October, 2016

Mr. Akinroyeje Kehinde Adeyemi
10 Ibadan Street
Off Irawo Bus Stop
Mile 14 Ikorodu Road
Lagos.

RE: APPROVAL FOR RESEARCH

1. The Health Service Commission is in receipt of your letter dated 21st September, 2016 as an addendum to the previous one submitted in July 2016 in respect of the above captioned subject.
2. Following the review of your request and as an addendum to our letter Ref. No. LSHSC/2222/Vol. XVIII/124 dated 26th August 2016; I have been directed to convey the Commission's approval for you to include Isolo General Hospital in your research titled ***'Survey of Brucellosis among susceptible professionals in Lagos Nigeria'*** as requested.
3. Kindly note that you are requested to submit a copy of your findings to the Commission as soon as the study is concluded.
4. Thank you.

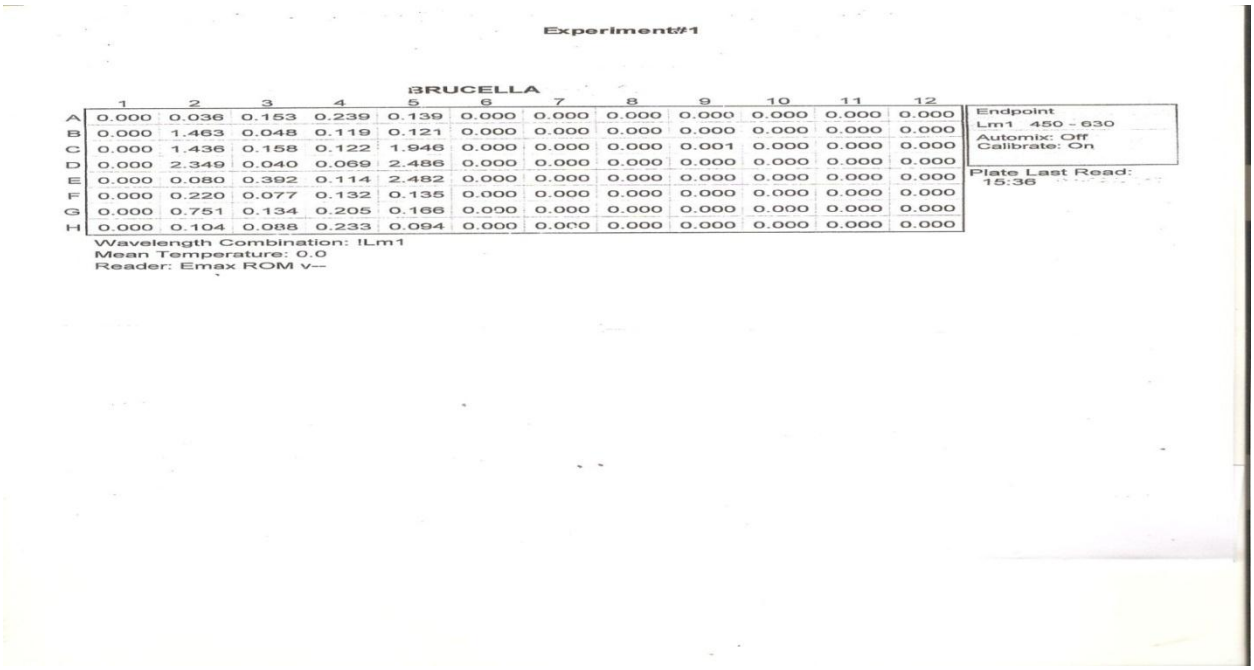
Dr. (Mrs.) A.M Onayiga
Director Medical Services
for: Permanent Secretary (HSC)

cc:
Medical Director, General Hospital Isolo

HEALTH SERVICE COMMISSION

1, Ganiu Smith Street, Lagos Island, Lagos. Telephone: 2637140, 8923056. Fax No. 01-2637140.
E-mail: hsc@lagosstate.gov.ng

Appendix E: print out of ELISA result sheet



Experiment#1

BRUCELLA

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.036	0.153	0.239	0.139	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	1.463	0.048	0.119	0.121	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	1.436	0.158	0.122	1.946	0.000	0.000	0.000	0.001	0.000	0.000	0.000
D	0.000	2.349	0.040	0.069	2.486	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.080	0.392	0.114	2.482	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.220	0.077	0.132	0.135	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.751	0.134	0.205	0.166	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.104	0.088	0.233	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Endpoint
Lm1 450 - 630
Automix: Off
Calibrate: On

