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DEPARTMENT OF CLINICAL MICROBIOLOGY

COMPARISON OF THREE DIAGNOSTIC METHODS IN DETECTING *BRUCELLA* INFECTION AMONG SLAUGHTERHOUSE WORKERS AT THE KUMASI ABATTOIR, GHANA

BY

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JULY 2015

COMPARISON OF THREE DIAGNOSTIC METHODS IN DETECTING *BRUCELLA* INFECTION AMONG SLAUGHTERHOUSE WORKERS AT THE KUMASI ABATTOIR, GHANA

A Thesis presented to the Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University Of Science And Technology, Kumasi, Ghana, in partial fulfillment of the requirement for the award of the degree of

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DECLARATION

I, Esimebia Adjovi Amegashie, author of this thesis, "Comparison of three diagnostic methods in detecting *Brucella* infection among Slaughterhouse Workers at the Kumasi Abattoir, Ghana" do hereby declare that, apart from references to past and current literature duly cited in thesis, the entire research work presented in this thesis was done by me as a student of the Department of Clinical Microbiology, KNUST.

It has neither in whole nor in part been submitted for a degree elsewhere.

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DEDICATION

I dedicate this work to God for the strength he gave me in every step of the way.

To my husband, Bright Kofi Amegashie, who believed in me when I needed it most, his exemplary leadership which I used as a template for my Christian and professional development.

To my mother, Diana Yawa Wormenor, the most important lady and epitome of my life and for her prayers.

And to the memory of my late father, Garnet Kofi Atise, for being my inspiration when he was alive.

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ABSTRACT

Brucellosis remains neglected in many countries despite its public health importance. The globally reported number of 500,000 cases per year is likely an underestimation of the actual figure. In Ghana, there remains paucity of Brucellosis data among high-risk populations such as slaughterhouse workers.

In hospital setting, laboratory diagnostic methodologies targeting brucellosis is not performed across Ghana. As a consequence, there is no randomized method with good specificity and sensitivity to be adopted for routine *Brucella* diagnostic purposes.

The aim of the study was to evaluate and compare diagnostic performance of Rose Bengal Plate test, ELISA and PCR used in diagnosing *Brucella* infection as well as its prevalence and risk factors associated with the infection among slaughterhouse workers.

A cross-sectional study was carried out at the Kumasi Abattoir with 220 participants randomly selected. Participants were interviewed about their knowledge on *Brucella* using a structured questionnaire. Blood samples were collected and serum extracted. The samples were tested for the presence of *anti-Brucella* antibodies using the Enzyme Linked Immunosorbent Assay (ELISA) and Rose Bengal Plate Test (RBPT). Extracted DNAs were amplified using the BCSP31-PCR assay.

From the 220 participants tested for antibodies against *Brucella spp*, 3 (1.4%) were positive in the Rose Bengal Plate test, 4 (1.8%) were positive in the anti-*Brucella* ELISA IgM, 21 (9.6%) were positive in the anti-*Brucella* ELISA IgG. PCR showed positive for 98 (44.5%) participants.

The sensitivity, specificity, positive predictive value, negative predictive value and Kappa value for Rose Bengal in comparison with PCR were 66.7%, 55.8%, 2.0%, 100% and 0.013 respectively while that for ELISA IgG in comparison with PCR were 85.7%,

71.3%, 18.4%, 98.5% and 0.212 respectively. Most of the anti-*Brucella* IgG seropositive (17/21) (OR 2.2; 95% CI 0.6-7.9; p=0.22) and PCR positive individuals (69/148) (OR 1.5; 95% CI 0.8-2.8; p=0.23) were working in the meat-processing unit.

Multivariate analysis showed Odds Ratio but statistically not significant associations for occupation (OR 1.32; 95% CI 0.64-1.15; p=0.34), assisting in birth of livestock (OR 1.29; 95% C I 0.54-3.11; p=0.34) and use of protective clothing (OR 1.54; 95% CI 0.86-2.76; p=0.147). Education (OR 0.87; 95% CI 0.79-2.19; p=0.284) showed a lower OR which was also statistically not significant.

The estimated prevalence among those at risk population was 44.5%. PCR method yielded the highest sensitivity and specificity among the applied methods. This method is especially helpful epidemiologically in high-risk workers who tested negative for serologic testing. ELISA method(Mantur *et al.*, 2006) can however be used in cases where PCR is not available.

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LIST OF ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Asaay
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IgA	Immunoglobulin A
PCR	Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
Fg	Ficogram
RBPT	Rose Bengal Plate Test
WHO	World Health Organization
WOAH	World Organization on Animal Health
LPS	Lipopolysaccharides
S-LPS	Smooth Lipopolysaccharides
IL-2	Interleukin-2
IL-4	Interleuken-4
IL-12	Interleuken-12
TNF-α	Tumour Necrosis Factor-alpha
IFN-γ	Interferon-gamma
Th1	T helper type 1
Th2	T helper type 2
STAT	Standard Tube Agglutination Test
EDTA	Ethylene Diamine Tetraacetic Acid
FPA	Florescent Polarization Assay
µl	Microliter
Rf	Rheumatoid factor
nm	Nanometer
°C	Degree Celcius
µM	Micromolar
UV	Ultra Violet
Tris-HCL	Tris- Hydro Chloric Acid

$MgCl_2$	Magnessium Chloride
Bp	Base pair
IU	International Unit

CHAPTER ONE

1.0 INTRODUCTION

Globally, brucellosis still remains neglected as a public health disease in livestock with serious zoonotic implication to humans. Of the six identified *Brucella* species, the most significant ones for causing zoonotic infection are *Brucella abortus, Brucella melitensis* and *Brucella suis* (Pappas *et al.,* 2006). Transmission of *Brucella* from infected livestock to humans can either be direct through contact with infected material, or indirect through consumption of contaminated animal products (Corbel, 1997). More than 500,000 new cases are reported annually though the World Health Organization suggests that this figure has been underestimated (Corbel, 2006; Pappas *et al.,* 2006).

Whereas in developed countries, massive eradication campaigns in the 1970s and 1980s resulted in the elimination of cattle brucellosis and a substantial decline in its incidence (Corbel, 2006), the same cannot be said for most developing countries. Occupational exposure predisposes shepherds, butchers, laboratory workers, veterinarians and abattoir workers to a high risk of *Brucella* infection through inhalation of contaminated aerosols, contact with conjunctival mucosa of infected animal fluids, and/or entry of the bacteria through skin lesions after contact with infected animals or their products (Cutler *et al.,* 2005).

In humans, all age groups have been documented to be at risk (Aworh *et al.,* 2013; Cutler *et al.,* 2005). A study in Nigeria has also shown males to be at higher risk than females because abattoir workers and butchers are male dominating (Aworh *et al.,* 2013).

In Ghana, very little is known about the burden of brucellosis among human populations. A study found no evidence of human brucellosis when Rose Bengal Test, an antibody screening test was employed in selected risk groups in the Akwapim South district of Ghana (Kubuafor *et*

al., 2000). In Nigeria, the endemicity of brucellosis was confirmed among slaughtered cattle, using the Enzyme Linked Immunosorbent Assay (ELISA) as the test method (Agasthya *et al.*, 2012).

Laboratory diagnosis is achieved either through blood culture, serological testing and Polymerase Chain Reaction (PCR) (Araj, 2010). The blood culture method is successful in only 60% due to high false negative rate, their slow growing nature that can take up to 45 days to grow (Corbel, 2006) and the cultivation requiring biosafety level three facilities that are rarely available in most developing countries (Araj, 2010; Pappas *et al.*, 2005).

Despite false positive or negative results in serological tests and cross reactions with other gram negative bacteria, ELISA is still recommended (Gall and Nielsen, 2004; Kubuafor *et al.*, 2000). ELISA allows for a better interpretation of the clinical situation as it measures immunoglobulin M (IgM) (Aworh *et al.*, 2013) (Pappas *et al.*, 2005); and immunoglobulins G and A (IgG and IgA) (Araj, 2010). The ELISA method has a higher sensitivity as compared to other serological methods such as Rose Bengal and Standard Agglutination Tests (Giambartolomei *et al.*, 2002). ELISA is an excellent diagnostic methodology especially for sero-surveys of brucellosis (Araj, 2010; Smits and Kadri, 2005).

Application of Polymerase Chain Reaction (PCR) is the quickest method for detection of *Brucella* by amplification of bacterial genome in blood sample, bone marrow, mucus or cerebrospinal fluid (CSF) (Al-Nakkas *et al.*, 2005; Navarro *et al.*, 2004, 2004; Nimri, 2003; Queipo-Ortuño *et al.*, 2005; Zerva *et al.*, 2001). PCR and real-time PCR (RT-PCR) assays have been used to directly detect *Brucella* from clinical specimens for the identification, diagnosis and differentiation of *Brucella spp.* (Probert *et al.*, 2004).

While PCR directly detects the DNA of the pathogen, serology is dependent upon the rising and falling titers of antibodies during the different phases of brucellosis (Araj, 2010). However, studies have shown that PCR has a higher sensitivity and specificity than serological tests and is able to detect as little as 30 femtograms (fg) of *Brucella* DNA, therefore a useful tool in confirming *Brucella* infection (Al-Attas *et al.*, 2000; Amin *et al.*, 2001; Guarino *et al.*, 2000; Gupta *et al.*, 2010; KANANI, 2007; PATEL, 2005).

1.1 PROBLEM STATEMENT

Many people live with their livestock. This behavior puts the people at risk of zoonotic diseases. Public health systems such as vaccination programmes against brucellosis are virtually non-existent in Africa (Pappas *et al.*, 2005). The scarce data on brucellosis are derived from small sero-epidemiological studies of patients with fever or high-risk populations (McDermott and Arimi, 2002). A study detected some occupational risk factors associated with the disease (Cutler *et al.*, 2005). However, neither the exact prevalence has yet been determined in Ghana nor have the associated occupational risk factors been assessed.

Studies carried out at the Kumasi Abattoir (Frimpong *et al.*, 2012) focused on animal supply, logistic activities and their challenges, but failed to address issues like prevalence and occupational risk factors associated with handling livestock.

In hospital setting, diagnostic methodologies (Culture, serological testing and PCR) targeting brucellosis are not part of the microbiological tests carried out in laboratories across Ghana. As a consequence, hardly had any of these studies looked at comparing the specificity and sensitivity pattern of the various diagnostic tests.

1.2 JUSTIFICATION

There is a need to obtain epidemiological data to assess the risk involved in the transmission of *Brucella* infection. Few studies have documented the prevalence of human brucellosis in sub-Saharan Africa (McDermott and Arimi, 2002) and especially among abattoir workers (Aworh *et al.*, 2013).

Diagnosis of human brucellosis is hindered by the difficulty in clinically differentiating it from other febrile-like infectious diseases such as malaria that is prevalent in sub-Saharan Africa. Therefore, laboratory testing is crucial for proper diagnosis of human and animal brucellosis (Al Dahouk *et al.*, 2002).

Brucella spp is antigen encoded which enters a host cell and induces an immune response leading to a rise in antibodies production, therefore being easily identified by serological testing while PCR detects the presence of the DNA in a host cell.

Sensitivity and specificity patterns for PCR and serological tests however vary for each test between laboratories and hence the need for standardization (Baddour and Alkhalifa, 2008).

Diagnosing *Brucella* infection with the appropriate tool, knowing its prevalence and associated risk factors will generate the interest of researchers to conduct large epidemiological studies. It may lead to the control of the infection among livestock rearers, butchers, abattoir workers and the general public.

1.3 RESEARCH QUESTION

1. What is the prevalence of *Brucella* infection among slaughterhouse workers using Rose Bengal Plate test, ELISA and PCR?
2. Which of the test methods (Rose Bengal Plate test, ELISA and PCR) has the highest sensitivity and specificity?

3. What are the occupational risk factors associated with *Brucella* infection?

1.4 AIM

The aim of the study was to compare Rose Bengal Plate test, ELISA and PCR used in diagnosing *Brucella* infection as well as its prevalence and risk factors associated with the infection among Slaughterhouse workers.

1.5 SPECIFIC OBJECTIVES

1. To determine the sensitivity, specificity and predictive values patterns of Rose Bengal plate test, ELISA test using PCR as the gold standard.
2. To determine the prevalence of *Brucella* infection among slaughterhouse workers at the Kumasi Abattoir using the best performing test.
3. To determine the occupational risk factors associated with *Brucella* infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY OF BRUCELLOSIS

Brucellosis is a zoonotic infection (Pappas *et al.*, 2006). In 1853, Jeffrey Allen Marston diagnosed the disease among British army troops serving in Malta (Brachman and Abrutyn, 2009). In 1887, David Bruce isolated gram negative coccobacilli, later known as *Brucella melitensis* from spleens of humans infected with brucellosis (Brachman and Abrutyn, 2009; Pappas *et al.*, 2006). Later, in the late 1890s, Bernard Bang isolated *Brucella abortus* from the placenta of cattle while investigating contagious abortion among cattle in Denmark (Pappas *et al.*, 2006). Thus the disease became known as Bang's disease. The disease was named 'undulant fever' as suggested by M.L Hughes after reviewing the clinical and pathological features of the disease in 1897 (Mantur and Amarnath, 2008; Pappas *et al.*, 2006).

Wright and Smith in 1897 detected antibodies to *Brucella melitensis* in human and animal sera by an agglutination test. This unraveled the zoonotic potential of the disease (Pappas *et al.*, 2006). Later, Zammit a young Maltese physician working with Mediterranean Fever Commission in 1905 confirmed it by isolating the organism from the milk and urine of goats. Thus he concluded that goats were the reservoir of *Brucella melitensis* and the consumption of the raw milk and cheese infects man (Brachman and Abrutyn, 2009; Pappas *et al.*, 2006).

Brucellosis has also many synonyms derived from the geographical regions in which the disease occurs e.g., Gibraltar fever, Cyprus fever and typhomalarial fever in 1810, mediterranean fever in 1861 and Malta fever in 1887 (Mantur *et al.*, 2007).

In 1914, the third member of the organism named *Brucella suis* was isolated from an aborted

swine fetus by Jacob Traum in the United States of America (Mantur *et al.*, 2007; Pappas *et al.*, 2006). In 1918, Alice Evans published data on the antigenic differences between *Brucella melitensis* and *Brucella abortus* shown by agglutination absorption test. She also confirmed with convincing evidence that *Brucella melitensis* and *Brucella abortus* could not be differentiated morphologically or by their cultural and biochemical reactions (Brachman and Abrutyn, 2009). Meyer and Shaw further confirmed Evan's observations and suggested the generic name *Brucella* in honour of Sir David Bruce (Brachman and Abrutyn, 2009).

In 1953, van Drimmelen isolated *Brucella ovis* in sheep. In 1957, Stoenner and Lackman identified *Brucella neotomae* in rodents (Pappas *et al.*, 2006). In 1964, Carmichael and Bruner identified *Brucella canis* in the canines (Brachman and Abrutyn, 2009; Lucero *et al.*, 2005; Pappas *et al.*, 2006). Two new *Brucella* species, provisionally called *Brucella pinnipediae* and *Brucella cetaceae*, have been isolated from marine hosts within the past few years (Pappas *et al.*, 2006).

In 2004, World Organization for Animal Health (WOAH) joint consultation on emerging zoonotic diseases held in Geneva defined an emerging zoonosis as a pathogen that is newly recognized or newly evolved or that has occurred previously but shows an increase in incidence or expansion in geographical, host or vector range (Coker *et al.*, 2011).

In November 2006, a panel of international experts met in Ioannina in Greece and made a number of therapeutic recommendations that included treatment of uncomplicated brucellosis using a combination of oral doxycycline for 6 weeks and parenteral streptomycin for 2 to 3 weeks or oral rifampicin for 6 weeks (Ariza *et al.*, 2007).

