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Assessment of brucella infection status in abattoir workers and animals destined for slaughter at Dodoma modern abattoir, Tanzania

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ASSESSMENT OF BRUCELLA INFECTION STATUS IN ABATTOIR WORKERS AND ANIMALS DESTINED FOR SLAUGHTER AT DODOMA MODERN ABATTOIR, TANZANIA

Denice Luwumba

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

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ABSTRACT

Brucellosis is one of the most widespread zoonosis of serious public health and an obstacle to food safety and security and, socio-economic development in most African countries. A cross-sectional study was conducted to determine the prevalence of brucellosis in abattoir workers and animals destined for slaughter; molecular characterization of *Brucella* species; and assess risk factors, knowledge, awareness and practices associated with transmission of human brucellosis at an abattoir setting. A total of 452 serum samples; 190, 200 and 62 from cattle, goats and humans, respectively were collected in animals and workers at Dodoma modern abattoir, Tanzania. The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3%, 1.5% and 1.6%, respectively based on Rose Bengal Plate Test. A semi-structured questionnaire was used to collect data for assessing the knowledge, awareness and practices as well as risk factors related to brucellosis exposure at the abattoir. Knowledge, awareness coupled with adherence to biosafety measures such as wearing protective gears minimizes significantly the risks of human exposure to brucellosis. On molecular characterization of *Brucella* species, an amplicon with 731 base pairs (bp) which is from *B. melitensis* was detected in one person working in the abattoir. The low prevalence of human brucellosis at the abattoir may be attributed to the institution of biosafety measures and training of the workers. Therefore more education on the disease to farmers, abattoir workers and general public as well as instituting control measures at the farm level and biosafety measures at abattoir setting is needed.

DECLARATION

I, Denice Luwumba do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation titled 'Assessment of brucella infection status in abattoir workers and animals destined for slaughter at Dodoma modern abattoir, Tanzania' is my own original work and has never been submitted for a degree in any other university.

..... 08-04-2019

Denice Luwumba **Date**

The above declaration is confirmed by

..... 08th April, 2019

Dr. Gabriel Mkilema Shirima **Date**

..... 08th April, 2019

Prof. Lughano J. M. Kusiluka **Date**

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CERTIFICATION

This is to certify that this dissertation by Denice Luwumba has been accepted in partial fulfilment for requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

..... 08th April, 2019

Dr. Gabriel Mkilema Shirima **Date**

..... 08th April, 2019

Prof. Lughano J. M. Kusiluka **Date**

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DEDICATION

I dedicate this piece of work to my lovely parents, Modestusi Luwumba and Fausta Mayanga who raised and nurtured me. I also dedicate this work to my beloved wife, Gabriela Mwilongo for her love and our children Gloria and Gian for their encouragement and patience.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
AMOS	Abortus, Melitensis, Ovis, Suis
C	Celsius
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CDC	Center for Disease Control and Prevention
c-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFU	Colony Forming Units
CI	Confident Interval
Df	Degree of Freedom
DNA	Deoxyribosenucleic acid
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FPA	Flouresence Polazation Assay
i-ELISA	Indirect Enzyme- Linked Immunosorbent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IS711	Insertion sequence 711
LPS	Lipopolysaccharides
LRT	Log Likelihood Ratio Test
MHCII	Major Histocompatibility two
MLVA	Multilocus Variable Number Tandem Repeat Analysis
MoLFD	Ministry of livestock and fisheries development
NH	Native Hapten
OD	Optical Density
OIE	World Animal Health Organization
OM	Outer Membrane
OMP	Outer Membrane Protein
O-PS	Oligo-Polysaccharides
OR	Odds Ratio

Bp	Base Pair
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
P^H	Measure of the molar concentration of hydrogen ions in the solution
RB51	Rough Strain
RBPT	Rose Bengal Plate Test
Rev1	*Brucella melitensis* vaccine
S19	Strain 19 *Brucella abortus* vaccine
SAT	Serum Agglutination Test
S-LPS	Smooth Lipopolysaccharides
TBE	Tris Borate Ethylenediamineacetic acid
TLR4	Toll Like Receptor Four
TMC	Tanzania Meat Company
TNF- α	Tumour Necrosis Factor-alpha
USD	United States Dollar
UV	Ultraviolet Light
WHO	World Health Organization
γIFN	Gamma Interferon
µl	Microlitre
µM	Micromole
χ^2	Chi-square
mAb	Monoclonal antibodies

CHAPTER ONE

INTRODUCTION

1.1 Background information

Brucellosis is an endemic bacterial zoonotic infection and a serious obstacle to public health, socio-economic development, food safety and security in most low and middle-income countries (Corbel, 2006; Dean *et al*., 2012b; Mcdermott *et al*., 2013). It affects most marginalized poor livestock keeping communities, therefore, its impacts is most seen in rural areas where people's livelihood depend heavily on livestock production or sales of dairy products. It always poses risk of human infection due to close contact between livestock and humans and consumption of unsafe animal products (Baddour, 2012). The impact of disease is very big as evidenced by a study conducted in India which estimated the cost of brucellosis on livestock production to be USD 3.4 billion (Singh *et al*., 2015). Brucellosis is classified as one of the eight neglected endemic zoonotic diseases which contribute to the continuation of poverty in developing countries (Mableson *et al*., 2014; WHO, 2006).

Brucellosis is caused by bacteria of the genus *Brucella* which affects a wide range of hosts that include domestic livestock, wild animals, marine mammals and human (Galińska and Zagórski, 2013). Animals contract brucellosis through ingestion of contaminated pastures, feed or water and transmission between animal to animal occur through licking of aborted foetuses or contact with infected abortion materials (Mai *et al*., 2012; Mcdermott and Arimi, 2002). The disease in domestic ruminants is caused by host-specific *Brucella* species including *Brucella abortus, Brucella melitensis* and *Brucella ovis* in cattle, goats and sheep, respectively (Whatmore, 2009). However, cross transmission may occur in settings where cohabitation is common.

Ordinarily, brucellosis is a reproductive disease which, in livestock cause reproductive losses due to abortions, stillbirths, infertility and birth of weak offspring that can have major impacts on productivity through the loss of replacement stock, reduced milk production and family income (Jergefa *et al*., 2009; Karimuribo *et al*., 2007). The *Brucella* species have been isolated from a number of wildlife species such as bison, elk, feral swine, wild boar, fox, hare, African buffalo, reindeer, and caribou, thus wild animals may act as reservoirs to both domestic animals and human (Godfroid *et al.,* 2011; Godfroid *et al*., 2013).

For a human to contract the disease, there must be brucellosis circulating in domesticated or wild animals and possible contamination with infected animals or materials from these animals (Osoro *et al*., 2015). The causative agents of the disease in humans are *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis.* Although, all four species cause brucellosis in humans with different infection intensities, *B. melitensis* is the most common species accounting for most of the severe cases of human brucellosis (Alshaalan *et al*., 2014; Pappas *et al*., 2006a). Other species such as *B. abortus* and *B. canis* cause mild form of the disease while *B. suis* causes more severe symptoms with longer duration (Franco *et al*., 2010; Xavier, *et al*., 2010). Brucellosis is transmitted indirectly from livestock to humans through consumption of infected unpasteurized dairy products, raw or undercooked meat and blood or through direct contact with infected animals, placentas, foetal membranes, vaginal discharges or aborted foetuses and inhalation of aerosolized particulates (Corbel, 2006; Makita *et al*., 2008; Ducrotoy *et al*., 2014).

Acute form of brucellosis in human causes intermittent fever, sweating, joint and low back pains, headaches, fatigue, weight loss and general weakness persisting for a long time (Dean *et al*., 2012a; Madut *et al*., 2018). These clinical signs resemble those of other febrile illnesses such as malaria, typhoid, tuberculosis, rheumatic fever and active lymphoma (Bouley *et al*., 2012; Crump *et al.*, 2013). According to the World Health Organization (WHO), the incidence of human brucellosis worldwide is estimated to be over a half million cases per year, however, this is an underestimation because symptoms of the disease are confused with other endemic febrile illnesses especially in developing countries where diagnostic facilities are a challenge (Pappas *et al*, 2006b; Bonfoh *et al*., 2012). For example, in Tanzania, most of the health facilities do not test for brucellosis because of low awareness of the disease by most of the human health practitioners (Kunda *et al*., 2007; Cash-Goldwasser *et al*., 2018). Therefore, for accurate diagnosis of brucellosis in humans, a good anamnesis of possible contact with animals is needed to rule out other possible febrille illnesses.