Plate Last Read:
15:36

Wavelength Combination: !Lm1
Mean Temperature: 0.0
Reader: Emax ROM v--

Appendix F: Approval letter from the Veterinary Division, Lagos State Ministry of Agriculture

LAGOS STATE GOVERNMENT

05/08/2016

THE UNIT HEAD

IKORODU SLAUGHTER SLAB,

IKORODU

RESEARCH WORK

Please assist the bearer Mr Akinroyeje Kehinde Adeyemi to carry out his research work in your Units

Yours

05/08/16

Dr. Macaulay R.M

DVS

MINISTRY OF AGRICULTURE

Block 23, 1st & 2nd Floor, The Secretariat, Alausa, Ikeja. P.M.B 21028
E-mail: agric@lagosstate.gov.ng

Appendix G: Package insert from the manufacturer for the ELISA Kit

VIRCELL
microbiologists

CE

BRUCELLA ELISA IgG

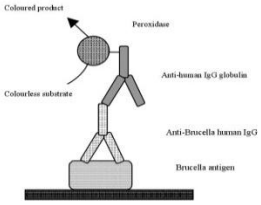
G1003: Indirect immunoenzyme assay to test IgG antibodies against Brucella in human serum. 96 tests.

INTRODUCTION:

Direct agglutination, Rose Bengal test, Coombs´test and ELISA are the most widely used techniques for the serologic diagnosis of brucellosis. Detection of IgG against Brucella lipopolysaccharide (LPS) is suitable for the diagnosis of all forms of the disease. The ELISA technique is sensitive and specific for the detection of IgG antibodies against Brucella.

PRINCIPLE OF THE TEST:

The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.



KIT FEATURES:

All reagents, except for the washing solution, are supplied ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

KIT CONTENTS:

1 VIRCELL BRUCELLA PLATE: 1 96-wells plate coated with LPS antigen of *Brucella abortus*,strain S-99.
2 VIRCELL SERUM DILUENT: 25 ml of serum dilution solution: a blue coloured phosphate buffer containing protein stabilizers and Proclin. Ready to use.
3 VIRCELL IgG POSITIVE CONTROL: 500 µl of positive control serum containing Proclin.
4 VIRCELL IgG CUT OFF CONTROL: 500 µl of cut off control serum containing Proclin.
5 VIRCELL IgG NEGATIVE CONTROL: 500 µl of negative control serum containing Proclin.
6 VIRCELL IgG CONJUGATE: 15 ml of anti-human IgG peroxidase conjugate dilution in a red-coloured Proclin-containing buffer. Ready to use.
7 VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
8 VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
9 VIRCELL WASH BUFFER: 50 ml of 20x washing solution: a phosphate buffer containing Tween®-20 and Proclin.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:
Precision micropipettes 5 and 100 µl.
Eight channel micropipette 100 µl.
ELISA plate washer.
Thermostatized incubator/water bath.
ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter.
Alternatively, an ELISA automated processor.
Distilled water.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

REAGENT	STABILITY AND STORAGE
1x washing solution	4 months at 2-8ºC
Rest of reagents	Refer to package label for expiration date (at 2-8ºC)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Do not let the plate dry between washing and reagent addition.
Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate.
Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the serum dilution, washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL ELISA references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Serum dilution solution, plate, conjugates and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells are coated with inactivated Brucella antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.

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7. Susbstrate solution may be irritant to skin and mucus. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
8. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay. To this purpose, VIRCELL counts with sets of samples reserved for evaluation in parallel with the manual technique. These sets of samples are available on request, as well as a list of commercial systems which have already been validated for use with the VIRCELL ELISA range.
9. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.