2.2 ETIOLOGY

Brucellosis is caused by a Gram negative bacteria of the genus *Brucella*, which are facultative intracellular coccobacilli that belong to the order Rhizobiales of the α2- Proteobacteriacea family (Garrity *et al.*, 2004). In spite of more than 94% similarity amongst the members of the genus (DelVecchio *et al.*, 2002) bacteria of the genus *Brucella* have different host preferences. They are therefore capable of causing disease in a variety of animal species, including humans.

The genus *Brucella* has been classified into six species according to differences in pathogenicity and host preference (Osterman and Moriyon, 2006) that is *Brucella melitensis, Brucella abortus, Brucella suis, Brucella ovis, Brucella canis and Brucella neotomae*. *Brucella melitensis, Brucella suis, Brucella abortus* and occasionally *Brucella canis* are considered the most pathogenic species for humans and have sheep and goat, pigs, cattle and dogs as preferential hosts, respectively (Godfroid *et al.*, 2005). *Brucella canis*, a pathogen of dogs, has a low zoonotic potential, while *Bruella neotomae* and *Brucella ovis* that infect desert rats and sheep respectively, are not associated with human disease (Godfroid *et al.*, 2005). Two new *Brucella* species, provisionally called *Brucella pinnipediae* and *Brucella cetaceae*, have been isolated from marine hosts within the past few years (Foster *et al.*, 2007; Pappas *et al.*, 2005).

Table 1 presents the zoonotic potential and host preference of the *Brucella* species.

Table 1: Zoonotic Potential and Host Preference of *Brucella* Species

Species	Level of Zoonotic Potential	Host Preference
Brucella melitensis	High	Sheep, goat
Brucella abortus	Moderate	Cattle
Brucella suis	Moderate	Pig
Brucella canis	Mild	Dog
Brucella ovis	Absent	Sheep
Brucella ceti	Mild	Cetaceans
Brucella pinnipedialis	Mild	Seals
Brucella microti	Absent	Common voles

2.3 EPIDEMIOLOGY

2.3.1 Prevalence of *Brucellosis*

Despite tremendous results achieved by developed countries in eradicating and controlling brucellosis, it still remains a problem in some of these countries and as well as in most developing countries (McDermott and Arimi, 2002). The United Kingdom and the United State

of America (USA) were considered to be free of brucellosis but were still reported in France, Italy and Ireland (Godfroid *et al.*, 2002).

Data on the sero-prevalence of human brucellosis in developing countries is very limited. The prevalence of brucellosis has been reported in the Middle East (Abdollahi *et al.*, 2010), Mediterranean region (Minas *et al.*, 2007), Northern and Sub-Saharan countries in Africa (Dean *et al.*, 2012; Pappas *et al.*, 2006; Schelling *et al.*, 2003) of which 5-55% and 8-46% were present in humans and animals respectively (Nabukenya *et al.*, 2013).

In the Mediterranean region, a prevalence of 32.49% was recorded among 100,000 inhabitants in Central Greece (Minas *et al.*, 2007). Other findings reported 4.1% in Brazil (Ramos *et al.*, 2008), among high-risk group using the Complement Fixation Test (CFT) with a sample size of 645, and 18% in the northern region of Turkey (Arvas *et al.*, 2013) among hospital patients using the Standard Tube Agglutination Test (STAT).

In the Middle East, using the Standard Tube Agglutination Test (STAT), a prevalence of 15% was reported in Saudi Arabia (Al-Sekait, 1999) among nomadic households with a sample size of 23,613. In Iran, prevalence of 0.5%-10.9% has been reported in different provinces (Sofian *et al.*, 2008).

Also, in Africa, brucellosis is highly prevalent in Uganda with 7 – 42.2% (Bernard *et al.*, 2005; Nabukenya *et al.*, 2013) in cattle and goats, posing a big threat to abattoir workers and consumers. Previous studies in humans reported sero-prevalence of 10%-13.3% in Uganda (Mutanda, 1998; Nabukenya *et al.*, 2013). A study in Uganda estimated a prevalence of 6–7% among herdsmen and consumers of raw milk and products (Nabukenya *et al.*, 2013). In Kampala, Uganda, of 150 patients with joint pain, general malaise, and/or constant headache,

73% were found to be suffering from malaria and 13.3% from brucellosis showing a scenario often leading to misdiagnosis (Makita *et al.*, 2011). In Egypt, incidence ranges from 0.28 to 70 per 100,000 population (Dean *et al.*, 2012; Jennings *et al.*, 2007) and 11% prevalence among hospital patients (Jennings *et al.*, 2007).

In Sub-Saharan Africa, a prevalence of 5.3% was reported in Nigeria among predisposed patients with pyrexia of unknown origin (Baba *et al.*, 2001), 9.8% in a study in Nigeria among abattoir workers with a sample size of 224 (Aworh *et al.*, 2013). A study carried out in a slaughterhouse located in the Lahore district in Pakistan reported a higher prevalence of 21.7% (Mukhtar and Kokab, 2008). Among hospital patients in Markudi, Nigeria, overall brucellosis prevalence was 7.6%, and 43.8% of these were abattoir workers and butchers (Mohammed *et al.*, 2011).

In Ghana, no evidence of human *Brucella* infection was found in selected risk groups in the Akwapim South district (Kubuafor *et al.*, 2000).

2.3.2 Transmission of Brucellosis

The transmission of *Brucella* infection depends upon numerous factors like food habits, methods of processing milk and milk products, social customs, farming practices, climatic conditions, socio-economic status and environmental hygiene (Corbel, 2006). Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurized animal milk and milk products, through direct contact with infected animal parts (such as the placenta, fetus, fetal fluids and vaginal discharges from infected animals), through ruptures of skin and mucous membranes and through the inhalation of infected aerosolized particles (Pappas *et al.*, 2005).

Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories (Corbel, 2006; Swai and

Schoonman, 2009). Consumption of unpasteurized dairy products, especially raw milk, soft cheese, butter, and ice cream are the most common means of transmission (Corbel, 2006; Cutler *et al.*, 2005).

In addition, laboratory-acquired *Brucella* infection due to accidental ingestion, inhalation and mucosal or skin contact is a major health hazard for the laboratory workers handling the cultures of the virulent or attenuated *Brucella* strains (Yagupsky and Baron, 2005). The disease has been recognized as one of the common laboratory- transmitted infections and has been reported to occur in clinical, research, and production laboratories (Kimman *et al.*, 2008; Yagupsky and Baron, 2005).

2.3.3 Risk Factors

Risk factors for human brucellosis include the handling of infected animals, handling aborted material without protective gear, ingestion of contaminated animal products such as unpasteurized milk and milk products (including cow, goat, and camel milk), meat, close contact and co-habitation of livestock with humans, history of travel to endemic areas and handling of cultures of *Brucella* spp. in laboratories (Al-Tawfiq and AbuKhamsin, 2009; Donev *et al.*, 2010). Other risks include contact with infected animals' secretions, and contact with infected secretions from animals into the conjunctiva (Young, 2006). This has become an occupational disease for veterinarians, slaughterhouse workers, dairy workers and laboratory workers (Godfroid *et al.*, 2005; Swai and Schoonman, 2009).

2.3.3.1 Handling of Infected Animals

Slaughterhouse workers, butchers, veterinarians, cattle farmers are directly in close contact with animals due to the nature of their jobs, therefore they easily come into contact with animals

infected with *Brucella* spp (Cutler *et al.*, 2005). In India, assessment of 165 serum samples of abattoir-associated personnel with dot-ELISA found 25.5% having been infected with *Brucella* (Mantur and Amarnath, 2008). Slaughterhouse workers are more prone to infection as compared to other occupations because they are exposed to carcasses and viscera of infected animals (Swai and Schoonman, 2009). They also get infected through cuts and wounds and splashing of infected blood and other fluid in the conjunctiva (Young, 2006) which was also confirmed by Young (Ramos *et al.*, 2008).

2.3.3.2 Handling of aborted materials and placenta

Aborted materials from livestock are among the common features of *Brucella* infection in livestock (Schelling *et al.*, 2003). During abortion, large numbers of *Brucella*e are released which may, in turn, cause infection to other animals in the herd (Kreeger *et al.*, 2002). This leads to the spread of infection among livestock farmers and handlers. The spread of infection normally comes about when these cattle farmers do not wear protective gears while assisting in the birth of animals or disposing off aborted materials. Results from other studies demonstrated an increased risk in association with assisted parturition and abortion (Bikas *et al.*, 2003; Schelling *et al.*, 2003; Young, 2006). In a study conducted in Greece, human trauma during animal delivery was found to increase the risk for contracting brucellosis (Bikas *et al.*, 2003). A study conducted in Saudi Arabia (Cooper, 1992) and Chad (Schelling *et al.*, 2003) showed that contact while assisting livestock during parturition and their placenta, respectively, was associated with *Brucella* transmission. Another finding from Tanzania reported that *Brucella* infection in humans was strongly associated with handling aborted foetuses and placenta of infected animals (Swai and Schoonman, 2009) which was also confirmed by Aworh and Co

(Aworh *et al.*, 2013). Study by Kozukeev and others however did not show handling aborted foetus as a risk factor for *Brucella* transmission (Kozukeev *et al.*, 2006).

2.3.3.3 Ingestion of contaminated animal products

Recent exposure to *Brucella spp* is normally seen in consumption of raw or inadequately pasteurized milk and meat or offal derived from infected livestock has been shown to be a major risk factor in the transmission of the infection (Corbel, 2006). Because of the variable manifestations of brucellosis, viable *Brucellae* can be seen in the milk and tissues of animals thus its transmission to humans (Yagupsky and Baron, 2005). Findings from most developing countries have confirmed this trend of infection from contaminated animal products (Aworh *et al.*, 2013; Cetinkaya *et al.*, 2005; Sümer *et al.*, 2003).

2.3.3.3.1 Consumption of unpasteurized milk and milk products

Consumption of fresh cheese and milk cream produced from unpasteurized milk has been reported to be a significant risk factor for *Brucella* infection in Jordan (Shaqra, 2000) and in Turkey (Cetinkaya *et al.*, 2005; Sümer *et al.*, 2003). Centikaya and others reported that 30 % of subjects consumed unpasteurized milk of which 9.5% were sero-positives while 70% consumed pasteurized milk of which 2.9% were sero-positives (Cetinkaya *et al.*, 2005). In Nigeria, Aworh and others reported that 39% of the 224 abattoir workers who drank unpasteurized milk were infected with *Brucella,* which was a highly significant risk factor.

2.3.3.3.2 Consumption of raw meat

The habit of eating raw meat, e.g. raw liver or other offal with spices was found to be an important epidemiological factor in contracting the disease (Pappas *et al.*, 2006). A study in

Nigeria and Tanzania reported that, abattoir workers who ate raw meat were infected with *Brucella* (Aworh *et al.,* 2013; Mfinanga *et al.,* 2003).

2.3.3.4 Close contact and co-habitation of livestock with humans

The nearness of livestock to human habitations is seen in the agricultural methods practiced in most African countries (Omer *et al.*, 2000; Swai and Schoonman, 2009). This has lead to the transmission of diseases from animals to humans. A study carried out by Omer and others identified the main risk factor among dairy farm workers and pastoralist to be in close contact with animal (Omer *et al.,* 2000).

2.3.3.5 Duration of Occupational Exposure

Length of service time for high-risk occupation has been found to be a risk factor in the transmission of *Brucella* infection. A study in Pakistan reported a rise in seropositivity to *Brucella* and was associated with the duration of occupational exposure with the exception of those with less than 1 year's job duration (Mukhtar and Kokab, 2008). A study in Nigeria also reported that Individuals who had worked in the abattoir for more than 5 years were more likely to be seropositive to human brucellosis (Aworh *et al.,* 2013).

2.3.3.6 Contact with infected animal's secretions

Human *Brucella* infection can be transmitted by inoculation through cuts and abrasions in the skin (Pappas *et al.,* 2006). A study carried out in Tanzania reported that slaughtering animals especially when the butcher has an injury was associated with being infected with *Brucella* among abattoir workers (Kunda *et al.,* 2007). Other studies have reported similar associations of persons with bruised skin or cuts and infection with *Brucella* (Aworh *et al.,* 2013; Young, 2006).

2.3.3.7 Handling of cultures of *Brucella spp* in laboratories

Routine bacteriologic procedures such as preparing, centrifuging, and vortexing of bacterial suspensions, performing subcultures and biochemical testing can create dangerous aerosols and the potential for accidental spillage (Noviello *et al.*, 2004). This may lead to the transfer of *Brucella* infection to laboratory scientists when proper lab practices are not implemented in their daily lab routine. Also *Brucella* is a highly pathogenic organism that must be handled in a Biosafety Level Three facilities which is not common in most African countries (Araj, 2010). Most laboratory-acquired brucellosis had been caused by the more virulent *Brucella melitensis* species (Memish and Mah, 2001; Noviello *et al.*, 2004; Sophie *et al.*, 2004; Yagupsky and Baron, 2005). Transmission of *Brucella abortus* (Noviello *et al.*, 2004) and the attenuated *Brucella abortus* 19 and *Brucella melitensis* Rev-1 vaccine strains has also been reported (Noviello *et al.*, 2004).

2.4 MICROBIOLOGY OF BRUCELLA SPP

2.4.1 Morphology of *Brucella spp*

Brucellae are gram-negative bacteria, small, non-motile, non-encapsulated coccobacilli. They function as facultative intracellular parasites. The bacterium is 0.5-0.7 in diameter and 0.6-1.5μm in length. They occur singly or in groups. They are also urease, catalase and oxidase positive (Young, 2006).

2.4.2 Culturing Characteristics

Brucella spp are aerobic with *Brucella abortus* requiring a carbon dioxide (5-10%) enriched atmosphere in order to grow well (Muhammad, 2009; Young, 2006). All strains grow over a

temperature range 20-40°C with best at 37°C in a medium enriched with animal serum and glucose. *Brucella spp* are mostly isolated from blood, pus and bone marrow of humans. Tryptone soya (tryptic soy) biphasic medium (Castaneda) is recommended for isolation *Brucella* species (Muhammad, 2009). Species are differentiated by production of urease, Hydrogen peroxide, dye sensitivity and cell wall antigens (Corbel, 2006). Multiplication is slow therefore needs enriched medium to support adequate growth. *Brucella* colonies are visible on suitable solid medium in 2-3 days but must be incubated for about 45 days to rule out positivity (Corbel, 2006). However, isolation rates of only 20-50% are reported even from experienced laboratory. The colonies of smooth strains are small, round, convex but some species like *Brucella canis* and *Brucella ovis* lack O chains of the LPS, giving a rough or mucoid variant form (Corbel, 2006).