Brucellosis is an occupational disease to veterinarians, slaughterhouse workers, butchers, farmers herders and laboratory personnel who commonly get into contact with infected animals or animal by-products in the course of their work (Gardner, 2005; Mirambo *et al*., 2018; Schneider *et al*., 2013). In fact, abattoir workers may be the most risky group compared to other occupations, because they are overexposed to carcasses, viscera, body fluids and

organs of infected animals (Mirambo *et al*., 2018; Mukhtar, 2010). Studies conducted in Tanzania have shown high seroprevalences of brucellosis both in animals destined for slaughter and personnel working in the slaughterhouses (Kiputa *et al*., 2008; Mirambo *et al*., 2018; Swai and Schoonman, 2009). Also, studies conducted elsewhere in Africa have shown similar trends in the slaughterhouses with human seroprevalence ranging from 12.5% to 75.2% (Agada *et al*., 2018; Cadmus *et al*., 2006; Nabukenya *et al*., 2013; Zakaria *et al*., 2018).

The possible risk factors that perpetuate the disease at abattoirs includes poor biosafety measures, slaughter of infected animals and poor infrastructures that lead to cross contamination. Therefore, improved biosafety measures coupled with modern infrastructure may reduce human exposure to brucellosis and other zoonotic pathogens.

1.2 Problem statement and justification

Dodoma modern abattoir receives animals for slaughter from different parts of the country produces meat for export and local consumption. Mwilawa *et al*. (2008) reported that almost 99% of the slaughter animals come from pastoral and agropastoral settings and 1% comes from ranches and dairy farms. This means that a large proportion of animals for slaughter originate from villages where disease control programmes and veterinary services provision are minimal or non-existent. It is true that availability of good veterinary services are the key in disease prevention and control and hence, lack of such services in the livestock keeping communities pose the greatest risk of widespread occurrence of diseases in the livestock population and human including exposure to zoonotic disease, particularly brucellosis (Swai and Schoonman, 2012). Also, the tendency of livestock keepers to sell infected animals unknowingly to the market or to other livestock keepers enhances transmission of brucellosis not only between households in the same village but also between villages or get to the slaughter houses thus, increasing the risk to abattoir workers (Holt *et al*., 2011). Moreover, a large number of animals slaughtered in each day poses an increased risk of exposure of abattoir workers to brucellosis because of handling contaminated carcasses or body fluids (Casalinuovo *et al*., 2016). Under poor hygienic conditions, and infrastructure settings, the environment and facilities of slaughterhouses may be highly contaminated thereby increasing the risk of exposure not only for staff involved in butchery and meat handling but also to the carcasses of other animals that are free from brucellosis. Under this scenario, brucellosis

exposure to slaughterhouse personnel have been reported in Tanzania ranging from 19.5% to 42.1% (Mirambo *et al*., 2018; Swai and Schoonman, 2009). However, little is known in the current modern abattoirs in Tanzania.

Dodoma modern abattoir is one of the abattoirs which is well-designed with separate sections from stunning to packaging, with minimum exposure and with specific personnel trained to comply with biosafety measures and practices such as wearing protective gears. This study intended to assess the role of modern abattoirs in minimising occupational exposures with reference to zoonoses particularly, brucellosis.

Therefore, findings from this study may inform policy on ensuring abattoirs infrastructures are improved and personnel are trained, provided with protective gears and observe biosafety principles to minimise occupational exposures.

1.3 Study objectives

1.3.1 General objective

The general objective of this study was to assess the role of modern abattoirs in minimising occupational exposure with reference to brucellosis that may influence policy.

1.3.2 Specific objectives

The specific objectives of the study were as follows;

(i) To determine the prevalence of brucellosis in abattoir workers and animals brought for slaughter at Dodoma modern abattoir.

(ii) To conduct molecular characterization of *Brucella* species.

(iii) To assess the risk factors associated with *Brucella* exposure in human at the modern abattoir.

1.3.3 Research questions

The research questions which this study wanted to address included the following;

(i) What is the prevalence of brucellosis in abattoir workers and animals destined for slaughter?

(ii) What is the main animal host species that transmits *Brucella* species to humans at slaughterhouse settings?

(iii) What is the main *Brucella* species from animals spilling over to humans?

(iv) What are the main risk factors associated with *Brucella* infection in abattoir workers?

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and nomenclature

The etiological agents of brucellosis are *Brucella* species; small, gram-negative, aerobic, facultative intracellular, coccobacilli, oxidase and urease positive bacteria (Whatmore, 2009). Currently, eleven *Brucella* species are known and are classified based on host species they affect or host preferences, differences in pathogenicity and phenotypic characteristics. They include six *Brucella* species initially recognized to infect cattle (*Brucella abortus*), goats (*Brucella melitensis*), sheep (*Brucella ovis*), pigs (*Brucella suis*), dogs (*Brucella canis*), rodents (*Brucella neotomae*) (Whatmore, 2009). The five other *Brucella* species include *Brucella microti* isolated from common vole, soil (Scholz *et al*., 2008), then from red fox (Scholz *et al*., 2009) and some marine mammals, mostly cetacean (*Brucella ceti*) and seals *Brucella pinnipedialis* (Foster *et al*., 2007). The latest novel species of *Brucella inopinata* was isolated from a human breast implant infection (Scholz *et al*., 2010) and *Brucella papionis* isolated from baboons that had delivered stillborn offspring (Whatmore *et al*., 2014). These two novel isolates have been described and formally published as the tenth and eleventh *Brucella* species, respectively although, their natural reservoir remains unclear (OIE, 2016).

Brucella has cytoplasm encased in a cell envelope made up of an inner membrane, a periplasm and an outer membrane (OM). The OM contains free lipids, outer membrane proteins (OMP) and a lipopolysaccharide (LPS). The LPS is the dominant OM molecule and is critical in *Brucella* virulence and as an antigen (Corbel, 2006). The *B. ovis*, *B. canis* have rough lipopolysaccharides made of a lipid A (containing two types of aminoglycose) linked to an oligosaccharide, other *Brucella* species *B. abortus*, *B. melitensis* and *B. suis*, have smooth lipopolysaccharides with an O-polysaccharide linked to the oligosaccharide as their surface antigens, which are used for detecting antibodies generated by the host during the infection (Liu, 2015). The rough *Brucella* species have a non-canonical structure which makes its lipid-A to convey a stealthy infection following the development of a poor innate immune response (Barquero-Calvo *et al*., 2007).

Antibodies against smooth *Brucella* species cross-react with antigen preparations from *B. abortus*, whereas antibodies against rough *Brucella* species cross-react with antigen preparations from *B. ovis* (Nielsen, 2002).

Brucella O-polysaccharides contain three basic epitopes: A epitope present in *B. abortus* 5 contiguous sugars in α 1- 2 linkage); M epitope occurring in *B.melitensis* and C epitope common to all smooth *Brucella* species (*i.e* A=M). They are distributed in various proportions among smooth *Brucella* species and biovars so that neither A nor M is characteristic of *B. abortus* and *B. melitensis*, respectively (Bundle and Mcgiven, 2017; Zaccheus *et al*., 2013).

Smooth brucella also produces a free polysaccharide called native hapten [NH], a soluble fraction proteins common to all except the S-LPS cross-reacting bacteria, so is very useful for discriminating *Brucella* spp. infections from false-positive serological reactions caused by *Stenotrophomonas maltophilia*, group N (0:30), *Salmonella* spp., *Vibrio cholerae*, *E.coli* 0:157, some *Escherichia hermanii* strains and *Yersinia enterocolitica* 0:9 (Aragon *et al*., 1996; Baljinnyam, 2016).

2.2 Epidemiology of brucellosis

2.2.1 Epidemiology of brucellosis in animals

Brucellosis is a disease with a world-wide distribution, with the highest incidence observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico (OIE, 2016). Currently, countries in central and southwest Asia record the greatest increase in cases (Pappas *et al*., 2006). However, several countries in Western and Northern Europe, Canada, Japan, Australia and New Zealand are considered to be free from the disease (Moreno, 2014; OIE, 2016).

Brucellosis is endemic and widespread in most parts of sub-Saharan African countries including Tanzania, with varying seroprevalences (Ducrotoy *et al*., 2017; Sagamiko *et al*., 2018; Matope *et al*., 2011). In Tanzania, the history of the disease dates back to 1927 when an outbreak of abortion was reported in Arusha region and the first laboratory confirmation of the disease was performed in 1928 (Kitaly, 1984; Mahlau and Hammond, 1962). Since then, the disease is prevalent in domestic and wild animals. Studies conducted in cattle in various regions of Tanzania have shown varying seroprevalence of brucellosis of 3.2-15.2% in

Northern zone (Mahlau, 1967; Mtui-Malamsha, 2001; Schoonman and Swai, 2010; Swai *et al*., 2005), 10.8% in Mwanza (Jiwa *et al*., 1996), 4.2% in Dodoma (Mdegela *et al*., 2005), 5.9-18.4% in Eastern zone (Asakura *et al*., 2018; Lyimo 2013; Rubegwa 2015; Weinhaupl *et al*., 2000), 6% in Kigoma (Chitupila *et al*., 2015) and 6.8-11.4% in Southern highlands (Assenga *et al*., 2015; Chota *et al*., 2016; Sagamiko *et al*., 2018). In goats and sheep, the seroprevalence of 4.6% and 3.4% have been documented (John *et al*., 2010) and 6.5% in a combined small ruminant population in Arusha and Manyara regions (Shirima, 2005).