SPECIMEN COLLECTION AND HANDLING:

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. **Do not use plasma.**

PRELIMINARY PREPARATION OF THE REAGENTS:

Only the washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

1.-Set incubator/water bath to 37±1ºC.
2.-Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3.-Shake all components.
4.-Remove the plate 1 from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed.
5.-Add 100 µl of serum diluent 2 to all wells. Add 5 µl of each sample, 5 µl of positive control 3, 5 µl of cut off control 4 (in duplicate) and 5 µl of negative control 5 into the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of serum diluent 2 and sample. Mix homogenously with the pipette and dispense 105 µl of each diluted sample to the wells 1.
6.-Cover with a sealing sheet and incubate at 37±1ºC for 45 min.
7.-Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 9 per well. Drain off any remaining liquid.
8.-Immediately add 100 µl of IgG conjugate solution 6 into each well.
9.-Cover with a sealing sheet and incubate in incubator/water bath at 37±1ºC for 30 min.
10.-Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 9 per well. Drain off any remaining liquid.
11.-Immediately add 100 µl of substrate solution 7 into each well.
12.-Incubate at room temperature for 20 minutes protected from light.
13.-Add immediately 50 µl of stopping solution 8 into all wells.
14.-Read with an spectrophotometer at 450/620 nm within 1 hour of stopping.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.
Optical densities (O.D.) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

CONTROL	O.D.
POSITIVE CONTROL	>0.9
NEGATIVE CONTROL	<0.55
CUT OFF CONTROL	<0.7 x(O.D. POSITIVE CONTROL)
	>1.5 x(O.D. NEGATIVE CONTROL)

INTERPRETATION OF RESULTS:

Calculate the mean O.D. for cut off serum.

Antibody index=(sample O.D./ cut off serum mean O.D.) x 10

INDEX	INTERPRETATION
< 9	Negative
9-11	Equivocal
>11	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 9 are considered as not having IgG specific antibodies against Brucella.
Samples with indexes above 11 are considered as having IgG specific antibodies against Brucella.

LIMITATIONS:

1.-This kit is intended to be used with human serum.
2.-The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3.-The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4.-This test will not indicate the site of infection. It is not intended to replace isolation.
5.-Lack of significant rise in antibody level does not exclude the possibility of infection.
6.-Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7.-Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8.-The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

PERFORMANCE

SENSITIVITY AND SPECIFICITY:

113 serum samples were assayed with BRUCELLA ELISA IgG against Coomb´s test.
The results were as follows:

	SAMPLE NR	SENSITIVITY	SPECIFICITY
IgG	113	98%	100%

Indeterminate values were omitted from the final calculations

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INTRA-ASSAY PRECISION:

3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions. The results were as follows:

SERUM	N	%C.V.
PC	10	1.11
NC	10	7.45
CO	10	4.02

C.V. Coefficient of variation

INTER-ASSAY PRECISION:

3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

SERUM	N	% C.V.
PC	10	1.68
NC	10	9.38
CO	10	4.71

C.V. Coefficient of variation

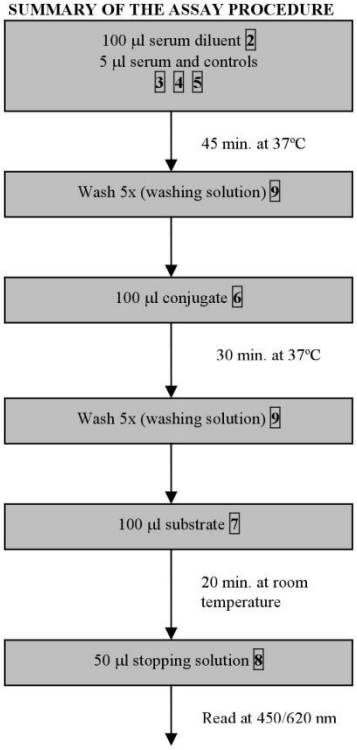
CROSS REACTIVITY AND INTERFERENCES:

16 samples known to be positive for other specimens of the syndromic group (*Salmonella typhi O*, *Salmonella typhi H*, cytomegalovirus, Toxoplasma and Epstein-Barr virus) were assayed for IgG testing. 3 samples known to be positive for antinuclear antibodies were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross-reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
8°C 2°C	Store at 2-8°C
Σ x	Contains sufficient for <X> tests
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS x	<X> wells

SUMMARY OF THE ASSAY PROCEDURE



LITERATURE:

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2. Araj, G. F., A. R. Lulu, M. Y. Mustafa, and M. I. Khateeb. 1986. Evaluation of ELISA in the diagnosis of acute and chronic brucellosis in human beings. J Hyg (Lond) 97:457-69.
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4. Baldi, P. C., S. E. Miguel, C. A. Fossati, and J. C. Wallach. 1996. Serological follow-up of human brucellosis by measuring IgG antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 22:446-55.
5. Foz, A., T. Pellicer, J. Comerma, and J. Ariza. 1985. Specificity of ELISA anti-immunoglobulin G conjugate in the diagnosis of human brucellosis. Eur J Clin Microbiol 4:138-9.
6. Magee, J. T. 1980. An enzyme-labelled immunosorbent assay for *Brucella abortus* antibodies. J Med Microbiol 13:167-72.
7. Pellicer, T., J. Ariza, A. Foz, R. Pallares, and F. Gudiol. 1988. Specific antibodies detected during relapse of human brucellosis. J Infect Dis 157:918-24.

For any question please contact:
technicalservice@vircell.com

REVISED: October-06

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Appendix H

INSTITUTIONAL REVIEW BOARD

NIGERIAN INSTITUTE OF MEDICAL RESEARCH

6, Edmond Crescent Off Murtala Muhammed Way, P.M.B. 2013 Yaba, Lagos.
Tel: 01-4823123, 01-7744723, 08050254484, 08033460947 Fax: 01-4823123, 234-1-3425171
E-mail: nimr_irb@yahoo.com *Website:* www.nimr-nig.org
Secretariat: Room 207, Biochemistry Division, Research Block, NIMR

5th May, 2014

PROJECT TITLE: **SURVEY OF BRUCELLOSIS AMONG SUSCEPTIBLE PROFESSIONALS IN LAGOS NIGERIA**

PROJECT №: **IRB/13/234**

APPROVAL LETTER

The above named proposal has been adequately reviewed; the protocol and safety guidelines satisfy the conditions of NIMR-IRB, policies regarding experiments that use human subjects.

Therefore the study under its reviewed state is hereby approved by Institutional Review Board, NIMR.

PROF. F. E. OKONOFUA
Name of IRB Chairman

.....
Signature of IRB Chairman & Date

MRS. O. A. NWOGBE
Name of IRB Secretary

.....
Signature of IRB Secretary & Date

This approval is given with the investigator's Declaration as stated below;
By signing below I agree/certify that:

1. I have reviewed this protocol submission in its entirety and that I am fully cognizant of, and in agreement with, all submitted statements.
2. I will conduct this research study in strict accordance with all submitted statements except where a change may be necessary to eliminate an apparent immediate hazard to a given research subject.
   - I will notify the IRB promptly of any change in the research procedures necessitated in the interest of the safety of a given research subject.
   - I will request and obtain IRB approval of any proposed modification to the research protocol or informed consent document(s) prior to implementing

such modifications.

3. I will ensure that all co-investigators and other personnel assisting in the conduct of this research study have been provided a copy of the entire current version of the research protocol and are fully informed of the current (a) study procedures (including procedure modifications); (b) informed consent requirements and process; (c) potential risks associated with the study participation and the steps to be taken to prevent or minimize these potential risks; (d) adverse event reporting requirements; (e) data and record-keeping; and (f) the current IRB approval status of the research study.

4. I will respond promptly to all requests for information or materials solicited by the IRB or IRB Office.

5. I will submit the research study in a timely manner for IRB renewal approval.

6. I will not enroll any individual into this research study until such time that I obtain his/her written informed consent, or, if applicable, the written informed consent of his /her authorized representative (i.e., unless the IRB has granted a waiver of the requirement to obtain written informed consent).

7. I will employ and oversee an informed consent process that ensures that potential research subjects understand fully the purpose of the research study, the nature of the research procedures they are being asked to undergo, the potential risks of these research procedures, and their rights as a research study volunteer.

8. I will ensure that research subjects are kept fully informed of any new information that may affect their willingness to continue to participate in the research study.

9. I will maintain adequate, current, and accurate records of research data, outcomes, and adverse events to permit an ongoing assessment of the risks/benefit ratio of research study participation.

10. I am cognizant of, and will comply with, current federal regulations and IRB requirements governing human subject research including adverse event reporting requirements.

11. I will make a reasonable effort to ensure that subjects who have suffered an adverse event associated with research participation receive adequate care to correct or alleviate the consequences of the adverse event to the extent possible.