2.4.3 Cell Envelope and Lipopolysaccharides (LPS) of *Brucella Spp*

The *Brucella spp* have cell envelopes composed of inner and outer membranes enclosing a periplasm with a peptidoglycan mesh and soluble components. The outer membrane contains the lipopolysacharide (LPS) that is the *Brucella* major virulence factor (Oliveira *et al.*, 2012). The LPS phenotype of *Brucella* species is either smooth or rough depending on the presence or absence of the surface exposed O-polysaccharides (O-PS) chain respectively. The O-PS plays a major role in virulence associated with smooth LPS (S-LPS) in that mutant smooth strains fail to survive in macrophages (Franco *et al.*, 2007; Xavier *et al.*, 2009). The LPS is smooth in *Brucella melitensis, Brucella abortus* and *Brucella suis* and rough in *Brucella canis* and *Brucella ovis* (Neta *et al.*, 2010). *Brucella spp* have a unique ability to inhibit phagosome maturation using the S-LPS to inhibit the phagosome–lysosome fusion although the exact mechanism of how the inhibition is achieved is not well understood (Neta *et al.*, 2010; Porte *et al.*, 2003). Formation of

the phagolysosme is paramount in the killing of engulfed bacteria. In addition, S-LPS establishes resistance to nitric oxide, free radicals and lysozyme, which are important antimicrobial mechanisms of macrophages and neutrophils (Fernandez-Prada *et al.*, 2003). Also, smooth LPS inhibit the synthesis of immune mediators and interfere in host release of inflammatory cytokines. This is due to its failure to be detected by pathogen recognition receptors of the innate immune system because of its low endotoxic properties (Lapaque *et al.*, 2006). Through this mechanism, it prevents stimulation of the innate immune system and therefore cannot facilitate the killing of the pathogens. The LPS alters the capacity of infected cell to present foreign antigens to CD4+ T cells, hence preventing attack and killing of infected cell by the immune system (Lecaroz *et al.*, 2006). In the same manner, smooth LPS is involved in the inhibition of apoptosis. Resistance to apoptosis of infected cells has been seen in patients with acute and chronic disease (Xavier *et al.*, 2010). Further more, *Brucella spp* do not activate the alternative complement system and have relatively low endotoxicity. This makes them poor inducers of some inflammatory cytokines such as tumor necrosis factor (TNF) and interferons (Xavier *et al.*, 2009). Interferon gamma activates macrophages to enhance killing of internalized bacteria (Porte *et al.*, 2003). The intracellular lifestyle makes the bacteria evade the immune system (antibodies) in the extracellular milieu.

2.4.4 Pathogenicity of Brucellosis

Brucella species are facultative intracellular bacteria that can invade and survive in both non-phagocytic and phagocytic cells with human beings as end hosts (Celli, 2006; Pizarro-Cerdá *et al.*, 1998). *Brucella* enters the host via ingestion or inhalation, or through conjunctiva or skin abrasions (Cutler *et al.*, 2005). After infecting the host, the pathogen becomes sequestered within

cells of the reticulo-endothelial system (Celli *et al.*, 2005). The mechanisms by which *Brucella* enters cells and evades intracellular killing and the host immune system are incompletely understood (Gorvel and Moreno, 2002). In depth study of *Brucella* spp genomes has failed to identify any of the classic virulence factors such as toxins, fimbriae, and capsules (Gorvel and Moreno, 2002). This finding raises the possibility that *Brucella spp* use unique and subtle mechanisms to evade host defences, penetrate host cells, alter intracellular trafficking to avoid degradation and killing in lysosomes, and modulate the intracellular environment to allow long-term intracellular survival and replication (Delrue *et al.*, 2004; Young, 2006). The smooth lipopolysaccharides (S-LPS) that cover the bacterium and proteins involved in signalling, gene regulation, and transmembrane transportation are among the factors suspected to be involved in the virulence of *Brucella* (Lapaque *et al.*, 2005).

An important aspect of *Brucella* infection is its ability to persist and replicate within phagocytic cells of the reticuloendothelial system as well as in non-phagocytic cells such as trophoblasts (Gorvel and Moreno, 2002; Pizarro-Cerdá *et al.*, 1998). This ability involves a temporary fusion of the *Brucella*-containing vacuole with the lysosome, and subsequent exclusion of the lysosomal proteins (Starr *et al.*, 2008). Following this process, the *Brucella*-containing vacuole becomes associated with the endoplasmic reticulum (Pizarro-Cerdá *et al.*, 1998). These endoplasmic reticulum-associated compartments are the niche for intracellular replication of *Brucella* in macrophages, epithelial cell lines and placental trophoblasts (Celli *et al.*, 2003). Once inside this compartment, the bacteria can establish chronic infection.

The host response against *Brucella* spp. involves the whole immune system, from innate to adaptive immunity (Golding *et al.*, 2001). Cytokines including IL-1, IL-12, IFN-gamma and TNF-alpha have shown to have an important role in the pathogenesis of brucellosis. The

Th1/Th2 balance is postulated to be involved in the susceptibility or resistance to the disease (Galanakis *et al.*, 2002; Pasquali *et al.*, 2001). Th1 cells are mediators of the effector mechanisms required for resistance to intracellular pathogens, while a Th2 cell response is detrimental in combating this type of infection (Yingst and Hoover, 2003). A Th1 response is essential for resolution of the primary infection caused by *Brucella* and the essential aspect of this response appears to be IFN-ã production (Yingst and Hoover, 2003). On the other hand, Th2 cytokine such as IL-4 evoke strong antibody responses (Romagnani, 2000). It has been long postulated that the outcome of the disease reflects the equilibrium developed between the bacterium and the human immune response.

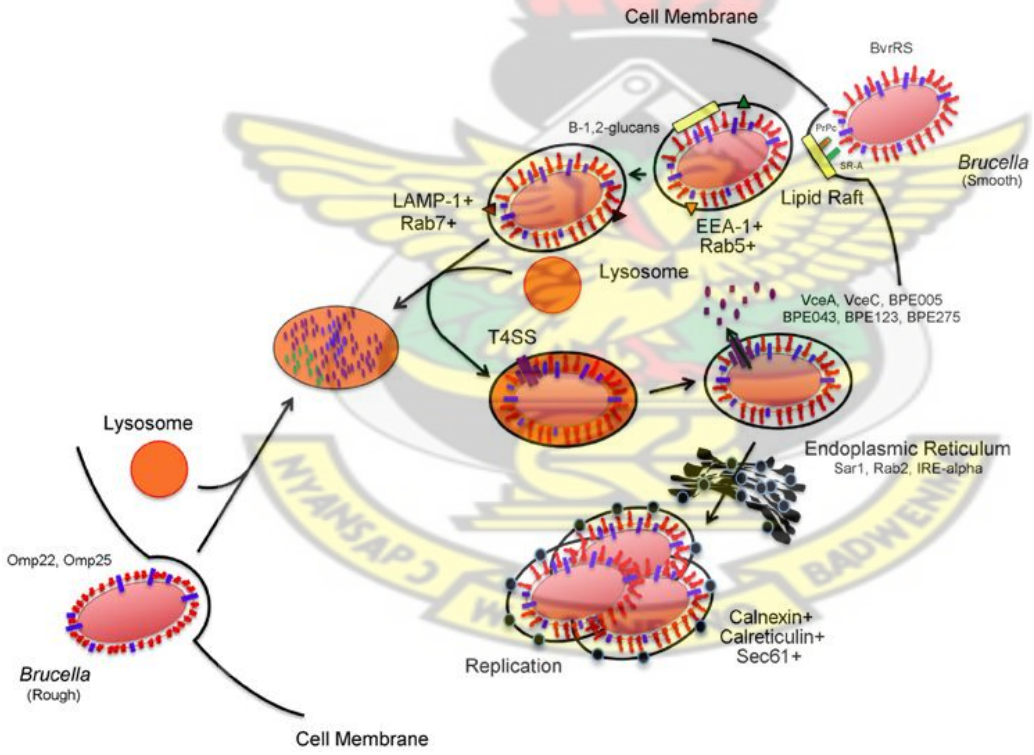

Figure 1: Host Response to Brucella infection in Humans

Brucella spp are internalized into a macrophage. Once internalized, *Brucella spp* are trafficked through a vesicle sharing markers with an early endosome. Later the *Brucella spp* are found in a compartment resembling a late endosome where a majority of the bacteria are killed. The

surviving bacteria will replicate in a vesicle sharing markers with the endoplasmic reticulum (Gomez *et al.*, 2013).

2.5 HOST-PATHOGEN INTERACTIONS AND DISEASE MANIFESTATION

Human brucellosis is considered one of the most important zoonotic diseases worldwide (Pappas *et al.*, 2006). Although *Brucella abortus*, *Brucella suis* and *Brucella canis* are potential agents of this disease, *Brucella melitensis* is considered the most virulent *Brucella* for humans (Corbel, 2006). Few organisms (10 to 100) are sufficient to cause a debilitating chronic infection (Franco *et al.*, 2007). In most cases, human infections occur through ingestion of contaminated milk and unpasteurized dairy products (Cutler *et al.*, 2005; Fugier *et al.*, 2009). However, occupational exposure of mucosa or skin abrasions to fluids and tissues from aborted fetuses of infected animals or carcass is also an important source of infection (Fugier *et al.*, 2009). The efficient transmission of *Brucella* via inhalation of contaminated dust or aerosols makes brucellosis one of the most common laboratory-acquired infections worldwide (Cutler *et al.*, 2005).

Brucella infection induces both humoral and cellular immunity (Young, 2006). Although humoral antibodies appear to play some role in resistance to *Brucella*, cell-mediated immunity appears to be the principal mechanism of recovery (Doganay and Aygen, 2003; Young, 2006). The serum antibody response to *Brucella* infection in humans is seen by an initial rise in antibody titers of the IgM class, followed in several weeks by a predominance of IgG antibodies (Young, 2006). Both IgM and IgG peak during the fourth week. After treatment, titers gradually come down, with a faster decrease of IgG antibodies than of IgM antibodies. In some cases, low titers of IgM antibodies may persist for months or years in the absence of an active infection (Xavier *et al.*, 2010; Young, 2006). The appearance of IgA with IgG for more than six months suggests the presence of chronic infection (Pappas *et al.*, 2005).

Human brucellosis is a life-threatening disease that may have variable clinical presentations (Colmenero *et al.*, 2002). After exposure to the bacteria, clinical manifestations may appear within 5 to 60 days (Young, 2006). Most infected patients present with acute disease consisting of general symptoms, such as fever, malaise, sweats and lymphadenopathy and/or hepatosplenomegaly (Mantur *et al.*, 2006).

However, a subset of patients may develop chronic brucellosis, a more severe form of the disease that can be associated with osteo-articular signs including spondylitis, arthritis and osteomyelitis, or genitourinary changes, such as orchitis, epididymitis, glomerulonephritis and kidney abscesses (Colmenero *et al.*, 2002). Life-threatening complications comprise, in descending order of frequency, neurobrucellosis, liver abscesses and endocarditis (Franco *et al.*, 2007).

2.6 CLINICAL PRESENTATION

2.6.1 Acute Brucellosis

Brucellosis is acute in about half the cases, with an incubation period of two to three weeks (Mantur *et al.*, 2006). In the other half, the onset is insidious, developing over a period of weeks to months. The lysis of the phagocytic cells releases the *Brucella* organisms and also results in the release of cellular debris and pyrogenic endotoxins (Geyik *et al.*, 2002). This is what causes an episode of fever (Geyik *et al.*, 2002). The processes of cell lysis and release of *Brucella* organisms and endotoxins occur repeatedly at different infected phagocytic cells leading to the undulant fever seen in human infections. Commonly patients feel better in the morning, with symptoms worsening as the day progresses (Geyik *et al.*, 2002).

The bacteraemia leads to bacterial colonization in numerous sites of the body. Therefore, the

acute disease symptoms are vague presenting with many signs and symptoms such as: weakness, undulant fever (pyrexia of unknown origin), headaches, pain involving muscles and joints (60% of cases – pain in the lumbar region of the spine), hot flushes, testicular pain in men, fine red rash (up to 5% of cases), enlarged liver and spleen (approximately 50–60% of cases), weight loss (Franco *et al.*, 2007; Mantur *et al.*, 2007).

Although symptoms and signs often occur in various combinations, one study reported fever as the only sign in 44% of patients with a positive blood culture for *B. melitensis* and fever with arthritis in another 42% (Memish *et al.*, 2000).

Symptoms on the part of the gastrointestinal tract are: stomach ache, diarrhea, nausea, vomiting, constipation, lack of appetite (Corbel, 2006). In addition, pregnant women can abort especially during the first trimester (Corbel, 2006). Rarely does breastfeeding result in transmission of disease to the breastfed infant (Çelebi *et al.*, 2007; Tikare *et al.*, 2008)

Acute brucellosis can progress to a more persistent disease with localized infections affecting one or more of the body organs or also lead to a nonspecific syndrome referred to as 'chronic fatigue syndrome (Ficht, 2003; Young, 2006). The acute phase can be cured when the right treatment is given and the patient follows treatment as indicated. It may also relapse into a sub-acute or chronic form when there is a treatment failure (Mantur *et al.*, 2006).

2.6.2 Sub-acute Brucellosis

All the majority of the symptoms typical of the acute course occurs here but more weakly expressed (Corbel, 2006). This normally occurs in patients who have relapsed because of incomplete or inappropriate antibiotic treatment. It may be an important cause of fever of unknown origin (Aygen *et al.*, 2002)

2.6.3 Chronic Brucellosis

The disease is termed chronic if an infected person has harbored it for more than one year from the time of diagnosis and treatment (Ergönül *et al.*, 2005). A majority of patients having chronic brucellosis have persistent disease caused by: inadequate treatment of the initial stage or focal disease in bone, liver, or spleen (Young, 2006).

About 20% of patients diagnosed as having chronic brucellosis complain of persistent fatigue, malaise, and depression which resemble chronic fatigue syndrome (Araj, 2010). These symptoms frequently are not associated with clinical, microbiologic, or serologic evidence of active infection (Young, 2006).

Chronic brucellosis may be both sero-positive and sero-negative. This can be detected by Burnet's reaction, PCR, and the isolation of *Brucella* rods from human autopsy material in which there occur: damage to the osteoarticular system of a degenerative character, enlargement or damage to the liver, non-specific neurological symptoms (Araj, 2010).

2.6.4 Sub-clinical or asymptomatic Brucellosis

Asymptomatic brucellosis often occurs in high-risk groups, including slaughterhouse workers, farmers, and veterinarians and is only detected by serological methods (Doganay and Aygen, 2003). More than 50% of abattoir workers and up to 33% of veterinarians have high anti-*Brucella* antibody titers but no history of recognized clinical infection (Doganay and Aygen, 2003). Also, children in endemic areas frequently have subclinical illness (Doganay and Aygen, 2003).

2.6.5 Localized Disease and Complications

Brucella organisms may localize in almost any organ but most commonly in bone, joints, central nervous system (CNS), heart, lung, spleen, testes, liver, gallbladder, kidney, prostate, and skin (Khorvash *et al.*, 2007; Mantur *et al.*, 2007). Localized disease may occur simultaneously at multiple sites (Aygen *et al.*, 2002). Localized complications often appear in a more chronic course of illness, although complications may occur with acute disease caused by *Brucella melitensis* or *Brucella suis.* (Aygen *et al.*, 2002; Doganay and Aygen, 2003).

2.6.6 Relapsing Infection

Up to 10% of patients with brucellosis experience relapse after antimicrobial therapy (Doganay and Aygen, 2003). Relapses occur usually 3 to 6 months after completion of therapy but may be seen up to 2 years after treatment (Doganay and Aygen, 2003). Relapses are associated frequently with antimicrobial resistance as well as the intracellular location of the organisms, which protects the bacteria from certain antibiotics and host defense mechanisms (Sauret and Vilissova, 2002). Relapsing infection is difficult to distinguish from re-infection in high-risk groups with continued exposure. Studies have shown that relapses are associated with inappropriate or insufficient antimicrobial therapy, positive blood cultures on initial presentation, and an acute onset of disease (Franco *et al.*, 2007; Nimri, 2003).

2.7 LABORATORY DIAGNOSIS OF HUMAN BRUCELLOSIS

New diagnostic tools have been developed that are capable of detecting infection caused by classic *Brucella* species and new strains (Franco *et al.*, 2007). Presently, the laboratory diagnosis of human brucellosis is based on the isolation of the bacteria from clinical samples such as

blood, bone marrow, cerebrospinal fluid and lymph nodes (Mantur *et al.*, 2006). This is followed by standard microbiological testing for isolation of live organism, detection of anti- *Brucella* antibodies using various serological tests, and the use of molecular methods for the detection of *Brucella* DNA (Araj, 2010; Mantur *et al.*, 2006).