2.2.2 Epidemiology of brucellosis in humans

Brucellosis is largely transmitted to human through direct contact with infected livestock, placenta material, vaginal discharge or indirect through consumption of contaminated unpasteurized milk and dairy products, raw meat, offals, blood or inhalation of aerosolized materials (Corbel, 2006; Liu, 2015; Makita *et al*., 2008; Shirima, 2005). In Tanzania, the seroprevalence of human brucellosis has been reported to range from 14.1% to 28.2% (Chota *et al*., 2016; Mngumi *et al*., 2016) in pastoral and agropastoral communities, 19.5-42% in slaughterhouse workers (Mirambo *et al*., 2018; Swai and Schoonman, 2009) and 6.2% in patients who presented at the hospital with suspected clinical signs of brucellosis (Kunda *et al*., 2007).

The study conducted in northern Tanzania showed that patients who presented to the hospital with fever, 3.5% were confirmed to have acute brucellosis and only 1.6% had malaria suggesting that the disease resembles other fever associated endemic diseases (Bouley *et al*., 2012; Crump *et al*., 2013). Similarly, a study conducted in Kilosa involving children reported a seroprevalence of 22.4% which indicates that the disease affects people of all ages (Chipwaza *et al*., 2015).

The most effective method for preventing human brucellosis is to control animal brucellosis; other control measures include consuming well-cooked meat, offals, blood, drinking pasteurized dairy products and people who handle animal tissues should protect themselves by wearing rubber gloves, goggles and gowns or aprons (CDC, 2012; Ducrotoy *et al*., 2017; Pérez-sancho *et al*., 2015).

2.3 Pathogenesis of brucellosis

The pathogenesis of brucellosis in humans and animals is related because common events occur during the interaction of the bacteria with susceptible cells in their preferred host (Liu, 2015). The incubation period of brucellosis in both animals and human varies greatly depending on the amount of the infective dose, virulence of the bacteria, previous exposure, innate resistance, age, sex, species and reproductive status of the animal (Bishop *et al.*, 1994). *Brucella* organisms gain entry to the body through ingestion, inhalation, abraded skin, or via the mucous membranes of the pharynx and alimentary tract. The organisms infect both phagocytes and non-phagocytic cells mainly macrophage, dendritic cell and trophoblast respectively while the non- phagocytic cells localise in the rough endoplasmic reticulum (Celli, 2006; Głowacka *et al*., 2018; Salcedo *et al*., 2013). After gaining entry into the body *Brucella* organisms are phagocytised by polymorphonuclear or mononuclear phagocytic cells, where they survive and replicate within these cells without being killed or survive intracellular killing (Carvalho *et al*., 2010; Corbel, 1999).

Brucella organisms use several mechanisms to avoid extracellular and intracellular killing or to suppress macrophage bactericidal responses (Corbel, 1999). These include production of inhibitors such as adenine and guanine monophosphate that inhibit phagolysosome fusion, degranulation and activation of the myelo-peroxidase-halide system and production of tumour necrosis factor-alpha *(TNF-a)* (Caron *et al.*, 1994; Corbel, 1997). The capacity of *Brucella* species to use pathways that avoid *TNF-a* production during infection may be an attribute of virulence (WHO 1998). Gamma interferon (γIFN) has also been found to be a potential activator of macrophages, by reducing *Brucella* growth, but it does not alone result in total elimination of the micro-organisms. The presence of lipopolysaccharides on the brucella surface membrane makes the brucella highly resistant to cationic bacterial peptides and also inhibits complement-mediated killing by blocking access of C1q to the outer membrane targets consequently enhancing survival in the extracellular environment (Pei and Ficht, 2011). The noncanonical structure (long-chain acyl groups on the lipid A) of the *Brucella* LPS in addition, results in a decreased inflammatory response through interaction with the toll-like receptor TLR4 which further enhances survival (Barquero-Calvo *et al*., 2007; Carvalho *et al*., 2010).

Furthermore, once brucellae invade the host cell, specific survival mechanisms are activated to avoid the host's natural defence mechanisms. The type IV secretion complex, the

BvrR/BvrS two-component regulatory systems and the LPS structure all enhance intracellular survival by preventing fusion of phagosome/endosome with the lysosome, controlling the expression of outer membrane proteins (OMPs) and preventing MHC II-peptide expression on the outer cell surface (Celli *et al*., 2003; Głowacka *et al*., 2018; Guzmán-Verri *et al*., 2001). In addition, the unique pathways to access various nutrients utilized by *Brucella* facilitate its long-term survival within the host (Roop *et al*., 2009). Although macrophages are the preferred phagocyte host cell target, the *Brucella* pathogen is also able to infect non-phagocytic cells, epithelial cells, male and female reproductive tissues, respiratory tissues and other tissue types (Xavier *et al*., 2009; Roop *et al*., 2009).

Following invasion in the host, the organisms are carried by neutrophils and macrophages to regional lymph nodes where they multiply, resulting in lymphadenitis followed by bacteraemia that may last for several months and, it may either resolve or recur (Bishop *et al*., 1994; Galińska and Zagórski, 2013). During bacteraemia, *Brucella* organisms are carried intracellularly or free in the plasma and localise in various organs such as the gravid uterus, udder, supramammary lymphnodes, spleen, testes, male accessory sex glands and in synovial structures (Bishop *et al.,* 1994; Galińska and Zagórski, 2013). The sugar alcohol, erythritol, present in the placenta, has been found to be a strong growth stimulant of *B. abortus,* thus accounting for its localisation in the gravid uterus (Carvalho *et al*., 2010). As the infection assumes a chronic form, bacteraemia becomes intermittent and tends to occur around parturition (Jubb *et al.,* 1991).

Following *Brucella* infection, both antibody-mediated and cellular-mediated responses which involve T-cells (CD4 and CD8 subsets) important in cell-mediated protection and smooth lipopolysaccharide for antibody production are activated (WHO, 1998). The early appearance of antibodies is related to the size and virulence of the inocula and host susceptibility. The IgM antibody is the first to be detectable in the serum as it is produced during the acute stage of infection, followed by IgG antibody production at the chronic stage (Berman, 1981). However, on the average antibody level reaches diagnostic titres by four weeks after exposure in heavy pregnant cows and at about 10 weeks after exposure in non-pregnant cows (Berman, 1981). A variation on the duration of antibody detection is dependent on the sex, age, stage of pregnancy and the virulence of the organism (Berman, 1981; Radostits *et al.,* 2000). As the disease advances, the level of IgM wanes and IgG become predominant. It has been shown that humoral response does not provide the main protective immunity but the

cell-mediated response that plays a major role in the defensive mechanism against *Brucella* organisms (Bishop *et al.,* 1994). Calves born from seropositive dams are passively immunized via the colostrum and this interferes with vaccination (Radostits *et al.,* 2000). Usually, the antibodies decline to undetectable levels though few remain immune for a long time (Radostits *et al.,* 2000*).*

Trophoblastic cells are key target cell of *Brucella* infection during late trimester of gestation in ruminants (Samartino and Enright, 1993). The growth of *Brucella* inside trophoblasts is apparently enhanced in the presence of high concentrations of steroid hormones and erythritol during the final late stages of gestation (Samartino and Enright, 1993; 1996). The capacity to replicate rapidly and extensively in the placenta cells can weaken the integrity of the placenta and cause infection of a fetus resulting in abortion or birth of weak offspring in animals (Xavier *et al*., 2010) and in humans (Salcedo *et al*., 2013). Moreover, hormonal changes in infected placentas may have an effect on the occurrence of abortion as an increase in prostaglandin 2α, estrogen and cortisol, and a decrease in progesterone levels mimic what happens during parturition (Gorvel and Moreno, 2002).

2.4 Clinical manifestations in animals and humans

Brucellosis in animals is characterized by late-term abortion, infertility and reduced milk production as a result of retained placenta, endometritis and some degree of sterility in both males and females (Radostitis *et al.,* 2000). For instance in cattle, the main characteristic clinical signs observed are abortion, retained placenta, stillbirths or birth of weak calves and decreased milk production in lactating cows (Jergefa *et al*., 2009; Matope *et al*., 2011). Accordingly, in highly susceptible non-vaccinated pregnant animals, storm abortion occurs in the late months of pregnancy. In male domestic ruminants, orchitis and epididymitis are important cardinal signs observed in the affected animals (Radostits *et al.,* 2006). In addition, hygroma involving one or more leg joints may be observed in all age groups, especially in chronic infections.