12. I will ensure that the conduct of this research study adheres to Good Clinical Practice guidelines

MR. AKINROYEJE K. A.
Principal Investigator's Name

Akinroyeje 07/05/2014
Principal Investigator's Signature and Date

Appendix I

ASSENT FORM

Project Title: Survey of Brucellosis Among People at Risk in Lagos Nigeria

Principal Researcher: Akinroyeje Kehinde Adeyemi (MSc Student, University of South Africa)

I am doing a research in order to know the number of people that have brucellosis infection (a disease that is common among people that work in animal farms, abattoirs and those that handle animals) in Lagos Nigeria. If you want to be part of this study, I want you to allow the medical personnel to collect blood samples from your body so as to carry out blood test in order to know if you have the disease. You will also fill a questionnaire as I want to know about things you do that can make you to be infected with the disease pathogen. You can do all these within an hour.

The medical personnel will collect about 5 ml of blood from your body. Hence, you may feel a little discomfort when the syringe needle is used on your body. However, I will make sure that the discomfort will be minimal. Also because of your likely fear of disease transmission to your body, I will ensure that only new syringe needle is used on your body.

This research will be of great benefit to you if you participate. It will help you know if you have brucellosis, which is a disease that makes somebody to have undulating fever and also affects the reproductive system. If you know your status about the disease, you can then go to the hospital where you will be treated.

I promise to keep all the information you fill in the questionnaire as well as the result of your blood test confidential. However, if the government/government agencies want to re-use the data that will be gotten from this research, I will give it to them.

Participation in this research is voluntary, and you can withdraw at anytime. You will not be penalized if you do not want to participate in the study or if you decide to withdraw at anytime. Your parents will know about this study also.

If you decide you want to be in this study, please write your name and append your signature

I ……. want to be part of this research study.

…… ……

Signature Date

If you have any question concerning this study, you may contact Dr. Adeshina Adeiga at Nigerian Institute of Medical Research (NIMR), Yaba, Lagos Nigeria. Tel: 08023026544 or 08097631483; email: aadeiga@yahoo.com. You may also contact Dr. Prudence Kayoka-Kabongo at UNISA on Telephone no +27827750754; email:kabonpnk@unisa.ac.za

If you have any question about your right or if there is any adverse consequence as a result of your participation in this study, please contact the Chairman, Institutional Review Board (IRB), Nigerian Institute of Medical Research, Yaba Lagos Nigeria or Chair of Department, Department of Agriculture and Animal Health, University of South Africa, South Africa.

WITNESSES

1. .....

2. .....

Appendix J

Specific jobs of hospital-based respondents

Professionals	Intermediate	Manual skilled	Unskilled
Teacher	students	hairdresser	Trader
Doctor	Customer care officer	Electrician	transporter
Accountant	Secretary	barber	Pastor
Nutritionist	Entrepreneur	caterer	attendants
Banker	Merchandizer	Mechanic	Business women
Marine dealer	Interior Designer	Artisan	Farmer
Civil servant	Movie producer	Fashion designer	Self employed
Travels & Tour consultants		Welder	Toll collector
Electrical engineer		Bricklayer	
Risk Manager		Photographer	
Nurse			
Credit support manager			
Academics			

Appendix K

Results of ELISA IgG test for sero-positive individuals on RBPT for brucellosis

Name	Sample Optical Density	Sample O.D/1.7485 (Mean O.D)= Antibody Index
1	0.08	0.46
2	0.22	1.26
3	0.751	4.3
4	0.104	0.6
5	0.153	0.88
6	0.048	0.28
7	0.158	0.9
8	0.04	0.23
9	0.392	2.24
10	0.077	0.44
11	0.134	0.77
12	0.088	0.5
13	0.239	1.37
14	0.119	0.68
15	0.122	0.7
16	0.069	0.4
17	0.114	0.65
18	0.132	0.76
19	0.205	1.17
20	0.233	1.33
21	0.139	0.8
22	0.121	0.69
23	1.946	11.13
24	2.486	14.22
25	2.482	14.2
26	0.12	0.69
27	1.611	9.21

NB: ELISA antibody index value of 11 and above means the individual is sero-positive to brucellosis; between 9-11 is equivocal while value less than 9 is negative.