Brucellosis mimics other infectious and non-infectious diseases, resulting in either delay in diagnosis of the disease or its misidentification (Mantur *et al.*, 2007). Diagnosis of the disease is greatly dependent on a patient's medical and epidemiological history, clinical signs, hematological and biochemical testing, radiological examination and most importantly on *Brucella*-specific laboratory tests (Mantur *et al.*, 2006; Seleem *et al.*, 2010). For correct and fast diagnosis of the disease, the disease-specific laboratory tests and knowledge of their weaknesses, proper analysis and correct evaluation of their results are needed (Al Dahouk and Nöckler, 2011).

2.7.1 Culture

Brucella isolation can be done in clinical samples, such as from the bone marrow, spleen, synovial fluid, cerebrospinal fluid and abscesses (Mantur *et al.*, 2006). However, the most common biological material used in the isolation of *Brucella* is whole blood (Araj, 2010; Mantur *et al.*, 2006). Culture is the gold standard for proving the disease, but is very difficult isolating the organism due to its slow growing nature, long incubation time and the production of infectious aerosols (Al Dahouk *et al.*, 2002). Due to these disadvantages, few laboratories diagnose *Brucella* infection making use of the culture method. Also, studies has shown that positive cultures occur in 10-70% of suspected cases, depending on the duration, localization of the infection, bacteraemia level, the isolation method, the type of *Brucella* species being isolated

and the disease phase (Al-Attas *et al.*, 2000; Mantur and Mangalgi, 2004). For acute infection, the proportion of positivity is between 40-90% and that of chronic is 5-20% (Corbel, 2006).

The conventional biphasic Ruiz-Castaneda culture method make use of both solid and liquid selective medium in the same bottle for isolating *Brucella spp* from suspected blood samples to minimize subculture (Araj, 2010; Mantur and Mangalgi, 2004). Often cultures become positive between one to three weeks of incubation but are kept for about six weeks to rule out positivity (Araj, 2010). For samples like milk, abscess and cerebrospinal fluid where *Brucella* organisms are likely to be lower, an enrichment liquid medium known as trypticase-soy broth (TSA) is used (Espinosa *et al.*, 2009). This should be incubated at 37°C in air supplemented with 5-10% CO_2 for up to 6 weeks with weekly subculture onto solid selective medium (Espinosa *et al.*, 2009; Moreno and Moriyón, 2006). Automated blood culture systems like Bactec (BD Diagnostics, Sparks, MD, USA) and BacTAlert (bioMerieux, Durham, NC, USA) is known to give higher yields than the conventional culture method and speed up the detection of bacterial growth (Araj, 2010). Growth is mostly recovered within 1 week of incubation and there is no need to incubate bottles longer than 10–14 days (Araj, 2010). The lysis centrifugation method, another automated culture system, has also replaced the conventional method (Cockerill *et al.*, 2004; Mantur and Mangalgi, 2004). The Lysis Centrifugation technique lyses the erythrocytes in a citrate solution, then isolate the *Brucella* bacilli by centrifuging the sample. This concentrates the bacilli and facilitates growth after subsequent plating (Sophie *et al.*, 2004). The Lysis Centrifugation method has been confirmed to have increased sensitivity and reduced culture time as compared to the conventional method (Durmaz *et al.*, 2003). *Brucella*-culturing methods must be carried out under a biosafety level 3 conditions because it is a microorganism with high virulence (Araj, 2010).

2.7.2 Serological diagnosis of brucellosis

Because of the difficulties in the process and low sensitivity of the isolation methods, laboratory diagnosis relies mainly on serological tests (Al Dahouk *et al.*, 2002). Variety of agglutination tests such as Rose Bengal Plate test (RBPT), Standard Tube Agglutination test (STAT) and Coombs' test are used in testing for *Brucella* infection. Other serological tests used for confirmation and sero-surveys are Enzyme Linked Immunosorbent Assay (ELISA) and Brucellacapt (Araj, 2010; Smits and Kadri, 2005). Florescent Polarization assay and Immunochromatographic Lateral Flow assay, a simplified version of ELISA are used as rapid test for point-of-care tests (Irmak *et al.*, 2004; Lucero *et al.*, 2003).

The major antigens of *Brucella* used in serological testing are the smooth lipopolysaccharide (smooth-S LPS) and internal-cytosolic proteins (Nielsen *et al.*, 2004). *Brucella* LPS is a strong immunogen but its epitopes cross-reacts with other Gram-negative bacteria (Yersinia enterocolitica O:9, Vibrio cholera O:1, Esherichia coli O:157, *Esherichia hermanni*, *Salmonella* O:30, *Stenotrophomonas maltophila*, *Francisella tularensis*, etc.), increasing the rate of false positivity (Nielsen *et al.*, 2000). False positives may also be caused by other illnesses such as salmonellosis, tularemia, cholera, lupus erythematosus and myeloma (Al-Attas *et al.*, 2000). False positives may also occur early on the course of the disease, or in case of focal infection (Alikhani *et al.*, 2013).

2.7.2.1 Evaluation of serological testing results

In endemic areas it is possible to detect low levels of antibodies in healthy individuals (Moreno and Moriyón, 2006). Special attention is required in setting the seropositivity cut-off values. In cases of chronicity and relapses, the cut-off values should be adjusted accordingly, while in endemic regions the limit should be higher than in non-endemic regions (Moreno and Moriyón,

2006). The antibody types detected in a patient's serum and the proper evaluation of each test assists greatly in diagnosis. The clinical symptoms and epidemiological information of the patient must always be taken under consideration when evaluating the results of tests (Mantur *et al.*, 2007).

2.7.2.2 Rose Bengal Plate Test (RBPT)

The Rose Bengal Plate test is often used as a screening test (Ruiz- Mesa *et al.*, 2005). The Rose Bengal Plate test is performed by mixing a drop of Rose Bengal reagent with equal volume of serum on a glass plate. Agglutination is read after 2-4 minutes. The sensitivity of RBPT is high (>99%) and false negative results are rarely seen. In unexposed population, false negative is rarely observed (Mantur *et al.*, 2006; Serra and Viñas, 2010). A study in Spain, has also reported a low specificity for RBPT in endemic areas and in patients with a long history of brucellosis (Serra and Viñas, 2010).With this challenge, the positive predictive value of the test is low and therefore all positive samples must be confirmed with a more specific test. To increase the specificity the test may be applied to a serial dilution (1:2 through 1:64) of the serum samples (Ruiz- Mesa *et al.*, 2005). The specificity of this test increases when higher dilutions agglutinate with titres at or above 1:8. In the acute phase, IgM antibodies dominate, thus agglutination tests have proven to be more sensitive. In the chronic phase, Rose-Bengal Plate test is negative or present low titers. For confirmation of RBPT, the STAT or ELISA can be used (Smits and Kadri, 2005).

2.7.2.3 Standard Tube Agglutination Test (STAT)

Standard Tube Agglutination Test (Giambartolomei *et al.*, 2002) developed by Wright and colleagues remains the most popular and easy test to perform. The STAT is performed by mixing

serial dilutions of serum between 1:20 through 1:256 with *Brucella* antigen in test tubes. After an overnight incubation, agglutination is read. A titre of 1:160 or above is considered positive alongside clinical findings (Nielsen and Yu, 2010). STAT can measure the total quantity of the agglutinating antibodies (IgG and IgM) (Nielsen and Yu, 2010). Drawbacks of this test include the inability to diagnose *B. canis* infections (Nielsen and Yu, 2010). Also, false-positive reactions can be seen in the STAT which occasionally result from cross reactions with antibodies to *Yersinia enterocolitica* O:9, *Francisella tularensis, Escherichia* O157, *Salmonella O:30, Vibrio cholerae O:1* (Pappas *et al.*, 2005).

On the other hand, false-negative reactions can also be seen in the STAT, early in the course of infection due to presence of blocking antibodies, or the "prozone" phenomenon (i.e., the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors) (Pappas *et al.*, 2005). Some of these shortcomings can be overcome by modifications such as the addition of EDTA, 2-mercaptoethanol, or antihuman globulin (Pappas *et al.*, 2005). The quantity of specific IgG is determined by treatment of the serum with 0.005M 2 mercaptoethanol (2ME), which inactivates the agglutinability of the IgM. However, many patients have low levels of agglutinating IgG antibodies and the results can easily be misinterpreted. STAT titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation; however, in endemic areas the titer of 1:320 is taken as the cut off (Araj, 2010). In chronic cases, STAT is negative or of lower titers (Araj, 2010). STAT has been reported to show lower sensitivity to other diagnostic methods when testing for *Brucella* infection. Hajia and others, reported a sensitivity of 38%, while Fallah and others reported 63% and 98% sensitivity and specificity, respectively as compared to 69% and 95% for ELISA (Fallah *et al.*, 2012; Hajia and Rahbar, 2006).

2.7.2.4 Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme linked immunosorbant assay (ELISA) has become very popular in its usage because of the excellent result it produces in testing for brucellosis (Baddour, 2012). It measures IgG, IgM, and IgA, which allows a better interpretation of the clinical situation (Araj, 2010). Enzyme-linked immunosorbent assays (ELISA) can detect antibody titers for every class of antibody separately, making this method useful for *Brucella* determination (Baddour and Alkhalifa, 2008). Disease progress can be monitored because there is the ability to examine IgG and IgA antibodies separately. The persistence of high titers of IgG and IgA for long periods is an indication of localized disease (Poester *et al.*, 2010).

Work carried out by Al-Attas and others showed a higher sensitivity of 83.6% for the ELISA method as compared to 46.9% for PCR (Al-Attas *et al.*, 2000) which has been consistent with findings in other studies (Gemechu *et al.*, 2011; Queipo-Ortuño *et al.*, 2009)

The specificity of ELISA, however, seems to be less than the agglutination tests especially when carried out in endemic areas or in people professionally exposed to *Brucella* (Amirzargar *et al.*, 2009). As the diagnosis of *Brucella* is based on the detection of antibodies against smooth LPS, the cut-off value needs to be adjusted to optimize the specificity when used in endemic areas (Smits and Kadri, 2005).

2.7.2.5 Florescence Polarization Assay (FPA)

The Fluorescence polarization assay (FPA) is a rapid test that is used as a point-of-care test. It is a simplified version of ELISA (Lucero *et al.*, 2003) that offers a valuable alternative to conventional serological tests. The test is analyzed by incubating the serum sample with *Brucella* O-polysaccharide antigen linked to a fluorescent probe. This fluorescent probe measures the size of the fluorescent-tagged antigen (Lucero *et al.*, 2003). The test does not require specific

training, is easy to interpret, and can be used at the bedside. The sensitivity of this test at the selected cut-off value is 96% for culture-confirmed brucellosis and the specificity is 98% (Lucero *et al.*, 2003). FPA can be used at all stages of the disease.

2.7.2.6 Immunochromatographic Lateral flow Assay (LFA)

Immunochromatographic *Brucella* IgM / IgG lateral flow assay is a simplified version of ELISA that is also used as a rapid point-of-care test (Elfaki *et al.*, 2005; Irmak *et al.*, 2004). This assay uses a composite strip having a nitrocellulose detection strip and a reagent pad. The nitrocellulose detection strip contains *Brucella* LPS antigen, as a *Brucella*-specific capture probe, and a reagent control applied in distinct lines (Mizanbayeva *et al.*, 2009). The reagent pad contains dried and stabilized detection reagent having a colloidal gold-conjugated antihuman IgG or IgM. Serum or blood sample is added to a sample well, followed by test liquid. The result is read based on positive or negative staining after 10–15 min by visual examination of the antigen and control lines in the test window (Mizanbayeva *et al.*, 2009). LFA assays have shown to be excellent for screening/surveillance of patients with brucellosis in endemic areas and as outbreak and field tests (Christopher *et al.*, 2010; Mizanbayeva *et al.*, 2009). Studies have shown that this test has high sensitivity and specificity for *Brucella* IgM and IgG (Christopher *et al.*, 2010). It has been suggested as a possible substitute for Coombs test and a better marker for disease progression (Casanova *et al.*, 2009). The flow assay may be used as a confirmatory test for the confirmation of Rose Bengal positive samples (Irmak *et al.*, 2004).

2.7.3 Molecular Detection

There has been a major advancement in all aspects of molecular diagnostics with regard to human brucellosis (Queipo-Ortuno *et al.*, 2008). Molecular diagnosis has been known to

minimize the risk associated with handling this potentially infectious specimen (Navarro *et al.*, 2004). Also studies has shown that molecular diagnosis increases the sensitivity, specificity and speed of testing, although some studies have reported only moderate sensitivity of 50% (Amirzargar *et al.*, 2009). Example can be seen in the study carried out by Hajia and others who reported a lower sensitivity level of 48.9% for PCR as compared to 84% for ELISA (Hajia and Rahbar, 2006). Several genus-specific PCR systems using primer pairs that target 16SRNA sequences and genes of different outer membrane proteins have been developed. Queipo-Ortuno and others found 100% sensitivity and 98.3% specificity by using a B4 / B5 primer and amplifying a 223-bp fragment of the *bcsp31* gene compared with 70% constituents of blood culture (Christopher *et al.*, 2010; Queipo-Ortuno *et al.*, 2008).

Incorporation of a robust DNA extraction method such as the diatom-guanidinium isothiocyanate method effectively removes the inhibitors commonly present in a variety of clinical specimens and may improve the sensitivity and reproducibility (Queipo-Ortuno *et al.*, 2008). However, as these PCR systems carry a high risk of contamination and require equipment for visualization, they are less suitable for routine diagnosis purposes (Queipo-Ortuno *et al.*, 2008). Hence, real time PCR systems have been developed to cater for the risk of contamination (Supriya *et al.*, 2010).

Real-time PCR is another diagnostic tool for detection of *B. abortus, B. melitensis and B. suis* biovar1. These PCR assays target the specific integration of IS711 elements within the genome of the respective *Brucella* species or biovar (Redkar *et al*, 2001).

Currently, a real-time multiplex PCR assay has been developed for rapid confirmatory identification of *Brucella* with speciation (Probert *et al.*, 2004). The genus, *Brucella abortus* and *Brucella melitensis* specific primers confirm the organism from isolates (Probert *et al*, 2004 and

Gee *et al*, 2004). PCR is also useful in species differentiation and biotyping of isolates (Elfaki *et al.*, 2005; Gopaul *et al.*, 2008) and assessing treatment efficacy (Nimri, 2003).

Although PCR is very promising, standardization of extraction methods, infrastructure, equipment and expertise are lacking and a better understanding of the clinical significance of the results is still needed (Navarro *et al*, 2004). Diagnosis of relapsing brucellosis is another challenge where PCR may prove to be useful (Mitka *et al.*, 2007).

There are some short nucleotide repeat sequences that are present in the *Brucella* genome showing a wide variation in the number of repeats between species and isolates (Gopaul *et al.*, 2008). PCR amplification of these variables repeat is more effective as compared to the conventional typing methods for species and biovar-identification (Gopaul *et al.*, 2008). Morta and coworkers recently evaluated the usefulness of a PCR-based assay in a post-treatment follow up and relapse of patients with brucellosis (Vrioni *et al.*, 2004). There are several PCR assays for the detection of *Brucella* DNA using pure culture, animal, and human clinical samples (Araj, 2010). However, the sensitivity and specificity of PCR for *Brucella* varies between laboratories, and hence standardization is required (Baddour and Alkhalifa, 2008).

2.8 TREATMENT OF HUMAN BRUCELLOSIS

Treatment of human brucellosis involves antibiotics that can penetrate macrophages and can act in the acidic intracellular environment (Solera *et al.*, 2004). The treatment of human brucellosis is controversial because it is a multisystem disease that may present with a broad spectrum of clinical manifestations (Mantur *et al.*, 2006). The location of the disease and the underlying conditions will determine the choice of regimen and duration of antimicrobial therapy (Mantur *et al.*, 2006).