Human brucellosis is debilitating disease that may have variable clinical presentations characterized by febrile illness and is often difficult to diagnose sorely from the clinical picture because of similarities to other febrile diseases such as malaria and typhoid fever

(Madut *et al*., 2018). After exposure to the bacteria, clinical manifestations may appear within 5 to 60 days (Young, 1995). The clinical signs manifested in humans affected with acute disease include irregular fever, sweating, arthralgia, low back pain, headaches, fatigue, general body malaise, weight loss, general weakness persisting for a long time, lymphadenopathy and or hepatosplenomegaly (Dean *et al*., 2012). However, few patients develop chronic brucellosis, a more severe form of the disease that can be associated with osteoarticular signs including spondylitis, arthritis and osteomyelitis, or genitourinary changes, such as orchitis, epididymitis, glomerulonephritis and kidney abscesses (Corbel, 2006). Besides, life-threatening complications are seen in some patients which include neurobrucellosis, liver abscesses, and endocarditis (Köse *et al*., 2014).

2.5 Methods of laboratory diagnosis of brucellosis

Brucellosis is diagnosed either by direct tests (bacteriological culture and molecular tests) or indirect tests (*in vivo* and *in vitro* serological tests) (Kaden *et al*., 2017; Poester *et al*., 2010).

2.5.1 Bacterial culture

The gold standard method for diagnosis of brucellosis is the direct bacteriological culture from body fluids and tissues (Yagupsky, 1999). Identification of bacteria is based on their morphology, staining and metabolic profile such as catalase, oxidase and urease tests (Godfroid *et al*., 2010). However, the limitations of this method are possible occupational exposure with the pathogen, laborious, needs biosafety level three laboratory facilities and potential for use of *Brucella* as a bioterrorism agent. Although bacterial culture is expensive and dangerous, it is unequivocal proof of active brucellosis.

2.5.2 Molecular methods

Polymerase chain reaction (PCR) assays can be applied to detect *Brucella* DNA in pure cultures and in clinical specimens such as serum, whole blood and urine samples, various tissues, cerebrospinal, synovial or pleural fluid, and pus (Al dahouk *et al*., 2013; Colmenero *et al*., 2010; Debeaumont *et al*., 2005; Queipo-Ortuño *et al*., 2006; Queipo-Ortuño *et al*., 2008). The PCR is more sensitive than blood cultures and more specific than serological tests (Al dahouk *et al*., 2013).

Molecular assays can be used to characterize brucellosis causing pathogen to species or biovars levels by using multiplex polymerase chain reaction (PCR which uses different primers combinations to identify the genus *Brucella* at the species level and partly, at the biovar level). The first species-specific multiplex PCR, called AMOS PCR assay (AMOS is an acronym from ‘‘abortus-melitensis-ovis-suis’’), comprised of five oligonucleotide primers for the identification of selected biovars of four species of *Brucella* has previously been described (Bricker and Halling, 1994). The five primers used include one single forward primer targeting the brucella-specific insertion element IS711, and four different reverse primers, each specific for a given species. These primers, specific for the various brucella species generate a PCR product of different sizes hence, species are differentiated based on PCR fragment sizes (Scholz and Vergnaud, 2013). In other words, AMOS-PCR is based on the polymorphism arising from species-specific localisation of the insertion sequence IS711 in the *Brucella* chromosome, and comprised five oligonucleotide primers that could identify without differentiating *B. abortus* bv. 1, 2 and 4 but could not identify *B. abortus* bv. 3, 5, 6, and 9. A multiplex AMOS PCR assay with three additional primers has been developed which is able to differentiate *B. abortus* vaccine strains S19 and RB51 from field strains (Ewalt and Bricker, 2000; Lopez-Goni *et al*., 2008). Further development of multiplex assay was made in which a new conventional multiplex PCR (Bruce-ladder) using eight primer pairs in a single reaction was developed and replaced AMOS PCR (Lopez-Goni *et al*., 2008). Later on, Bruce ladder was enhanced to cover novel species such as *B. microti* and *B. inopinata* (Mayer-scholl *et al*., 2010). Although the enhanced Bruce-ladder allows accurate species delineation of all existing species, it is not possible to differentiate the species at the biovar level or below (Scholz and Vergnaud, 2013).

Currently, a novel multiplex PCR assay which differentiates between all present recognized brucella species, including new species *B. ceti* (formerly named ‘*B. maris*’ or ‘*B. cetaceae*’), *B. pinnipedialis* (formerly named ‘*B. maris*’ or ‘*B. pinnipediae*’), *B. microti* and *B. neotomae* and which also allows accurate differentiation of certain biovars of *B. abortus* and *B. suis* is available (Huber *et al*., 2009; Ocampo-Sosa *et al*., 2005). Kang *et al*. (2011) have developed another advanced multiplex PCR which is able to discriminate between *B. suis* and *B. canis* and between *B. suis* and *B. microti* in just one step, and between the vaccine strains *B. abortus* S19, *B. abortus* RB51 and *B. melitensis* Rev.1. This test could also allow the

differentiation of the two marine mammal species, but this deserves further validation on field strains (OIE, 2016).

Real-time PCR is the fastest method for brucella detection in clinical samples. The merit of this method is that, there is no need to analyze the PCR products by agarose gel electrophoresis (Yu and Nielsen, 2010), its high sensitivity in comparison to the conventional PCR (Redkar *et al*., 2001) and reduced sample contamination but expensive especially in resource-poor countries as it needs Taqman probes. The high sensitivity of real-time PCR can detect as few as five bacteria per reaction (Al Dahouk *et al*., 2007; Navarro *et al*., 2006). Moreover, real-time PCR enables high-throughput screening of clinical samples and produces results within a few hours which enhance early treatments of the patient.

A number of methods which are important for adding useful epidemiological information have also been described such as multilocus sequencing scheme (Whatmore and Gopaul, 2011) and several typing schemes based on the use of MLVA (multiple locus variable number of tandem repeats analysis) (Flèche *et al*., 2006; Smirnova *et al*., 2013). Depending on the particular markers chosen, these methods allow isolates to be identified at the species level and provide epidemiological information at the subspecies level.

2.5.3 Serological methods

These are mainly based on the detection of antibodies against the smooth surface lipopolysaccharides (LPS) which are immunodominant antigens of *Brucella* but for the specific detection of *Brucella ovis* and *Brucella canis* infection antibodies against rough LPS of *Brucella* are used. Importantly, depending on the sensitivity and specificity serological tests can be used to screen for or confirm brucellosis. Most of the serological tests are simple, fast, sensitive and less expensive, however, there are chances of having false positive and false negative results because of its oversensitivity especially in vaccinated animals, cross-reaction with a number of other gram-negative bacteria in particular *Yersinia enterocolitica* O:9 and blocking effect or prozone, respectively (Muñoz *et al*., 2005). Therefore, no single serological test is appropriate in all epidemiological situations and in all animal species; all tests have limitations especially when screening individual animals. Consideration should be given to all factors that impact on the relevance of the test method and test results to a

specific diagnostic interpretation or application (OIE, 2016). The commonly used tests in low and middle-income countries are as follows.

(i) Rose Bengal Plate Test (RBPT)

This is the most commonly used serological method of brucellosis diagnosis, which is a simple and rapid spot agglutination test. The test involves mixing of drops of stained buffered antigen at P^H of 3.6-3.7 and serum on a plate and any resulting agglutination signifies a positive reaction (Alton *et al*., 1988). The results are received within four minutes. Rosebengal plate test is an agglutination test that is based on the reactivity of antibodies against smooth lipopolysaccharide (LPS). It uses a suspension of *B. abortus* smooth cells stained with the Rose Bengal dye, buffered to pH 3.65. At neutral p^H, this test can measure the presence of IgM, IgG1 and IgG2. However, IgM appears to be the most active. At acidic condition agglutinating, non agglutinating antibodies are detected and no blocking effects observed. The test is an excellent screening test but may be oversensitive for diagnosis in vaccinated individual animals and occasionally blocking effect (prozone) make strongly positive sera appear negative in RBPT resulting to false positive and false negative, respectively (Díaz *et al*., 2011). RBPT allows the diagnosis of larger proportions of brucellosis cases therefore, should be performed as a first test in any suspicious case of brucellosis. The titer of the test greater than 1:4 is highly indicative of infection this is because as a dilution increase the number of positive samples remains almost the same in infected population while that of in contact population decreases.

(ii) Indirect Enzyme Linked Immuno Sorbent Assay (iELISA)

The method is based on the specific binding of antibodies present in the test sample with immobilized antigen. The binding event is visualized using chemically or enzymatically derived fluorescent, luminescent or colourimetric reaction. Antibodies against smooth LPS are used, which have a common significant disadvantage as it leads to false-positive results and thus reduces the specificity of the test (Muñoz *et al*., 2005; Weynants *et al*., 1996). This is because the O-polysaccharides of *Brucella* are similar to that of *Yersinia enterocolitica* and other gram-negative bacteria (Gall and Nielsen, 2004). Therefore, because of this possibility of getting false positive results, the indirect enzyme-linked immunosorbent assay follow after using sensitive tests such as Rosebengal plate test.

(iii) Competitive ELISA (c-ELISA)

The c-ELISA assay uses the monoclonal antibody (mAb) specific for one of the epitopes of brucella species. In the test serum which has no anti-*brucella* antibody, the mAb binds to the polysaccharide epitope of the S-LPS antigen and is indicated by colour development. It measures the competitive binding between anti-*Brucella* LPS monoclonal antibodies and the host's antibody onto the same reactive site. If the test serum contains brucella specific-antibodies (positive), they compete with the mAb for the epitope sites and inhibit the mAb binding to the O-polysaccharide portion of S-SLP antigen and the subsequent colour formation. Serum obtained from animals vaccinated with smooth brucella vaccines do not compete with the mAb because of their specificity and lower affinity, leading to negative reaction (OIE, 2013). Therefore, this assay is able to distinguish antibodies elicited from brucella infected animals, animals vaccinated with smooth brucella vaccines *e.g.* Strain 19 and animals infected with cross-reacting gram-negative bacteria. This assay is internationally validated and one of confirmatory test for brucellosis (OIE, 2013).

The competitive ELISA (c-ELISA) with smooth brucella LPS as antigen is used for the detection of antibodies against brucella in serum samples from cattle, sheep, goats and pigs. This test is capable of differentiating vaccine antibody response from actual infections, and its sensitivity varies from 92 to 100%, whereas the specificity ranges from 90 and 99% (Godfroid *et al*., 2010; Perrett *et al*., 2010). The test partly solves the problem of false positives because the specific epitopes of *Brucella* O-polysaccharides are used as antigens but its sensitivity is significantly lower than the iELISA (Muñoz *et al*., 2005; Praud *et al*., 2012; Weynants *et al*., 1996). Besides, the test has the advantage of measuring different classes of reactive antibodies including IgG, IgA and IgM, thus, it has a greater ability to differentiate between acute infections and relapsing infection, importantly is the best test for detecting brucella antibody in the cerebrospinal fluid in cases of neurobrucellosis (Araj, 2010; Alshaalan *et al*., 2014).

(iv) Complement Fixation Test

This test is used to detect anti-brucella antibodies that are able to activate complement (Hill, 1963). The complement system consists of a complex series of proteins which when triggered by an antigen-antibody complex react in a sequential manner to cause cell lysis. The test is difficult to standardize and hence, it is being replaced by primary enzyme-linked immunosorbent assays (Poester *et al*., 2010). The complement fixation test is widely used but

it is complex to perform, and so requires good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents (OIE, 2016).

(v) Fluorescence polarization assay (FPA)

The FPA is a simple technique for measuring antigen and antibody interaction and may be performed in a laboratory setting or in the field. It is a homogeneous assay in which analytes are not separated and therefore, very rapid. The assay is based on the physical principle of the mass-dependent change of the molecule's rotation speed in a liquid medium. The smaller the molecule, the faster it rotates and the depolarization of a polarized beam of light occurs (Samartino *et al*., 1999). In FPA the serum sample is incubated with a specific brucella antigen, conjugated with a fluorescent label then, in case there are anti brucella antibodies in the serum the large fluorescently labelled antigen-antibody complex is formed, which can easily be distinguished from the unbound antigen negative control. FPA method has a high specificity but less sensitivity than iELISA (Mcgiven *et al*., 2003).

(vi) Lateral flow assay

This is an immunochromatographic brucella Ig M or Ig G lateral flow assay which is a simplified version of the ELISA test and has a great potential as a rapid point-of-care assay since have higher sensitivity than Rosebengal plate test (Elshemey *et al*., 2014; Quintero *et al*., 2018; Shome *et al*., 2018). The test has high sensitivity and specificity for brucella Ig M and Ig G. The test has a control antibody incorporated in it which forms lines or band when the problem serum is inoculated to see if contains brucella antibodies Ig M or Ig G (Quintero *et al*., 2018). It uses a drop of blood obtained by finger prick and can be done as a bedside procedure in a human or near animal side in animals. The test is rapid, simple and easy to interpret (Christopher *et al*., 2010; Shome *et al*., 2018). The test is a good screening test, gives a lot of information, however, it is expensive compared to other screening tests such as Rose Bengal plate test.

(vii) Serum agglutination test

This test is based on the reactivity of antibodies against the smooth lipopolysaccharide of brucella. As is in most of serological tests for brucella detection, excess of antibodies may results to false negative reaction due to prozone or blocking effect which can be corrected by applying a serial dilution of 1:2 through 1:64 of the serum samples for the purpose of

increasing the test specificity (Afify *et al*., 2013). The test is best performed at a near neutral pH, which makes it more efficient in detecting Ig M antibody; hence, it is best used to detect acute infections. Conversely, it is less effective for detecting IgG which is produced at a later stage of infection, does not detect non agglutinating antibodies resulting in low assay specificity (Corbel, 1972; Nielsen *et al*., 1984). Due to this fact, the serum agglutination test, despite being sensitive, is generally not used as a single test, but rather it is used in combination with other tests. Other shortcomings of the test include false positive and false negative results (Poester *et al*., 2010). For this reason, the test is only suitable for herd testing, rather than for testing individual animals. Furthermore, the presence of post-vaccination antibody can confuse the results (Corbel and Brinley-Morgan, 1984). The SAT does not detect antibodies to *B. canis* and *B. ovis* because these rough strains of the organism do not have OPS on their surfaces (Poester *et al*., 2010).

(viii) Slow agglutination test

The test is based on the sedimentation of the complexes of Ig M antibodies with brucella cell antigens (Alton *et al*., 1988). The reaction requires an overnight incubation at 37˚C hence, slow agglutination test, lacks specificity and sensitivity although it is inexpensive and easy to perform, hence, not commonly used nowadays.

(ix) Coombs test

This is the most suitable and sensitive test for confirmation of relapsing patients with persistent disease (Christopher *et al*., 2010). If serum agglutination test yields negative results due to the presence of blocking antibodies or non-agglutinating antibodies, Coombs test may be used instead after performed serum agglutination test as it has the advantage of detecting non-agglutinating antibodies. Agglutination can be determined visually, as for SAT, by using an agglutinoscope or a drop on a slide examined under the microscope (Araj, 2010). Coombs test is used for the detection of incomplete, blocking or no-agglutinating IgG in the sera.

(x) Immunocapture agglutination test; Brucella Capt

This new anti-Brucella immunocapture agglutination assay detects agglutinating and non agglutinating antibodies and has high sensitivity (Odemir *et al*., 2011). It has been suggested as a possible substitute for the Coombs test and a better marker for disease activity (Odemir *et al*., 2011)

(xi) Dipstick assay

The Ig M dipstick assay detects Ig M antibodies to the smooth LPS. It offers higher sensitivity and easier manipulation than Ig M ELISA to detect Ig M antibodies to brucella species (Taleski, 2010). Ig M dipstick assay could be used as a rapid and simple alternative to the ELISA Ig M for the serodiagnosis of patients with acute brucellosis (Asfaw Geresu and Mamo Kassa, 2015).

(xii) 2-Mercaptoethanol

The 2-mercaptoethanol is a confirmatory test that allows selective quantification of Ig G anti-brucella due to inactivation of Ig M in the test sample. Production of Ig G is usually associated with chronic infection, and therefore, a positive result with this test is a strong indicator of brucellosis (Geresu and Kassa, 2015). Nevertheless, this test has some drawbacks due to the toxicity of mercaptoethanol, which requires a fume hood for its manipulation, and the possibility of Ig G degradation caused by the 2-mercaptoethanol, which may result in false negative results (Poester *et al*., 2010).

(xiii) Milk ring test

The milk ring test is based on agglutination of antibodies secreted into the milk. This test allows screening of a large number of cattle by using milk samples from tanks or pools from several cows. The test is useful for monitoring cattle herds or areas free of brucellosis so it is classified as a surveillance or monitoring test (OIE, 2016). A positive result indicates the presence of infected cattle in the herd so the test should be followed by an individual serological test in the entire herd. False-positive reactions may occur in cattle vaccinated less than 4 months prior to testing, in samples containing abnormal milk such as colostrum or in cases of mastitis. Therefore, it is not recommended to use this test in very small farms where these problems have a greater impact on the test results (OIE, 2016).

2.6 Prevention and control of brucellosis

The measures to control brucellosis are based primarily on vaccination when the prevalence is high, in the initial steps of the control strategies and test-and-slaughter programmes; when the disease prevalence is already very low and economic and technical resources are available in the final stages of the eradication process prior to the achievement of the disease-free status (Liu, 2015). These measures such as vaccination, test and slaughter programmes, and

herd depopulation alone are not fully effective in eradicating the disease in a country, so addition of other harmonizing prevention measures, such as control of animal movements, use of surveillance systems and adequate laboratory support are needed (Ducrotoy *et al*., 2017; Pérez-sancho *et al*., 2015).