The World Health Organization issued guidelines for the treatment of human brucellosis (Pappas

et al., 2005). The guidelines outline two regimens, both using doxycycline 100 mg twice daily for a period of six weeks. This is in combination with either streptomycin (1gm/day intramuscular) for two weeks treatment or rifampicin 600 to 900 mg once daily for six weeks. Both combinations are the most popular treatments worldwide, although they are not used universally (Corbel, 2006).

The streptomycin regimen is slightly more efficacious in preventing relapse (Ariza *et al.*, 2007; Mantur *et al.*, 2006). This may be related to the fact that rifampicin down-regulates serum doxycycline levels (Colmenero *et al.*, 2002). However, parenteral administration of streptomycin mandates either hospital admission or the existence of an adequate health care network- both of which are often absent in areas of endemic disease (Mantur *et al.*, 2006). On the other hand, the use of rifampicin in areas in which brucellosis is endemic, where tuberculosis is also usually endemic, raises concern about the development of community resistance to rifampin (Pappas *et al*, 2005).

Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar result to that of the classic regimens (Karabay *et al*, 2004). Although quinolones have been used and will continue to be used, the cost of this approach remains a major drawback (Solera *et al.*, 2004). The action of macrolides is attenuated in the acidic phagolysosomal environment, and thus these agents are not useful in brucellosis (Pappas *et al.*, 2005).

Childhood brucellosis can be successfully treated with a combination of two drugs; doxycycline 4 mg / kg / day and rifampicin 10 mg/kg /day orally for six weeks (Pappas *et al.*, 2005). Co-trimoxazole 8 mg / 40 mg/kg/day can be used for children < 8 years of age (Mantur *et al.*, 2004). Rifampicin has proven to be safe in treating brucellosis during pregnancy (Pappas *et al.*, 2005).

There is a general need for combined treatment, since all mono-therapies are characterized by

unacceptably high relapse rates (Pappas *et al.*, 2005; Solera *et al.*, 2004). Some authors advise that gentamicin (5 mg/kg/day intramuscularly) be administered concomitantly for the initial five to seven days of therapy in order to prevent relapse (Pappas *et al.*, 2005). Relapses occur at a rate of about 10% and are often milder in severity than the initial disease and can be treated with a repeated course of the usual antibiotic regimens (Ozbay and Inanmis, 2005; Pappas *et al.*, 2005). Most complications of brucellosis can be adequately treated with standard regimens. However in some complications like spondylitis, osteomyelitis, neurobrucellosis and endocarditis, surgery combined with antimicrobial therapy is the best approach (Mantur *et al.*, 2006).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY AREA

The study was done at the Kumasi Abattoir, which is located at Kaase a suburb of Kumasi. Kaase is both a residential and industrial area of the Kumasi Metropolitan Area. Livestock to be slaughtered at the Kumasi Abattoir are mainly transported from the Brong Ahafo and Northern Regions of Ghana. Some animals are transported from neighboring Burkina Faso, Mali and Niger. The Kumasi Abattoir was established in 1997 with grants from the Government of Ghana and the Canadian International Development Agency (CIDA). It commenced operations in 1998. There is a cattle market at the abattoir premises. At the cattle market are kraals, which are used to house the animals for sale. A total of 250 cattle and 150 sheep and goats and 100 pigs are slaughtered daily at the abattoir. After slaughter and dressing, the carcasses are distributed to meat shops and cold stores in and around Kumasi (Frimpong *et al*., 2011).

3.2 STUDY POPULATION

There are about 145 workers employed by the Kumasi Abattoir. These workers include kill floor workers, veterinarians, marketing staff, security staff and administrative workers. The kill floor workers are the line operators, slaughterers, butchers and meat processors.

There are also about 195 auxiliary workers on the premises of the Kumasi Abattoir. These auxiliary workers are not employees of the abattoir, but are involved directly or indirectly with abattoir operations. These workers are meat sellers, livestock farmers and livestock traders, cowboys, loaders and drivers. Therefore in addition to the 145 abattoir workers, a total of 340

participants were targeted for the study. Workers at the abattoir were between the ages of 16 and 78.

3.3 STUDY DESIGN

This was a cross sectional study to compare three diagnostic methods in detecting *Brucella* infection among slaughterhouse workers. It was also meant to determine the exposure factors associated with *Brucella* infection such as demographics, occupational information, handling of livestock, consumption of animal and animal products and knowledge on disease transmission.

3.4 SAMPLING

We used a random sampling method to select the study participants after providing a sampling frame of all workers at the Kumasi abattoir. Participants were divided into three groups based on the nature of their job and sampled on the basis of probability proportional to size method as follows: 1. Working in meat processing 2. Contact with animals and 3. Others. Others included administrative workers, plumbers, security officers and mechanics.

3.5 SAMPLE SIZE AND STUDY POWER

We included a total of 220 participants assuming a default study power of 80%, an alpha of 5% and an assumed prevalence of *Brucella* among the study population of 17.5%. This would detect *Brucella* to as close as 2% within the study population if present. 54 were from the 'animal contact' group, 148 from the 'meat processing' group and 18 were from the 'others' group.

3.6 ETHICAL APPROVAL

The study was approved by the Joint Committee on Human Research Publication and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi-Ghana. Formal permission

was also obtained from the head of the abattoir. Informed consent was obtained from each participant.

3.7 INCLUSION CRITERIA

Workers who are directly or indirectly involved in abattoir operations and present at the abattoir at time of visit were included in the study.

3.8 EXCLUSION CRITERIA

Individuals at the abattoir at the time of visit who are not abattoir workers were excluded from the study.

3.9 FIELD WORK (DATA COLLECTION)

Data collection started from May 2013 and ended in August 2013. The aim and objectives of the study were explained to workers at the abattoir in the local dialect. After obtaining their informed consent, a structured questionnaire was administered to them.

3.10 BLOOD SAMPLE COLLECTION AND PREPARATION

A total of 4 ml of venous blood was taken with the help of a tourniquette and dispensed into a serum vacutainer. The blood samples were placed in a cold box and transported to the laboratories of Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) for analysis. The blood samples were centrifuged at 4,000 × g for 5 minutes to obtain sera. The sera were dispensed into two different 2mls labeled eppendorf tubes and stored at -20°C for serological and molecular analysis, respectively.

3.11 LABORATORY ASSAYS FOR BRUCELLA DETECTION

These were:

1. ELISA B*rucella* IgM assay
2. ELISA *Brucella* IgG assay
3. *Brucella* Rose Bengal Plate Test
4. *Brucella* DNA extraction and
5. *Brucella* DNA amplification

3.11.1 ELISA *Brucella* IgM assay

This assay was to determine the presence of human immunoglobulin M (IgM) in serum for *Brucella* infection.

3.11.1.1 Procedure for the determination of *Brucella* IgM in serum

Using a micropipette, 200µl of Rheumatoid factor absorbent was placed in 2ml eppendorf tube with 800µl of dilution buffer. This was mixed thoroughly to form Rf-dilution buffer. Ten microliters of serum was pipetted into a 2ml eppendorf tube and 1000µl of Rf-dilution buffer was added to serum and mixed thoroughly. Hundred microliters of diluted serum was pipetted into the microtiter plate. And then, 100µl of Rf-dilution buffer was pipetted into the well for substrate blank. Microtiter plate was incubated for 60 minutes at 37°C in a moist chamber. After incubation, 200µl of washing solution was pipetted into microtiter plate to wash out unbound antigens. Washing was repeated four times and the microtiter plate was inverted over paper towel to mop excess moisture. Afterwards, 100µl of *Brucella* IgM conjugate was added to the wells to bind to the antibody-antigen complex. The microtiter plate was incubated for 30 minutes at 37°C in moist chamber. After incubation, 200µl of washing solution was used to wash microtiter plate wells to remove unbound conjugate. Hundred microliters of substrate solution

was then added to wells to react with conjugated enzyme to produce a colour derivative of the substrate. The microtiter plate was incubated for 30 minutes at 37°C in moist chamber. After incubation, 100µl of stopping solution was added to the wells to stop the reaction. The microtiter plate was mixed gently. A yellow colour was generated which was proportional to the level of antibody bound. Optical Density (OD) of the test was read within 60 minutes at 405nm against substrate blank using the SPECTRA MAX 190 microplate reader. The actual antibody concentration was computed from the OD using a Microsoft excel-based evaluation software tool, SERION *evaluate* (Institut Virion\Serion GmbH, Germany). This was carried out by a four parameter logistic-log model (4 PL) and is based on the formula:

$$OD = \mathbf{A} + \frac{\mathbf{D} - \mathbf{A}}{1 + e^{\mathbf{B}(\mathbf{C} - \ln conc.)}}$$

Where parameters A, B, C, and D were representative for the exact shape of the curve:

1. Lower asymptote …… parameter A
2. Slope of the curve ……parameter B
3. Turning point ……...parameter C
4. Upper asymptote …….parameter D

A concentration of <15Uml^{-}1 was considered as negative, 15-20Uml^{-1} as borderline and >20Uml^{-1} as positive.

Figure 2: Reagents used in detecting anti-Brucella IgM and IgG by ELISA method.

3.11.2 ELISA *Brucella* IgG assay

This assay was to determine the presence of human immunoglobulin M (IgG) in serum for *Brucella* infection.

3.11.2.1 Procedure for the determination of *Brucella* IgG in serum

Using micropipette, 10µl of serum was placed into a 2ml eppendorf tube and 1000µl of dilution buffer was added to serum and mixed thoroughly. Hundred microliters of diluted serum was pipetted into the microtiter plate. Then, 100µl of dilution buffer was pipetted into the well for substrate blank. Microtiter plate was incubated for 60 minutes at 37°C in a moist chamber. After incubation, 200µl of washing solution was pipetted into microtiter plate to wash out unbound antigens. Washing was repeated four times and the microtiter plate was inverted over paper towel to mop excess moisture. Afterwards, 100µl of *Brucella* IgG conjugate was added to the wells to bind to the antibody-antigen complex. The microtiter plate was incubated for 30 minutes at 37°C in moist chamber. After incubation, 200µl of washing solution was used to wash

microtiter plate to remove unbound conjugate. Hundred microliters of substrate solution was added to wells to react with conjugated enzyme to produce a colour derivative of the substrate. The microtiter plate was incubated for 30 minutes at 37°C in moist chamber. After incubation, 100µl of stopping solution was added to the wells to stop the reaction. The microtiter plate was mixed gently. A yellow colour was generated which was proportional to the level of antibody bound. Optical Density (OD) of the test was read within 60 minutes at 405nm against substrate blank using the SPECTRA MAX 190 microplate reader. The actual antibody concentration was computed from the OD using a Microsoft excel-based evaluation software tool, SERION *evaluate*. This was carried out by a four parameter logistic-log model (4 PL) and is based on the formula:

$$OD = \mathbf{A} + \frac{\mathbf{D} - \mathbf{A}}{1 + e^{\mathbf{B}(\mathbf{C} - \ln conc.)}}$$

Where parameters A, B, C, and D were representative for the exact shape of the curve:

1. Lower asymptote …… parameter A
2. Slope of the curve ……parameter B
3. Turning point ……...parameter C
4. Upper asymptote …….parameter D

A concentration of <20Uml^{-}1 was considered as negative; 20-30Uml^{-1} as borderline and >30Uml^{-1} as positive.

Figure 3: SPECTRA MAX 190 microplate reader for reading test absorbance (Pelkin Elmer, USA)

3.11.3 *Brucella* Rose Bengal Plate Test (RBPT)

The test determines serum IgG to *Brucella melitensis*, *Brucella abortus*, *Brucella bovis* or *Brucella suis* infections. The *Brucella* antigen reacts with serum IgG antibodies to form agglutination.

3.11.3.1 Procedure for the determination of *Brucella* Rose Bengal Plate Test in serum

Using a micropipette, 30µl of serum was placed on sterile card and 30µl of buffered antigen was added. The solution was mixed using sterile sticks. The card was rotated to mix. After 4 minutes, agglutination observed was recorded as positive. No agglutination was recorded as negative.

3.11.4 DNA Extraction

DNA from all serum samples was extracted using a commercial purification system with columns (Genotype DNA Isolation Kit; Hain Lifescience GmbH, Nehren, Germany).

3.11.4.1 Procedure for DNA Extraction

Fifty microliters of serum was pipetted into a well-labeled eppendorf tube. Fifty microliters of Lysis buffer and 10µl of Proteinase K were also added and the tube was vortexed for at least 5 seconds. Sample was incubated at 56°C for 5 min and centrifuged at 800rpm in a thermal mixer. Hundred microliters of Binding Buffer B6 was added and mixed by pipetting up and down four times. Lysate was transferred into a spin column with a receiver tube. Afterwards, the lid of the spin column was closed and centrifuged for 1 min at 13,000 x g. After centrifuging, the lid was opened and 300µl Wash Buffer 1 was added to spin column and centrifuged for 30 seconds at 13,000 x g. After centrifuging, the receiver tube with filtrate was discarded and the spin column was placed into a new 2.0ml receiver tube. The lid was opened and 750µl Wash Buffer 2 added and centrifuged for 13,000 x g for 30 seconds. The receiver tube with filtrate was discarded. The spin column was then placed into a new receiver tube and centrifuged for 2 min at 13,000 x g to eliminate residual ethanol. After centrifuging, receiver tube with filtrate was discarded. The spin column was transferred into a 1.5ml labeled eppendorf tube and 200µl prewarmed Elution Buffer D was added. It was incubated for 1 min at room temperature. The DNA was eluted by centrifuging for 1 min at 6,000 x g.

3.11.5 DNA Amplification.

The BCSP31-PCR assay was used to amply *Brucella* DNA.

3.11.5.1 The BCSP31-PCR assay

This assay amplified a 223bp sequence gene encoding an immunogenic outer membrane protein of 31 kDa *Brucella abortus* antigen conserved in all *Brucella* species making use of Primers B4 (TGG CTC GGT TGC CAA TAT CAA) and B5 (CGC GCT TGC CTT TCA GGT CTG).

A master mix scheme was performed in a reaction volume of 50µl. The reaction volume contained 10µl DNA template, 5µl 10x Buffer, 3.5µl MgCl2 (25mM), 1.5µl deoxynucleoside triphosphate (dNTP), a 1µl of the primer B4 (10 pmol) and 1µl of the primer B5 (8 pmol), 0.5 µl of Hotstar *Taq* polymerase and 27.5 µl of RNAse free water.

PCR cycling conditions used consisted of an initial 15 min incubation step at 95°C, followed by 38 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, with a final incubation step at 72°C for 10 min.

3.12 GEL ELECTROPHORESIS

Amplified products were detected by fluorescence after electrophoresis in 2% agarose gel stained with ethidium bromide (1 µg/ml) under UV light. Positive control (*Brucella melitensis* 16M) and negative control (RNAse free water) were added to validate the test results.

3.13 STATISTICAL ANALYSIS

Data entry and data cleaning were done using *EPI Info 3.4.5*. Descriptive statistics were presented in tables, graphs and proportions using *Microsoft Excel 2007*. *Stata version 12.0* was used to estimate the impact of the predictor variables on the outcome variable. Odds ratio and their 95% confidence intervals (95%-CI) were also estimated. Associations with a P-value ≤0.05 were considered significant. The sensitivity, specificity, positive predictive value, and negative predictive value for each of the serological tests in comparison with PCR assay were calculated using *Stata version 12.0.*

CHAPTER FOUR

4.0 RESULTS

Almost all participants were males (218 males, 2 females). The median age of the participants was 36.7 years (SD ± 11.4, range 16-78 years). Of the 220 sera tested for antibodies against *Brucella spp,* 3 (1.4%) were positive in Rose Bengal Plate test, 4 (1.8%) in anti-*Brucella* ELISA IgM, 21 (9.6%) in anti-*Brucella* ELISA IgG and 3 (1.4%) in both anti-*Brucella* IgM and IgG tests, respectively. PCR was positive in 98 (44.5%) study participants (Table 2 and figure 1).