Modified live vaccines are available against *Brucella* spp. *B. abortus* S19 (smooth strain) or RB51 (rough strain) and *B. melitensis* Rev.1 are proven effective vaccines against *B. abortus* in cattle and *B. melitensis* in small ruminants, respectively (Elberg, 1996). The S19 strain is the main vaccine used to control bovine brucellosis in many countries for more than 50 years now. Female calfhood vaccination administered intramuscularly or subcutaneously using S19 with full doses 3.5×10^{10} colony forming unit (CFUs) has demonstrated to protect bovine against *B. abortus* infection for the whole productive lifespan (Manthei, 1959; Thakur *et al*., 2012). However, adult vaccination may be recommended in high prevalence areas in which a rapid impact on disease spread is anticipated and in large herds in which test and slaughter strategies are not feasible. In fact, adult vaccination is an emergency measure as may lead to abortion in pregnant cows because of the abortifacient effect of S19 (Olsen and Stoffregen, 2005). Therefore, the use of reduced dose and subconjunctival route is recommended during mass vaccination as this alternative method produces protection without a persistent antibody response and reduces the risks of abortion and excretion in milk (Chand *et al*., 2014). Also, vaccination with RB51 vaccine (rough strain with a minimal expression of O-PS) for the control of *B. abortus* infection has been regarded as an alternative to S19 (Sanz *et al*., 2010). However, its true usefulness and ability to induce a potential degree of protection equivalent to that induced with the S19 vaccine are still under discussion.

In small ruminants, Rev. 1 is the best currently available vaccine to immunize sheep and goats against *B. melitensis*. The vaccine is administered by the sub-conjunctiva route in young (<6 months) female animals at doses of $0.5\text{-}2 \times 10^{9}$ colony forming units CFU/animal. The immunization in high prevalent areas at full doses by the conjunctiva route during the pre-breeding period and late lambing season would be the most suitable approach for whole flock vaccination because it may prevent reproductive failures due to Rev 1 vaccination (Blasco, 2010). Rev 1 is not safe for use in pregnant animals since high rates of abortion are induced in pregnant animals even with reduced doses of vaccine administered via sub-conjunctiva route because of the abortifacient effect of Rev. 1 (OIE, 2016). Like with other live brucella vaccines, Rev 1 is virulent for humans and thus care must be taken to avoid the

risk of contaminating the environment or causing human infection especially to veterinarians and livestock keepers (Liu, 2015; OIE, 2016). Currently, there is no vaccine available for immunization of humans, pigs, or wildlife (Godfroid *et al*., 2011; Godfroid *et al*., 2013). Then, in the absence of a human brucellosis vaccine, prevention of human zoonotic brucellosis depends predominantly on the control of the disease in animals (Godfroid *et al.,* 2005; Pappas *et al*., 2006).

In the developed countries, animal brucellosis control has been successfully achieved through a combination of mass vaccination, test and slaughter programmes, effective disease surveillance and animal movement control while that in humans has greatly been controlled through milk pasteurization, and health education (Mcdermott and Arimi, 2002; Pappas *et al*., 2006). In Israel, eradication of brucellosis in the 1950s was associated with test and cull policy coupled with fully dose subcutaneous S19 vaccination of all replacement females (Banai *et al*., 2018). However, in developing countries, these strategies have not worked out due to inadequate financial resources to compensate livestock keepers whose animals are slaughtered during screening programs and for buying vaccines (Godfroid *et al*., 2011). The disease has been eradicated in Japan, Canada, Australia, New Zealand and several Northern and Central European countries, yet it remains an uncontrolled problem in regions of high endemic such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (OIE, 2016).

Control and eradication of the infection in animal reservoirs is the most rational approach for preventing human brucellosis and through proper handling and disposal of an aborted foetus, foetal membranes and discharges as well as drinking pasteurized milk (Ducrotoy *et al*., 2017). Brucella pathogen hides inside the macrophages, therefore, treatment of brucellosis requires antibiotics which adequately penetrates intracellularly and active in the acidic environment where the bacteria reside (Pappas *et al*., 2006). In humans, treatment with rifampicin at a dosage of 600-900 mg combined with doxycycline at 200 mg daily are used to treat infections to encounter treatment failure due to monotherapy (Corbel, 2006). However, for effective clearance of infection with this combination of drugs needs a long treatment of about six weeks which can have negative impacts on health of human and use of rifampicin as a combination drug is not feasible in Africa as may results into more complicated *Mycobacterium tuberculosis* resistance as the drug is used to treat tuberculosis (Pappas *et al*., 2006). Control of the disease in animals, proper handling of infected or suspected materials,

drinking pasteurized milk and eating properly cooked meat are the best methods to prevent human brucellosis in both rural and urban settings.

Currently, in Tanzania test and slaughter policy is not practised in the control and prevention of brucellosis because of inadequate financial resources and breakdown of public veterinary services (TLP, 2006). National disease control programmes in low and middle-income countries are unlikely to succeed when national veterinary services are weak, uncoordinated and livestock movements are uncontrolled (Swai and Schoonman, 2012). For example, in Tanzania vaccination for bovine brucellosis using *B. abortus* S19 previously practised in state-owned dairy farms, or government-owned ranches were stopped since the 1980s due to financial resources constraints (Shirima, 2005). With this situation, animals that are owned by poor livestock farmers remain susceptible to brucellosis due to their owners' inability to meet the cost of buying vaccines for disease prevention, control and finally transmission to a larger number of animals and humans (FAO, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted at Dodoma modern abattoir in Dodoma municipality which is located between latitudes 6.00° and 6.30° South, and Longitude 35.30° and 36.02° East. Dodoma is the capital city for the United Republic of Tanzania, which is growing very fast both in human population and economic activities resulting in increased demands for meat as a source of protein. Dodoma modern abattoir started to operate since 2004 under the Government of Tanzania but in 2008, it was privatized to Tanzania Meat Company with Government owning 49% of its shares. Dodoma abattoir is the largest and most modern one in the central zone, currently the only in the country slaughtering animals for export. Animals slaughtered at Dodoma modern abattoir originated mainly from Dodoma and neighbouring regions of Iringa, Singida and Manyara as well as some from Lake and Western regions of Kigoma, Tabora, Mwanza, Shinyanga and Geita where brucellosis has been reported to be prevalent in livestock (Chitupila *et al*., 2015). Dodoma modern abattoir slaughters an average of 700 cattle and 4900 goats per week. Meat from goats is mainly exported to Comoro, Iraq, Vietnam, Oman, Qatar, Kuwait and United Arabs Emirates while beef is consumed locally (Masika Personal communication, 2018).

3.2 Study design

A cross-sectional study design was used to investigate and establish *Brucella* species circulating between animals destined for slaughter and abattoir workers through serology and molecular typing and assess risk factors associated with human exposure of brucellosis for better management of the disease at risky settings. A semi-structured questionnaire was used to gather baseline information, risk factors associated with human exposure at the working environment and the knowledge of the disease and other zoonotic diseases.

3.3 Sampling and sample size

A systematic random sampling method was used to select cattle and goats. By using a sampling fraction of 20%, every fifth animal was sampled (Cadmus, *et al*., 2006). Daily,

before starting sample collection all the animals to be slaughtered in that same day were arranged in the crush and five animals counted from which the first animal was randomly selected, then every fifth animal was systematically sampled (Cadmus, *et al*., 2006).

The sample size of animals was determined using the formula:
$N=1.96^2P(1-P)/d^2$ (Naing, *et al*., 2006).
Where; N=Sample size P=Expected prevalence d=Precision level
By using the expected prevalence (P) of 12%, precision level (d) of 0.05 and confidence level of 95%. Thus, 324 samples were to be collected from all animal species.
All abattoir workers above 18 years who have worked for at least one year and consented were sampled. Therefore 62 out of 120 abattoir workers agreed to participate in the study and to be bled

3.4 Data and sample collection

3.4.1 Collection of blood and tissue samples

A total of 390 animals destined for slaughter involving 200 goats and 190 cattle were bled for brucellosis screening and 38 supramammary lymphnodes were collected from pregnant cows. Furthermore, 62 human blood samples were collected from the abattoir workers who consented to participate in the study. The inclusion criteria of individuals to participate included age of at least 18 years and working in the abattoir for at least one year. However, prior to bleeding of animals, verbal consent was sought from livestock traders. Sterile plain vacutainer tubes and EDTA tubes were used to collect approximately 5 ml of blood from the jugular vein in animals and brachial vein in humans. While bleeding individual animal information such as breed, age, sex and geographical origin were recorded. Apart from blood samples supramammary lymphnodes were also collected in pregnant animals after slaughter.

All blood and tissue samples were stored in a cool box with ice packs and transported to the Tanzania Veterinary Laboratory Agency station in Dodoma where blood samples were left at room temperature for six hours to allow separation of clear serum, plasma. The tissues, serum and plasma samples were stored at -20°C before analysis.