Table 2: Results of ELISA IgM, ELISA IgG, Rose Bengal and PCR Tests

VARIABLE	ELISA		Rose Bengal	PCR
	IgM	IgG		
Negative (%)	216 (98.2%)	199 (90.4%)	217 (98.6%)	122 (55.5%)
Positive (%)	4 (1.8%)	21 (9.6%)	3 (1.4%)	98 (44.5%)

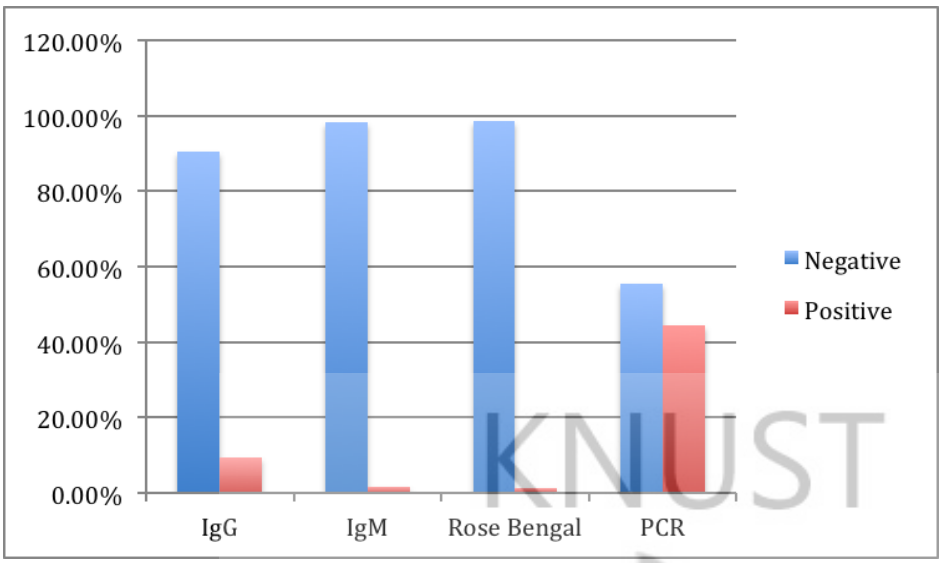

Figure 4: The percentage of *Brucella* positive and negative cases by ELISA IgM, ELISA IgG, Rose Bengal and PCR Tests.

Table 3 shows the true positives, true negatives, false positives and false negatives of Rose Bengal Test, ELISA IgM and ELISa IgG in comparison with PCR. Of the 3 that showed positive for Rose Bengal Plate test, 2 were also positive for PCR. All the four that showed positive for ELISA IgM were also positive for PCR. Of the 21 that showed positive for ELISA IgG, 18 were also positive for PCR (Table 3).

Table 3: Comparison of PCR results with Rose Bengal, ELISA IgM and ELISA IgG results for the diagnosis of *Brucella* infection.

VARIABLE		ROSE BENGAL		ELISA IgM		ELISA IgG	
		Neg	Pos	Neg	Pos	Neg	Pos
PCR	Neg	121	1	122	0	199	3
	Pos	96	2	94	4	80	18

Table 4 shows the sensitivity, specificity, negative and positive predictive values and Kappa values for Rose Bengal, ELISA IgM and IgG using PCR as the gold standard. The lower positive predictive value for Rose Bengal, ELISA IgM and IgG in comparison with PCR were 2.0%, 4.1% and 18.4%. The Kappa value of 0.013, 0.045 and 0.212 were calculated for Rose Bengal, ELISA IgM and ELISA IgG (Table 4).

Table 4: Comparison of the Sensitivity, Specificity, Positive and Negative Predictive Values for Rose Bengal, ELISA IgM and IgG using PCR as the gold standard.

	Rose Bengal	**ELISA IgM**	**ELISA IgG**
Sensitivity	66.7%	100%	85.7%
Specificity	55.8%	56.8%	71.3%
PPV	2.0%	4.1%	18.4%
NPV	99.2%	100%	98.5%
Kappa Value	0.013	0.045	0.212

Table 5 shows the anti-*Brucella* IgG seropositives among the abattoir workers in relation to their demographic characteristics. The group termed Animal contact include cowboys, loaders, cattle sellers, drivers etc. Meat processing group include workers like Slaughterers, Butchers, Line operators etc. The group termed 'Others" include mechanics, plumbers and administrative workers etc.

Table 5: Anti-*Brucella* IgG seropositivity stratified by demographic characteristics among s Slaughterhouse Workers

	Total	No. of Pos (%)	OR	95% CI	P-value
AGE					
< 30 years	52	1 (2.0)	Reference		
30-39 years	84	10 (11.9)	6.6	0.8-53.4	0.076
40-49 years	57	7 (12.3)	7.0	0.8-59.0	0.074
50+ years	27	3 (11.1)	6.4	0.6-64.8	0.117
OCCUPATION					
Animal contact	54	3 (5.6)	Reference		
Meat processing	148	17 (11.5)	2.2	0.6-7.9	0.220
Others	18	1 (5.6)	0.9	0.1-10.1	0.987
EDUCATION					
Illiterate	74	11 (14.9)	Reference		
Primary	53	5 (9.4)	0.6	0.2-1.8	0.372
Secondary	68	4 (8.7)	0.3	0.1-1.0	0.048
Post-secondary	25	0	N/A		
DURATION ON JOB					
< 10 years	74	7 (9.5)	Reference		
10-19 years	100	10 (10.0)	1.1	0.4-3.0	0.894
20+ years	46	4 (8.7)	0.9	0.3-3.3	0.899

The age distribution of the 21 IgG seropositive individuals was as follows: 1/52 (2%) was under 30 years, 10/84 (11.9%) were 30-39 years old (OR 6.6; 95% CI 0.8-53.4; p=0.08) and 7/57

(12.3%) were 40-49 years old (OR 7.0; 95% CI 0.8-59.0; p=0.07) while 3/27 (11.1%) were older than 50 years (OR 6.4; 95% CI 0.6-64.8; p=0.12) (Table 5).

Additionally, most anti-*Brucella* IgG seropositive individuals (17/21) were working in the meat-processing unit (OR 2.2; 95% CI 0.6-7.9; p=0.22). 14.9% were illiterate while 9.4% (OR 0.6; 95% CI 0.2-1.8; p=0.37) and 8.7% (OR 0.3; 95% CI 0.1-1.0; p=0.05) had attained primary and secondary education respectively. Furthermore, 10% (OR 1.1; 95% CI 0.4-3.0; p=0.84) of these IgG positive study respondents had worked for 10-19 years and 8.7% for more than 20 years (OR 0.9; 95% CI 0.3-3.3; p=89) (Table 5).

Figure 5 shows a picture of agarose gel electrophoresis pattern of amplified DNA with negative and positive controls, which fluoresces under UV light with a band size of 223bp using primers B4/B5.

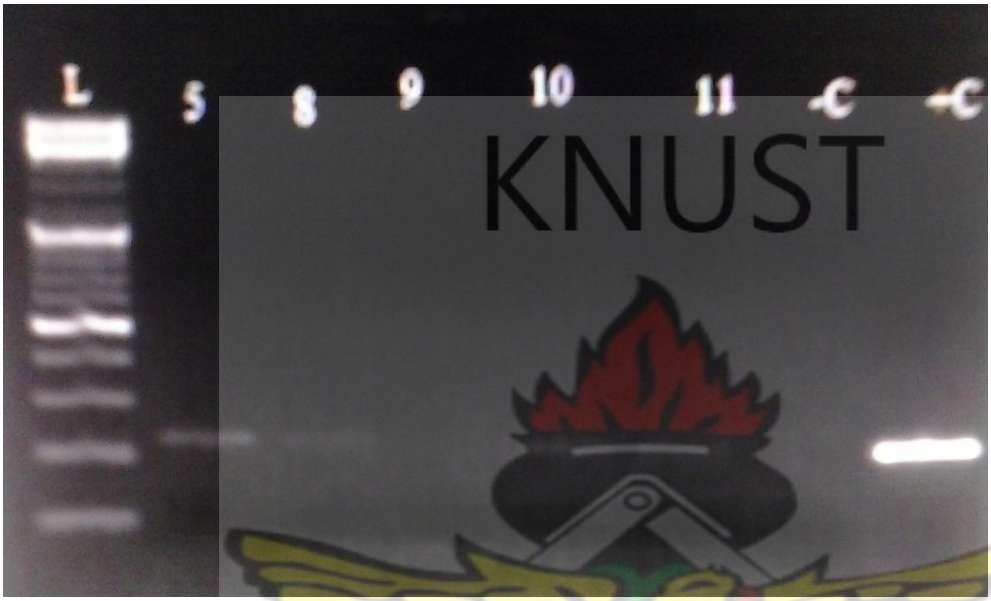

Figure 5: Agarose gel Electrophoresis pattern of Brucella bcsp31 gene 223bp specific PCR product amplified with primer B4/B5.

L: DNA molecular weight Ladder 100bp

9: Negative field samples

5,8,10,11: Positive field samples

-C: Negative Control

+C: Positive Control

Table 6 shows PCR positives among the slaughterhouse workers in relation to their demographic characteristics.

Table 6: Demographic Characteristics of PCR positivity among Slaughterhouse Workers

	Total	**No. of Pos (%)**	**OR**	**95% CI**	**P-value**
AGE					
>30 years	52	26 (50)	Reference		
30-39 years	84	30 (35.7)	0.6	0.3-1.1	0.101
40-49 years	57	25 (43.9)	0.8	0.4-1.7	0.521
50+years	27	17 (62.9)	1.7	0.7-4.4	0.274
OCCUPATION					
Animal Contact	54	20 (37.0)	Reference		
Meat processing	148	69 (46.6)	1.5	0.8-2.8	0.226
Others	18	9 (50)	1.7	0.6-4.9	0.334
EDUCATION					
Illiterate	74	36 (48.6)	Reference		
Primary	53	20 (37.7)	0.6	0.3-1.3	0.223
Secondary	68	35 (51.5)	1.1	0.6-2.2	0.737
Post-secondary	25	7 (28)	0.4	0.2-1.1	0.076
DURATION ON JOB					
<10 years	74	33(44.6)	Reference		
10-19 years	100	42(42)	0.9	0.5-1.7	0.733
20+ years	46	23(50)	1.2	0.6-2.6	0.564

The age distribution of the 98 *Brucella* PCR positive individuals showed that 26/52 (50%) were under 30 years while 25/57 (43.9%) were 40-49 years old (OR 0.8; 95% CI 0.4-1.7; p=0.52)

Also, most of the *Brucella* PCR positive individuals (69/98) were working in the meat-processing unit (OR 1.49; 95% CI 0.8-2.8; p=0.23). 48.6% were illiterate while 51.5% (OR 1.12; 95% CI 0.6-2.2; p=0.74) had attained secondary education. Furthermore, 42.0% (OR 0.90; 95% CI 0.5-1.7; p=0.73) of these *Brucella* PCR positive study respondents had worked for 10-19 years and 50% (OR 1.2; 95% CI 0.6-2.6; p=0.564 worked for 20 years and more (Table 6).

Table 7 shows the risk factors associated with Brucella infection among Slaughterhouse workers. Risk factors assessed are listed in the table below with their associated Odds ratio (OR), P-values and 95% confidence interval.

Table 7: Risk Factors associated with *Brucella* infection

IgG	**Odds Ratio**	**P-value**	**95% CI**
Age	1.00	0.842	0.98-1.02
Occupation	1.32	0.337	0.67-1.15
Education	0.87	0.284	0.79-2.19
Duration on Job	1.02	0.241	0.99-1.05
Assisting in birth	1.29	0.546	0.54-3.11
Use of Protective clothing	1.54	0.147	0.86-2.76
Consumption of meat	0.96	0.964	0.14-6.36
Consumption of milk	1.00	0.822	0.54-2.18
Knowledge on disease	0.95	0.908	0.39-2.30

Table 8 shows the relationship between the different occupations of the slaughterhouse workers and knowledge about *Brucella* infection. 53.2% of the meat processing group, 20.9% of the animal contact group and 3.2% of the 'others' group did not know about *Brucella* infection and its transmission modes. Only 2.3% of the animal contact group, 7.7% of the meat processing group and 1.8% of the 'others' group knew bout *Brucella* infection and its transmission modes (Table 8).

Table 8: Relationship between occupation and knowledge about *Brucella* infection.

	Knowledge about *Brucella* infection			
Occupation	**No**	**Yes**	**Unknown**	**Total**
Animal Contact	46 (20.9%)	5 (2.3%)	3 (1.4%)	54
Meat Processing	117 (53.2%)	17 (7.7%)	14 (6.4%)	148
Others*	7 (3.2%)	4 (1.8%)	7 (3.2%)	18
Total	**170**	**26**	**24**	**220**

CHAPTER FIVE

5.0 DISCUSSION

Brucellosis is a disease placing many people at risk but certain occupations such as abattoir workers, veterinarians, butchers, cattle rearers, farmers etc are considered to be of higher risk of acquiring this infection (Kunda *et al.*, 2007). In Ghana, however, brucellosis diagnosis in animals and people is not routinely done.

The diagnostic performance of PCR, ELISA and Rose Bengal tests in diagnosing *Brucella* infection among slaughterhouse workers were compared. In this study, among the applied methods used for detecting Brucellae, the Rose Bengal test gave the lowest prevalence of 1.4%. The low performance of Rose Bengal test has been reported elsewhere (Ruiz- Mesa *et al.*, 2005; Serra and Viñas, 2010). A study carried out in Ghana using the Rose Bengal test (Kubuafor *et al.*, 2000), found no evidence of human *Brucella* infection in selected risk groups in the Akwapim South district of Ghana.

The ELISA method was used to detect anti-*Brucella* IgM (recent infection) and anti-*Brucella* IgG (past infection). These tests gave prevalence of 1.8% and 9.6% respectively. The IgG prevalence of 9.6% in this present study is similar to that in Nigeria (Aworh *et al.*, 2013) among abattoir workers where a prevalence of 9.8% was obtained. Our overall sero-prevalence obtained for the IgM (1.8%) was lower compared to an Iranian study amongst slaughterhouse workers (Nikokar *et al.*, 2011) where prevalence of 6.9% was obtained.

Though human brucellosis has been poorly studied in Africa, the infection has been reported in studies carried out in some African countries. A sero-prevalence of 3.8% was reported in nomadic pastoralists from Chad (Schelling *et al.*, 2003). El-Ansary and others reported a sero-

prevalence of 1% among occupational contacts, including butchers, slaughterhouse workers, milkers, and cow attendants in eastern Sudan (El-Ansary *et al.*, 2001). Eritrea reported a sero-prevalence of between 3.0% and 7.1% (Omer *et al.*, 2002). Studies of febrile patients in a large hospital in Kampala, Uganda, yielded 13.3% (Mutanda, 1998), while in eastern Nigeria, 5.2% of those screened were seropositive (Baba *et al.*, 2001).

PCR in this study detected *Brucella* infection in 44.5% of the study participants giving a higher detection rate than ELISA and Rose Bengal. Findings by Hajia and Rahbar (2006) have reported a prevalence of 48.9% for PCR as compared to 84% for ELISA in Iran. Amirzargar and others reported a prevalence of 50% for PCR in Iran (Amirzargar *et al.*, 2009; Hajia and Rahbar, 2006). However a lower prevalence of 7% was reported for PCR in a study carried out in India (Gemechu *et al.*, 2011). Elfaki *et al.*, (2005) detected *Brucella* infection of 40% and 70% by culture and PCR respectively. They concluded that detection of antibody against *Brucella* is not always related to disease condition and that it has to be followed up by either culture or PCR.