3.4.2 Questionnaires

A semi-structured questionnaire was used to assess the knowledge of abattoir workers about brucellosis as well as their attitudes and practices that lead to potential exposure. Social and demographic factors such as sex, age, marital status, education level, period of time worked at the abattoir, types of activities within and outside the abattoir were gathered. The questionnaire was pre-tested and adjusted to improve clarity before actual data collections.The questionnaire was administered in Swahili language because most of the workers were conversant with the language.

3.5 Laboratory analysis

3.5.1 Blood samples processing

Blood samples were centrifuged at 3000g for 20 minutes to obtain clear serum and plasma. These were decanted into1.5 ml cryovials and stored at -20 °C prior to conducting serology and molecular tests.

3.5.2 Rose Bengal Plate Test

Four hundred fifty-two (452) serum samples were screened for anti-brucella antibodies using Rose Bengal buffered antigen (ID Vet, France). The test procedure was performed as recommended by OIE (2016)**.** Briefly, 30 µl of buffered antigen and 30 µl of test serum were pipetted in each well on the white tile plate and then mixed thoroughly. The white tile plate was rocked for four minutes while observing the degree of agglutination. The sample was classified as positive if agglutination was observed and negative if there was no agglutination.

3.5.3 DNA extraction

DNA extraction was performed by using Zymo Quick DNA™ Miniprep Plus kit (Zymo Research, USA) for blood and tissue extraction according to manufacturers' instructions. The DNA was extracted from plasma, serum and supramammary lymphnodes with concentration and purity measured using a NanoDrop Lite spectrophotometer (Thermo Scientific, USA). The quality of the DNA was checked using 1% agarose gel electrophoresis. The DNA was stored at -20°C before further tests were carried out.

3.5.4 Multiplex polymerase chain reaction

The real time multiplex polymerase chain reaction, the AMOS-PCR (*B. abortus, B. melitensis, B. ovis and B. suis* PCR) was used as described previously (Bricker & Halling, 1994) but with minor modifications of the assay environment. Briefly, PCR assay reaction mixture consisted of the following; Master mix (SYBR® green/Rox qpcr master mix 2x, Thermo Fischer Scientific), the four reverse primers (0.2 µM each) of *B. abortus, B. melitensis, B. ovis, B. Suis*, forward primer IS711-specific primer and the nuclease free water. A total of 5 µl DNA template was added per 20 µl reaction mixtures to make a final volume of 25 µl reaction mixture.

The PCR was performed with real time PCR (QuantiStudio™ 6 flex, Applied Biosystem®). The amplification was started with initial denaturation at 95°C for 10 minutes; followed by 45 cycles comprising of denaturation at 95°C for 15 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds. The PCR products were incubated for a further 5 minutes at 72°C to allow elongation of products before storage at 4°C. The agarose gel was prepared by dissolving the agarose powder in 1% TBE (Tris Borate Ethylenediaminetetraacetic acid), stained with EZ vision (Amresco, fountain parkway solon, OH USA). The PCR products were mixed with the loading dye, loaded into the 1.5% agarose gel immersed in the electrophoresis tank containing running buffer TBE and then the band were separated by electrophoresis at 100 V for one hour. Finally, the bands were visualized in UV transilluminator and photographed using a camera.

3.5.5 Ethical consideration

The ethical clearance for conducting this study was granted by the Medical Research Coordinating Committee of the National Institute for Medical Research; reference number NIMR/HQ/R8a /Vol. IX /1627. Permission to conduct the study at the abattoir was sought and granted by the General Manager of the Tanzania Meat Company (TMC). Permission to conduct the study in Dodoma municipality was sought and granted by District Livestock Officer for animal sampling and District Medical Officer for human sampling, respectively. In addition, the verbal consent was sought from the livestock traders after explaining to them the aim and benefit of the study. The research was conducted in compliance with the Tanzania Animal Welfare Act 2008.

3.5.6 Data analysis

Data were stored and cleaned in Microsoft Excel 2007 spreadsheet and analyzed using R software version 3.5.2 ("Kite-Eating Tree" Copyright (C) 2017-The R Foundation for Statistical Computing platform). Descriptive statistics, particularly frequencies and percentages were computed for both animals and humans. Furthermore, categorical dichotomous variables were computed and compared using Chi-square test at the critical probability of $\alpha \leq 0.05$. Moreover, a logistic regression model was used to study the association between exposure variables and prevalence of brucellosis and to find out which risk factor(s) best predicted the likelihood of brucella seropositivity. The multivariate analysis was constructed by backward elimination method involving all statistically significant variables from univariate analysis. The model fit was tested using the likelihood ratio test (LRT) at ≤ 0.05 and chosen based on the lowest Akaike information criterion (AIC). The association of a particular variable was expressed using odds ratio (OR) with a 95% confidence interval (CI).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seroprevalence of brucellosis in abattoir workers and animals

A total of 452 sera were collected and tested in this study comprising of cattle (190), goats (200) and humans (62) samples, respectively. The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3 %, 1.5% and 1.6% based on Rose Bengal plate test, respectively (Table 1).

Table 1: **The seroprevalence of brucellosis in abattoir workers and animals destined for slaughter at Dodoma modern abattoir**

	Rose Bengal Plate Test (RBPT)	
Sample source	N (Positive)	Prevalence (%)
Cattle	190 (14)	7.3
Goats	200 (3)	1.5
Human	62 (1)	1.6
Overall	452 (18)	3.98

4.1.2 Demographic characteristics of participants

A semi-structured questionnaire was administered to 49 (79%) out of 62 abattoir workers who participated during blood collection to assess the knowledge, awareness of brucellosis, other zoonotic diseases and risk factors associated with brucellosis exposure. The remaining participants did not participate in the questionnaire survey because they were retrenched before the study was completed. The participants' age ranged from 21 to 59 years with an overall mean of 31 (± 8.087) years with the majority 49.4 % (n =22) being between 20–30 age group. 59.2 % (n = 29) of the participants had post-primary education. The length of time the participants worked at the abattoir ranged from 1 to 14 years with an average of 5 (± 2.79). years (Table 2). Majority of participants (32.7%) were working in the skinning section followed by cleaners (25.6%). The results showed that 75.5% of the participants had heard of brucellosis and 61.2% had knowledge of zoonoses and their means of transmissions to human.

Table 2: Demographic characteristics of workers at Dodoma modern abattoir

Variables	Levels	Frequency N=49	Percentage (%)
Age	18-30	22	44.9
	31-40	19	38.8
	41-50	6	12.2
	>50	2	4.1
Sex	Male	27	55.1
	Female	22	44.9
Level of education	Primary	20	40.8
	Secondary	23	46.9
	Certificate	1	2
	Diploma	4	8.2
	University	1	2
Marital status	Married	33	67.3
	Single	15	30.6
	Widow	1	2
Section at work	Carcass splitting	1	2
	Cleanliness	13	26.5
	Evisceration	7	14.3
	Meat inspection	6	12.2
	Skinning	16	32.7
	Slaughtering	1	2
Duration of work	1-3 years	9	18.4
	4 – 6 years	30	61.2
	7-9 years	9	18.4
	10-12 years	2	4.1
	>12 years	3	6.1

4.1.3 Molecular characterization of *Brucella* species from abattoir workers and animals

Detection of *Brucella* spp. in serum and plasma samples with *B.abortus* and *B. melitensis* specific primers (IS711) was performed in both animal and human samples. In pregnant cow supramammary lymphnode was also tested for presence of *Brucella* DNA. The amplicon with molecular size of 731bp was produced in a sample obtained from human serum and no band was recorded from animal samples in both serum and plasma samples and in lymphnodes tissue samples (Fig. 1).