From this study, a sensitivity of 66.7%, specificity of 55.8%, Positive Predictive Value of 2.0% and Negative Predictive value of 99.2% for Rose Bengal in comparison with PCR tests and the sensitivity of 85.7%, specificity of 71.3%, Positive Predictive value of 18.4% and a Negative Predictive value of 98.5 % for ELISA IgG in comparison with PCR were reported. The lower Positive Predictive values and Kappa values for Rose Bengal test and ELISA IgM indicate that these methods are poor in diagnosing *Brucella* infection among these high-risk groups. Kappa value of 0.212 for ELISA IgG indicates that ELISA IgG is fairly good in diagnosing *Brucella* infection, therefore, a better alternative in cases where PCR is not available. However, it is advisable to run both ELISA IgM and IgG to get conclusive results since the absence of one does not give a true indication of *Brucella* infection.

These findings are consistent with reports from other studies making PCR a gold standard in diagnosing *Brucella* infection (Navarro *et al.*, 2004; Queipo-Ortuño *et al.*, 1997). Queipo-Ortuno and others also found 100% sensitivity and 98.3% specificity by using a B4 / B5 primer and amplifying a 223-bp fragment of the *bcsp31* gene compared with 70% constituents of blood culture (Queipo-Ortuno *et al.*, 2008).

The higher positivity of ELISA IgG for *Brucella* diagnosis was in the age groups 30-49 years. However, PCR positive results were evenly distributed among all age groups studied. This suggests that all age groups may be infected with *Brucella.* A study carried out in Turkey indicated age 50 and above having a higher risk of infection than lower age groups (Cetinkaya *et al.*, 2005).

Individuals in the slaughterhouse have different job descriptions giving them different degrees of exposure to the disease. In this present study, workers were selected according to their job type. Most of the ELISA positive IgG (17/21) and PCR positive (69/148) were workers in the meat-processing unit. These were the line operators, slaughterers, butchers, carcass carriers and meat processors. PCR results suggest that working in the meat processing group gives higher risk of *Brucella* infection (OR=1.32), as compared to those working in the animal contact group (eg: cowboys, loaders, cattle handlers etc). The meat processing workers are responsible for dissecting animals, removing internal organs, processing meat into other products and also selling meat at the Abattoir. This category of workers are often in close contact with animal fluids which are great risks for contracting *Brucella* infections (Cutler *et al.*, 2005). These workers can also be infected through cuts and abrasion on bare hands, inhalation of aerosols in the slaughtering area or splashing of infected fluid into their eyes (Kunda *et al.*, 2007). This finding is consistent with studies conducted in Greece (Minas *et al.*, 2007) and in Tanzania (Swai

and Schoonman, 2009) that indicated that the majority of cases with brucellosis were attributed to direct contact with animals and their products.

Findings in this present study showed a reduced risk of infection among those with higher education ie secondary and post secondary for ELISA IgG results. With PCR results, however, participants who had completed secondary education showed an increased risk of infection. This is so as a higher percentage (51.5%) of participants who had completed secondary education had *Brucella* infection. A continuous work at the Abattoir without frequent public health education can cause workers to disregard the proper codes and ethics in hygienic practices. A study among abattoir workers in Pakistan reported an increased positivity among lower educated workers as compared to higher educated workers (Mukhtar and Kokab, 2008)

Brucella can be transmitted via air and the minutest concentration of the organism is viable to cause an infection therefore workers who have worked for a year still have the chance of being infected (Mukhtar and Kokab, 2008). Longer service time workers were exposed repeatedly to body fluids of potentially infected animals and therefore had a higher chance of being infected repeatedly (Swai and Schoonman, 2009). An Iranian study (Karimi *et al.*, 2003) highlighted a strong association between *Brucella* infection and duration of occupational exposure. Also a study in Nigeria reported that abattoir workers who had worked for more than 5 years were more than likely to be exposed to *Brucella* infection (Aworh *et al.*, 2013). A long-term cumulative exposure by workers to *Brucella* infected livestock or to a contaminated environment increases the chance of workers getting infected (Pappas *et al.*, 2006).

Assisting in the birth of livestock showed that the more one assists in delivery, the higher the risk of infection though statistically not significant (P=0.546). This normally comes about when

workers do not wear protective clothing while assisting in birth (Bikas *et al.*, 2003; Schelling *et al.*, 2003). Many studies reported that the use of protective clothing is one way of preventing the spread of *Brucella* infection (Mantur and Amarnath, 2008; Swai and Schoonman, 2009). From this present study, workers who used protective clothing had an increased risk of infection (OR=1.54) because they do not change used worn out and dirty clothing frequently (Memish and Balkhy, 2004).

From this study those who consumed meat and milk products have a similar risk of being infected as those who did not consume these products though statistically not significant. Undercooked (boiled or grilled) meat types are likely means of transmission of *Brucella* from processed meat (Aworh *et al.*, 2013; Cetinkaya *et al.*, 2005; Sümer *et al.*, 2003).

Of concern is that only 9.3% of the participants working in the animal contact (5/54) and 11.5% of those with meat processing (17/148) reported any knowledge about *Brucella* infection. The odds ratio of 0.95 also indicates that those who know about *Brucella* transmission have a lower risk of being infected. There is the need for targeted public health education on *Brucella* including its transmission routes in slaughterhouses and the use of best protective practices. The education material needs to be designed for all workers so they know about the risk and are able to protect themselves. Additionally, protective clothing such as headgears, gloves, boots and others need to be provided for them.

5.1 LIMITATIONS

The study could have benefited from including additional slaughterhouses across the country thereby increasing the sample size, power and representativeness. The study could have

benefited from the inclusion of other diagnostic methods thereby getting to know their performance.

5.2 CONCLUSION

PCR method yielded the highest sensitivity and specificity among the applied methods. This method is especially helpful epidemiologically in high-risk workers who tested negative for serologic testing. ELISA method can however be used in cases where PCR is not available.

In this study, prevalence of *Brucella* infection was 44.5%, an indication that the infection is an occupational hazard among slaughterhouse workers at the Kumasi abattoir. Workers categorized within the meat processing groups like line operators, slaughterers, butchers, carcass carrier etc. showed the highest risk of infection. This is because workers in this group adopt poor personnel hygienic measures and have frequently higher exposure to blood, body secretions and tissues of the infected slaughtered animals. Education and longer duration on the job, age, assisting in birth of livestock, no knowledge on disease transmission were also associated with a higher risk of infection although not significant.

We recommend intensive educational programmes to be conducted for the slaughterhouse workers on modes of transmission and prevention of *Brucella* infection in their work environment. Such programmes need to be targeted for workers that have the highest risks and designed in a way that messages can be understood by staff with lower education including the illiterate. Additionally, workers need to be provided with the required protective devices to limit risk and spread of *Brucella* infection.

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APPENDIX

Table 9: PROTOCOL WORKSHEET FOR IGM AND IGG ELISA QUANTIFICATION

SW001	SW002	SW003	SW004	SW005	SW006	SW007	SW008	SW009	SW010	SW011	SW012
										POS	POS
										NEG	BLK

SW001-SW012 -Test samples

POS: Positive control

NEG: Negative Control

BLK: substrate blank

DATA ON EDUCATION WITH DIAGNOSTIC METHODS

code	Illiterate	Primary	Secondary	Postsecon.	IgM Results	IgG Results	Rose Bengal Results	PCR assay
SW001	NO	NO	YES	NO	**0.81**	**0.76**	Neg	Pos
SW002	NO	NO	YES	NO	**1.92**	**2.71**	Neg	Pos
SW003	NO	NO	YES	NO	**0.80**	**1.21**	Neg	Pos
SW005	NO	NO	NO	YES	**15.12**	**21.87**	Neg	**Pos**
SW004	NO	NO	YES	NO	**1.06**	**2.68**	Neg	Pos
SW006	YES	NO	NO	NO	**2.10**	**86.67**	Neg	**Pos**
SW007	NO	NO	YES	NO	**25.82**	**118.63**	Neg	Pos
SW008	NO	NO	NO	YES	**3.16**	**3.57**	Neg	Pos
SW009	NO	NO	YES	NO	**1.18**	**1.11**	Neg	Neg
SW010	NO	NO	NO	YES	**0.65**	**6.26**	Neg	Pos
SW011	NO	NO	NO	YES	**0.98**	**1.00**	Neg	Pos
SW011	NO	NO	YES	NO	**0.36**	**2.27**	Neg	Neg
SW013	NO	NO	YES	NO	**1.01**	**1.63**	Neg	Neg
SW014	NO	YES	NO	NO	**8.92**	**1.44**	Neg	Neg
SW015	NO	NO	YES	NO	**6.09**	**1.94**	Neg	Neg
SW016	NO	YES	NO	NO	**0.60**	**1.55**	Neg	Pos
SW017	YES	NO	NO	NO	**2.57**	**113.38**	Neg	**Pos**
SW018	NO	YES	NO	NO	**0.80**	**2.11**	Neg	Neg
SW019	YES	NO	NO	NO	**18.66**	**151.03**	Pos	**Pos**
SW020	NO	YES	NO	NO	**7.64**	**2.35**	Neg	Neg
SW021	YES	NO	NO	NO	**2.37**	**0.87**	Neg	Pos
SW022	NO	YES	NO	NO	**0.77**	**2.59**	Neg	Neg
SW023	NO	YES	NO	NO	**1.56**	**2.00**	Neg	Pos
SW024	YES	NO	NO	NO	**5.42**	**43.54**	Neg	**Pos**
SW025	YES	NO	NO	NO	**1.70**	**1.25**	Neg	Pos
SW026	YES	NO	NO	NO	**6.11**	**1.55**	Neg	Pos
SW027	NO	YES	NO	NO	**6.10**	**20.70**	Neg	**Pos**

SW028	YES	NO	NO	NO	**3.78**	**3.55**	Neg	Pos
SW029	YES	NO	NO	NO	**2.74**	**72.56**	Neg	**Pos**
SW030	NO	NO	NO	YES	**0.36**	**3.21**	Neg	Pos
SW031	NO	NO	YES	NO	**5.94**	**12.51**	Neg	Pos
SW032	YES	NO	NO	NO	**4.76**	**2.60**	Neg	Pos
SW033	NO	YES	NO	NO	**94.49**	**79.77**	Pos	**Pos**
SW034	YES	NO	NO	NO	**0.55**	**3.34**	Neg	Pos
SW035	YES	NO	NO	NO	**4.51**	**0.88**	Neg	Pos
SW036	NO	NO	YES	NO	**3.75**	**0.96**	Neg	Neg
SW037	NO	NO	NO	YES	**0.66**	**0.68**	Neg	Pos
SW038	YES	NO	NO	NO	**2.01**	**1.40**	Neg	Pos
SW039	YES	NO	NO	NO	**1.65**	**2.27**	Neg	Neg
SW040	NO	NO	YES	NO	**1.89**	**2.10**	Neg	Neg
SW041	YES	NO	NO	NO	**0.66**	**1.19**	Neg	Neg
SW042	NO	NO	YES	NO	**1.02**	**2.30**	Neg	Pos
SW043	NO	NO	YES	NO	**2.86**	**3.18**	Neg	Neg
SW044	YES	NO	NO	NO	**5.88**	**56.51**	Neg	**Pos**
SW045	NO	YES	NO	NO	**0.68**	**1.94**	Neg	Neg
SW046	NO	YES	NO	NO	**10.00**	**39.75**	Neg	**Pos**
SW047	NO	NO	NO	YES	**0.43**	**0.79**	Neg	Pos
SW048	NO	NO	YES	NO	**0.89**	**4.70**	Neg	Pos
SW049	YES	NO	NO	NO	**0.67**	**1.42**	Neg	Neg
SW050	NO	NO	YES	NO	**3.61**	**1.80**	Neg	Neg
SW051	YES	NO	NO	NO	**1.32**	**1.58**	Neg	**Pos**
SW052	NO	YES	NO	NO	**0.57**	**1.46**	Neg	Neg
SW053	NO	NO	YES	NO	**1.65**	**1.14**	Neg	Pos
SW054	NO	YES	NO	NO	**2.15**	**1.02**	Neg	Neg
SW055	NO	YES	NO	NO	**13.20**	**3.01**	Neg	Pos
SW056	NO	NO	NO	YES	**10.61**	**1.25**	Neg	Neg
SW057	YES	NO	NO	NO	**1.98**	**1.40**	Neg	Neg
SW058	NO	NO	YES	NO	**1.47**	**0.91**	Neg	Neg
SW059	YES	NO	NO	NO	**1.81**	**3.36**	Neg	Neg

SW060	NO	YES	NO	NO	**1.50**	**1.48**	Neg	Pos
SW061	YES	NO	NO	NO	**2.81**	**1.40**	Neg	Pos
SW062	NO	YES	NO	NO	**2.32**	**3.64**	Neg	Neg
SW063	NO	YES	NO	NO	**2.80**	**1.79**	Neg	Neg
SW064	YES	NO	NO	NO	**1.12**	**1.74**	Neg	Neg
SW065	NO	NO	YES	NO	**0.74**	**0.80**	Neg	Neg
SW066	NO	NO	NO	YES	**6.53**	**1.67**	Neg	Neg
SW067	NO	NO	YES	NO	**0.61**	**1.33**	Neg	Neg
SW068	NO	YES	NO	NO	**0.86**	**4.46**	Neg	Neg
SW069	NO	YES	NO	NO	**2.73**	**1.26**	Neg	Neg
SW070	NO	NO	YES	NO	**0.65**	**3.37**	Neg	Neg
SW071	YES	NO	NO	NO	**0.64**	**3.70**	Neg	Neg
SW072	NO	NO	YES	NO	**1.35**	**2.49**	Neg	Neg
SW073	NO	NO	NO	YES	**0.61**	**3.67**	Neg	Neg
SW074	YES	NO	NO	NO	**1.36**	**1.66**	Neg	Neg
SW075	NO	NO	YES	NO	**0.46**	**2.00**	Neg	Neg
SW076	NO	NO	YES	NO	**3.94**	**3.38**	Neg	Neg
SW077	NO	NO	YES	NO	**0.36**	**1.26**	Neg	Pos
SW078	NO	NO	YES	NO	**1.83**	**1.04**	Neg	Pos
SW079	NO	YES	NO	NO	**1.18**	**0.96**	Neg	Neg
SW080	NO	YES	NO	NO	**0.93**	**1.03**	Neg	Neg
SW081	YES	NO	NO	NO	**1.20**	**1.19**	Neg	Neg
SW082	NO	YES	NO	NO	**0.75**	**2.52**	Neg	Neg
SW083	NO	NO	NO	NO	**0.96**	**2.21**	Neg	Pos
SW084	NO	NO	NO	NO	**0.61**	**1.83**	Neg	Neg
SW085	NO	NO	NO	NO	**0.13**	**0.31**	Neg	Neg
SW086	NO	NO	NO	YES	**0.50**	**0.90**	Neg	Neg
SW087	YES	NO	NO	NO	**1.69**	**2.71**	Neg	Neg
SW088	NO	YES	NO	NO	**1.22**	**0.93**	Neg	Neg
SW089	YES	NO	NO	NO	**1.43**	**1.95**	Neg	Neg
SW090	NO	YES	NO	NO	**0.94**	**0.79**	Neg	Neg
SW091	YES	NO	NO	NO	**1.46**	**1.21**	Neg	Neg

SW092`	NO	YES	NO	NO	**1.04**	**1.76**	Neg	Neg
SW093	YES	NO	NO	NO	**31.91**	**3.59**	Neg	**Pos**
SW094	NO	YES	NO	NO	**11.11**	**11.17**	Neg	**Pos**
SW095	YES	NO	NO	NO	**1.33**	**59.28**	Neg	**Pos**
SW096	NO	NO	YES	NO	**0.35**	**2.58**	Neg	Neg
SW097	NO	NO	YES	NO	**1.16**	**1.52**	Neg	Neg
SW098	NO	YES	NO	NO	**4.94**	**6.48**	Neg	Neg
SW099	NO	NO	YES	NO	**0.93**	**2.68**	Neg	Neg
SW100`	NO	NO	YES	NO	**1.52**	**0.83**	Neg	Neg
SW101	YES	NO	NO	NO	**1.15**	**0.14**	Neg	Neg
SW102	YES	NO	NO	NO	**35.41**	**341.50**	Neg	**Pos**
SW103	YES	NO	NO	NO	**2.69**	**19.35**	Neg	Neg
SW104	YES	NO	NO	NO	**1.84**	**1.42**	Neg	Pos
SW105	YES	NO	NO	NO	**2.12**	**47.09**	Neg	Pos
SW106	YES	NO	NO	NO	**1.87**	**1.70**	Neg	Neg
SW107	YES	NO	NO	NO	**1.10**	**0.84**	Neg	Pos
SW108	YES	NO	NO	NO	**1.53**	**2.21**	Neg	Neg
SW109	YES	NO	NO	NO	**2.68**	**1.14**	Neg	Neg
SW110	YES	NO	NO	NO	**2.91**	**2.76**	Neg	Neg
SW111	NO	YES	NO	NO	**5.13**	**1.00**	Neg	Neg
SW112	YES	NO	NO	NO	**2.20**	**1.85**	Neg	Neg
SW113	NO	NO	NO	YES	**0.54**	**0.70**	Neg	Pos
SW114	NO	YES	NO	NO	**2.28**	**1.34**	Neg	Neg
SW115	NO	YES	NO	NO	**1.67**	**0.70**	Neg	Pos
SWII6	NO	NO	NO	YES	**0.34**	**0.37**	Neg	Neg
SW117	NO	NO	YES	NO	**4.07**	**43.19**	Neg	**Pos**
SW118	NO	NO	YES	NO	**5.80**	**0.37**	Neg	Pos
SW119	NO	NO	YES	NO	**1.11**	**0.80**	Neg	Pos
SW120	YES	NO	NO	NO	**1.04**	**2.74**	Neg	Pos
SW121	YES	NO	NO	NO	**0.89**	**0.83**	Neg	Neg
SW122	YES	NO	NO	NO	**1.52**	**0.78**	Neg	Pos
SW123	YES	NO	NO	NO	**0.42**	**1.37**	Neg	Pos

SW124	NO	NO	YES	NO	**5.36**	**3.93**	Neg	Pos
SW125	YES	NO	NO	NO	**0.73**	**0.85**	Neg	Neg
SW126	NO	NO	YES	NO	**0.96**	**3.35**	Neg	Pos
SW127	NO	NO	YES	NO	**9.45**	**12.04**	Neg	Pos
SW128	NO	YES	NO	NO	**1.19**	**2.61**	Neg	Pos
SW129	NO	NO	YES	NO	**0.65**	**2.47**	Neg	Pos
SW130	YES	NO	NO	NO	**0.57**	**0.42**	Neg	Neg
SW131	YES	NO	NO	NO	**2.30**	**3.58**	Neg	Pos
SW132	NO	NO	YES	NO	**3.19**	**2.23**	Neg	Neg
SW133	YES	NO	NO	NO	**1.70**	**1.88**	Neg	Neg
SW134	YES	NO	NO	NO	**11.02**	**6.75**	Neg	Neg
SW135	NO	NO	NO	YES	**0.68**	**1.93**	Neg	Neg
SW136	YES	NO	NO	NO	**1.36**	**1.60**	Neg	Neg
SW137	NOI	YES	NO	NO	**0.33**	**5.58**	Neg	Neg
SW138	NOI	NO	YES	NO	**1.54**	**0.74**	Neg	**Pos**
SW139	NOI	YES	NO	NO	**0.58**	**0.95**	Neg	Neg
SW140	NOI	NO	NO	YES	**1.32**	**3.85**	Neg	**Pos**
SW141	YES	NO	NO	NO	**1.18**	**1.57**	Neg	Pos
SW142	NO	NO	YES	NO	**0.55**	**2.83**	Neg	Neg
SW143	YES	NO	NO	NO	**0.55**	**7.45**	Neg	Pos
SW144	YES	NO	YES	NO	**1.35**	**1.77**	Neg	Pos
SW145	NO	NO	YES	NO	**0.69**	**2.07**	Neg	Neg
SW146	YES	NO	NO	NO	**0.23**	**1.33**	Neg	Neg
SW147	NO	NO	YES	NO	**1.6**	**0.91**	Neg	Pos
SW148	NO	NO	NO	YES	**1.64**	**2.37**	Neg	Neg
SW149	NO	YES	NO	NO	**0.58**	**1.25**	Neg	Neg
SW150	NO	NO	YES	NO	**1.58**	**1.04**	Neg	Neg
SW151	YES	NO	NO	NO	**0.24**	**48.03**	Neg	**Pos**
SW152	NO	NO	YES	NO	**0.34**	**3.72**	Neg	Neg
SW153	YES	NO	NO	NO	**0.76**	**0.85**	Neg	Pos
SW154	YES	NO	NO	NO	**0.98**	**0.72**	Neg	Neg
SW154	NO	NO	YES	NO	**1.28**	**0.74**	Neg	Neg

SW156	NO	YES	NO	NO	**0.2**	**0.85**	Neg	Neg
SW157	NO	YES	NO	NO	**1.05**	**0.73**	Neg	Pos
SW158	NO	YES	NO	NO	**0.41**	**1.29**	Neg	Pos
SW159	NO	NO	YES	NO	**1.92**	**1.62**	Neg	Neg
SW160	YES	NO	YES	NO	**5.53**	**0.85**	Neg	Pos
SW161	YES	NO	NO	NO	**0.82**	**16.98**	Neg	Pos
SW162	NO	NO	NO	YES	**4.17**	**1.86**	Neg	Pos
SW163	YES	NO	NO	NO	**0.78**	**3.41**	Neg	Pos
SW164	NO	YES	NO	NO	**3.02**	**0.68**	Neg	Pos
SW162	NO	NO	YES	NO	**0.79**	**0.83**	Neg	Neg
SW166	NO	NO	YES	NO	**0.81**	**1.31**	Neg	Neg
SW167	YES	NO	NO	NO	**3.7**	**15.54**	Neg	Neg
SW168	YES	NO	NO	NO	**0.58**	**11.46**	Neg	Pos
SW169	NO	NO	NO	NO	**1.86**	**3.06**	Neg	Pos
SW170	NO	YES	NO	NO	**0.43**	**1.52**	Neg	Pos
SW171	NO	NO	YES	NO	**0.55**	**1.09**	Neg	Pos
SW172	NO	NO	YES	NO	**4.6**	**2.54**	Neg	Neg
SW173	YES	NO	NO	NO	**0.7**	**2.02**	Neg	Pos
SW174	YES	NO	NO	NO	**1.35**	**1.43**	Neg	Pos
SW175	NO	NO	YES	NO	**0.51**	**2.09**	Neg	Pos
SW176	YES	NO	NO	NO	**0.33**	**0.78**	Neg	Pos
SW177	YES	NO	NO	NO	**0.54**	**1.61**	Neg	Pos
SW178	YES	NO	NO	NO	**1.8**	**1.47**	Neg	Pos
SW179	NO	YES	NO	NO	**10.67**	**2.02**	Neg	Pos
SW180	NO	NO	NO	YES	**0.64**	**1.24**	Neg	Neg
SW181	NO	NO	NO	YES	**1.28**	**1.04**	Neg	Neg
SW182	NO	YES	NO	NO	**5.62**	**9.64**	Neg	Neg
SW183	NO	NO	YES	NO	**3.52**	**24.78**	Neg	**Neg**
SW184	NO	YES	NO	NO	**9.12**	**50.69**	Neg	Pos
SW185	NO	NO	NO	YES	**0.76**	**1.66**	Neg	Neg
SW186	NO	NO	NO	YES	**2.34**	**1.82**	Neg	Neg
SW187	NO	NO	NO	YES	**1.01**	**1.10**	Neg	Neg

SW188	NO	NO	YES	NO	**3.16**	**11.94**	Neg	Neg
SW189	NO	YES	NO	NO	**1.34**	**2.19**	Neg	Neg
SW190	NO	YES	NO	NO	**0.98**	**1.09**	Neg	Neg
SW191	NO	NO	NO	YES	**1.16**	**0.69**	Neg	Neg
SW192	NO	NO	YES	NO	**1.92**	**1.06**	Neg	Neg
SW193	NO	NO	NO	YES	**2.36**	**1.20**	Neg	Neg
SW194	NO	YES	NO	NO	**0.75**	**1.23**	Neg	Neg
SW195	YES	NO	NO	NO	**1.49**	**7.89**	Neg	Neg
SW196	NO	NO	YES	NO	**1.48**	**7.96**	Neg	Neg
SW197	YES	NO	NO	NO	**1.10**	**1.58**	Neg	Neg
SW198	NO	NO	NO	YES	**1.86**	**1.16**	Neg	Neg
SW199	NO	NO	YES	NO	**2.78**	**2.18**	Neg	Neg
SW200	NO	YES	NO	NO	**1.68**	**2.26**	Neg	Pos
SW201	NO	YES	NO	NO	**2.23**	**49.67**	Neg	**Pos**
SW202	YES	NO	NO	NO	**3.98**	**2.16**	Neg	Neg
SW203	NO	YES	NO	NO	**12.11**	**6.00**	Neg	Neg
SW204	NO	NO	YES	NO	**6.97**	**16.13**	Neg	Neg
SW205	NO	NO	YES	NO	**0.94**	**0.64**	Neg	Neg
SW206	NO	NO	YES	NO	**2.19**	**8.71**	Neg	Neg
SW207	NO	NO	YES	NO	**4.85**	**63.40**	Neg	Pos
SW208	NO	NO	YES	NO	**1.42**	**175.32**	Neg	**Pos**
SW209	NO	NO	YES	NO	**3.92**	**3.97**	Neg	Neg
SW210	NO	NO	YES	NO	**2.16**	**1.10**	Neg	Neg
SW211	NO	NO	YES	NO	**4.32**	**84.68**	Pos	**Neg**
SW212	NO	NO	YES	NO	**0.88**	**1.05**	Neg	Pos
SW213	NO	YES	NO	NO	**5.57**	**17.92**	Neg	Neg
SW214	YES	NO	NO	NO	**8.10**	**4.65**	Neg	Pos
SW215	YES	NO	NO	NO	**6.84**	**28.15**	Neg	**Pos**
SW216	NO	YES	NO	NO	**1.11**	**4.78**	Neg	Neg
SW217	YES	NO	NO	NO	**12.77**	**131.18**	Neg	**Neg**
SW218	NO	NO	YES	NO	**0.97**	**1.82**	Neg	Pos
SW219	NO	YES	NO	NO	**0.63**	**6.82**	Neg	Neg

SW220	NO	YES	NO	NO	**7.04**	**92.01**	Neg	Neg

QUESTIONNAIRE

SERO-PREVALENCE AND OCCUPATIONAL RISK FACTORS OF BRUCELLA INFECTION AMONG SLAUGHTERHOUSE WORKERS AND BUTCHERS IN KUMASI, GHANA

Demographic Data

1. Respondent code (ID): …….. **2.** Sampling date (DD/MM/YY): ……....

3. Age: .....years

4. Gender: ☐ Male; ☐ Female

5. Marital Status: ☐ Single; ☐ Married; ☐ Separated; ☐ Divorced; ☐ Co-Habitation;

6. Religion: ☐ Christian; ☐ Muslim; ☐ Traditional; ☐ Other.

6a. If other, specify? _____

7. Occupation: ☐ Animal keeper; ☐ Loader; ☐ Slaughterer; ☐ Cleaner; ☐ Driver; ☐ Butcher; ☐ Veterinarian ; ☐ Other.

7a. If other, specify? _____

8. Duration at Job: .....years and months

9. Educational Status: ☐ Illiterate; ☐ Primary; ☐ Secondary; ☐ Post secondary

Occupational Information

11. Which of the following livestock do you handle at the abattoir? (more than 1 tick possible)

☐ Cattle; ☐ Sheep; ☐ Goats; ☐ Other.

11a. If other, specify? _____

12. Do you use protective gear when slaughtering animals? ☐ Yes; ☐ No; ☐ Don't know.

12a. If yes, specify. ☐ Head gear; ☐ Gloves; ☐ Boots; ☐ Apron; ☐ Other

12a1. If other, specify? _____

12b. If yes, how often do you change your working gear per week? _____

13. Do you use protective gear when selling meat? ☐ Yes; ☐ No; ☐ Don't know.

13a. If yes, specify. ☐ Head gear; ☐ Gloves; ☐ Boots; ☐ Apron; ☐ Other

13a1. If other, specify? _____

13b. If yes, how often do you change your working gear per week? _____

Handling of Livestock

14 Do you rear livestock? ☐ Yes; ☐ No; ☐ Don't know

14a. If yes, please specify. ☐ Cattle; ☐ Sheep; ☐ Goats; ☐ Other.

14a1. If other, specify? _____

15. Do you assist in the birth of livestock? ☐ Yes; ☐ No; ☐ Don't know

15a. If yes, do you use protective gear when assisting in the birth of livestock?

☐ Yes; ☐ No; ☐ Don't know

15a1. If yes, which of the following do you use? ☐ Head gear; ☐ Gloves; ☐ Boots; ☐ Apron; Other (specify).....

15a2. If yes, how often do you change your working gear per week? _____

Consumption of Animal Products

16. Do you eat meat? ☐ Yes; ☐ No; ☐ Don't know.

16a. If yes, do you eat raw meat? ☐ Yes; ☐ No; ☐ Don't know

16a1. If yes, which animals do you eat raw**.** ☐ Cattle; ☐ Sheep; ☐ Goats

Other (Specify) .....

16b. If yes, how often do you eat raw meat? ☐ Rarely; ☐ Sometimes; ☐ Often.

16. Do you drink milk? ☐ Yes; ☐ No; ☐ Don't know.

16a. If yes, how often do you drink milk? ☐ Rarely; ☐ Sometimes; ☐ Often.

16b. If yes, where do you usually buy milk? ☐ Farm; ☐ Shop; ☐ Market; ☐ Other.

16b1. If other, where? _____

16c. If from farm, which animal milk? .....

16c1. If from farm, was it pasteurised? ☐ Yes; ☐ No; ☐ Don't know

17. Do you consume other milk products? ☐ Yes; ☐ No; ☐ Don't know

17a. If yes, specify?.....

17b. If yes, how often do you consume them? ☐ Rarely; ☐ Sometimes; ☐ Often.

History of Disease

18. Have you heard of *Brucella* infection? ☐ Yes; ☐ No; ☐ Don't know;

19. Do you think you could have been exposed to *Brucella*? ☐ Yes; ☐ No; ☐ Don't know

20. Have you had any of the following signs and symptoms?

☐ Headache; ☐ Joint pain; ☐ Recurrent fever; ☐ Abdominal pain; ☐ Testicular pain; ☐ Chronic fatigue; ☐ Night Sweats; ☐ Weight loss; ☐ Diarrhoea; ☐ Backache ☐ Skin rash; ☐ Joint swelling; ☐ Loss of appetite; ☐ Cough

20a.If any of the boxes were ticked for 20 above, when did it manifest? .....

20b. If yes, did you seek any health care? ☐ Yes; ☐ No; ☐ Don't know

20b1. If yes, where? ☐ Traditional healer; ☐ Village health volunteer; ☐ Health Centre; ☐ District Hospital; ☐ Teaching Hospital

21. Did you receive vaccination before starting your job? ☐ Yes; ☐ No; ☐ Don't know

21a. If yes specify the type of vaccination? .....