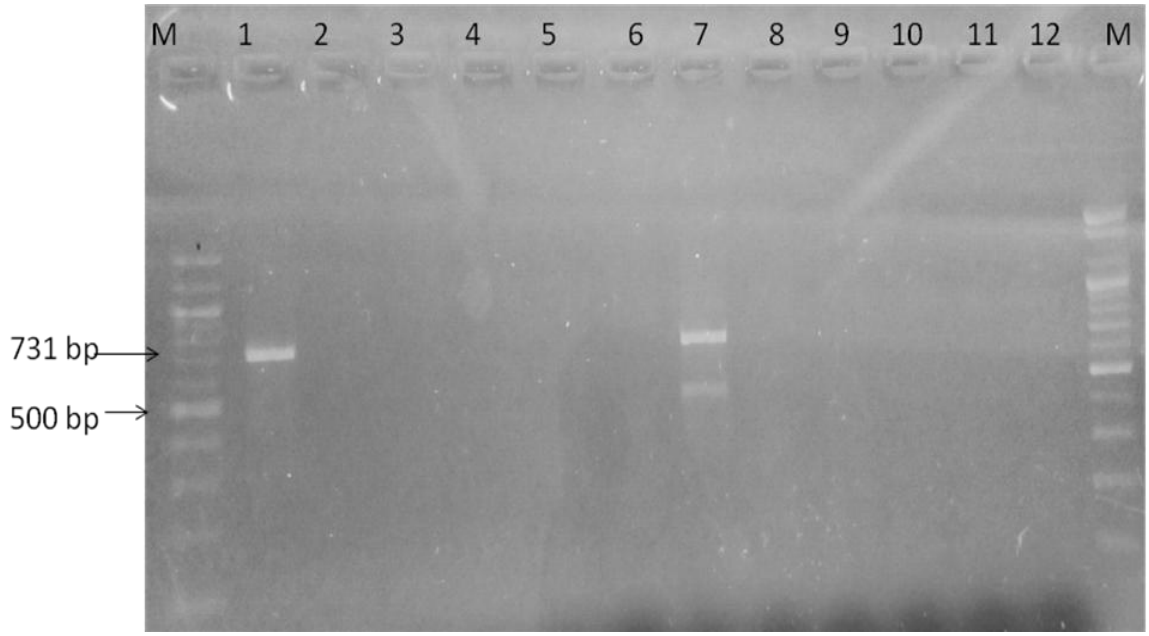

Figure 1: Identification of Brucella by PCR and agarose gel electrophoresis. Lanes: M - 100bp ladder marker; 1- Human sample; 2, 3, 4, 5-Goat samples; 6 Negative control; 7 -Positive control B. melitensis antigen; 8,9,10,11,12 - Cattle samples.

4.1.4 Work-related risk factors and practices that lead to transmission of human brucellosis

The respondents were asked to determine risk factors associated with human brucellosis transmission. In this study, there was no risk factor which was statistically associated with human seropositivity (Table 3). However, it was observed that all personnel wore protective apron, boots, gloves and some face masks.

Table 3: Work-related risk factors for human brucellosis transmission

Variable	Level	N	Seropositive (%)	Seronegative (%)	Odds ratio 95%CI	P-value
Sex	Female	22	1 (4.5)	21 (95.5)	0 (0.0,31.8)	0.449
	Male	27	0	27 (100)		
Age	20-30	22	0	22 (100)		0.551
	31-40	19	1 (5.3)	18 (94.74)		
	41-50	6	0	6 (100)		
	51-60	2	0	2 (100)		
Education	Primary	20	1 (5)	19 (95)		0.5306
	Secondary	23	0	23 (100)		
	Tertiary	6	0	6 (1000		
Occupation	Carcass splitting	1	0	1 (100)		0.6735
	Chilling	1	0	1 (100)		
	Cleanliness	13	1 (7.7)	12 (92.3)		
	Evisceration	7	0	7 (100)		
	Meat inspection	6	0	6 (100)		
	Skinning	16	0	16 (100)		
	Slaughtering	1	0	1 (100)		
Duration of work	≤ 3 years	9	0	9 (100)		0.4694
	4 – 6 years	29	1(3.4)	28 (96.6)		
	7-9 years	9	0	9 (100)		
	10-12 years	2	0	2 (100)		
	>12 years	3	0	3 (100)		

4.1.5 Eating habits

The respondents were asked if they consume raw animals' products and all of them responded that they did not eat raw blood, meat and offals from cattle, goats and sheep neither did they eat soup mixed with blood nor milk.

4.1.6 Proportion of abattoir workers with knowledge and awareness of brucellosis and other zoonoses

The results revealed that 76% of the abattoir workers had heard about brucellosis and among them, 69.4% reported to know that the disease affects both human and animals. In this study, it was revealed that most of the abattoir workers (61.2%) had knowledge on zoonoses. Other zoonoses mentioned included anthrax (6.1%), Rift Valley fever (4.1%), tuberculosis (2%) and helminths (6.1%). Furthermore, 59.2% of the workers had knowledge on how humans can acquire the infection from animals. They reported that consuming raw animal products and direct contact with sick animals or contaminated materials were the risk factors for the disease (Table 4).

Table 4: Proportion of abattoir workers with knowledge and awareness of brucellosis and other zoonoses

Variables	Levels	Frequencies(N=49)	Percentage
Knowledge on zoonoses	Yes	30	61.2
	No	19	38.8
Zoonotic diseases mentioned	Anthrax	3	6.1
	Brucellosis	21	42.9
	Rift Valley fever	2	4.1
	Tuberculosis	1	2
	Helminthosis	3	6.1
	Do not know	19	38.8
Knowledge on disease transmission	Yes	29	59.2
	No	20	40.8
Means of transmission	Drinking raw milk	1	2
	Eating infected meat	8	16.3
	Eating raw meat	1	2
	Removing retained placenta without wearing gloves	1	2
	Through wounds	1	2
	Touching aborted foetus without gloves	6	12.2
	Touching infected meat	5	10.2
	Touching blood	5	10.2
	Touching animals	1	2
	Do not know	20	40.8
Heard about brucellosis	Yes	37	75.5
	No	12	24.5
Affects only human	Yes	1	2
	No	48	98
Affects only animals	Yes	2	4.1
	No	47	95.9
Affects both animals and human	Yes	34	69.4
	No	15	30.6

4.1.7 Knowledge, awareness of abattoir workers on brucellosis and other zoonotic diseases

In assessing the knowledge and awareness of abattoir workers on brucellosis and other zoonotic diseases, it was observed that there was a significant difference in the awareness on brucellosis among sex (p=0.001), occupation (p=0.0048), duration of work (p=0.05) groups while there was no significant difference in the education (p=0.519) and age categories (p=0.181) (Table 5). The participants were 14 times more aware of brucellosis compared to other zoonotic diseases (OR 14, 95%CI 2.47,160.37, p=0.00046) However, there was no statistically significant difference in the knowledge of participants on other zoonotic diseases among sex, age, education, occupation and duration on the job categories ($p > 0.05$).

Table 5: **Knowledge, awareness of abattoir workers on brucellosis and other zoonotic diseases**

Variable	Level	N	Brucellosis (yes)N (%)	χ^2 (df)	*P*-value	Zoonosis (yes)N(%)	χ^2 (df)	*P*-value
Sex	Female	22	22 (100)			16 (72.73)		
	Male	27	15 (55.56)	10.56 (1)	**0.001**	14 (51.85)	1.43 (1)	0.231
Age	18-30	22	16 (72.73)			11(50)		
	31-40	19	13 (68.42)			11(57.89)		
	41-50	6	6 (100)			6 (100)		
	>50	2	2 (100)	28.7 (23)	0.181	2 (100)		
Education	Primary	20	13 (65)			11 (55)		
	Secondary	23	18 (78.26)			13 (56.52)		
	Tertiary	6	6 (100)	3.23 (4)	0.519	6 (100)	4.34 (4)	0.362
Occupation	Carcass splitting	1	1 (100)			1 (100)		
	Chilling	1	0 (0)			0 (0)		
	Cleanliness	13	12 (92.31)	23.7 (9)	**0.0048**	8 (61.54)	11.33(9)	0.254
	Evisceration	7	7 (100)			5 (71.43)		
	Herding	1	1 (100)			1 (100)		
	Meat inspection	6	6 (100)			6 (100)		
	Skinning	16	6 (37.5)			7 (43.75)		
	Slaughtering	1	1 (100)			1 (100)		
	Supervisor	1	1 (100)			0 (0)		
Duration of work in years	≤3	9	4 (44.44)	18.8(10)	**0.05**	4 (44.44)	13.77(10)	0.179
	4-6	29	20 (68.97)			14 (48.28)		
	7-9	9	8 (88.89)			7 (77.78)		
	10-12	2	2 (100)			2 (100)		
	>12	3	3 (100)			3 (100)		

4.2 Discussion

Brucellosis is a zoonotic disease which affects both animals and humans that come into contact with infected live animals, their products such as blood, milk, meat or fetus and placental fluids. Therefore, abattoir workers are among the most risky group for acquiring brucellosis because they frequently come in contact with the carcasses and fluids during their routine activities at the slaughter facilities.

The seroprevalence of 7.3% in cattle destined for slaughter observed in this study is an indication that brucellosis is prevalent in areas where these animals originates. All cattle slaughtered at the abattoir were obtained from the livestock markets in central zone regions of Dodoma and Singida. The prevalence recorded in this study is lower than those reported by other authors in Tanzania such as Swai and Schoonman (2012) and Kiputa *et al*. (2008), respectively. A similar trend of high prevalence was observed in some African countries (Ayoola *et al*., 2017; Cadmus *et al*., 2006; Joseph *et al*., 2015) and these could be attributed to different animal management systems, awareness of cattle keepers about the disease and efficiency of disease control practices.

The seroprevalence of 1.5% in goats brought for slaughter observed in this study is low however, has a major implications in the epedemiology of human brucellosis because the *B. melitensis* which cause brucellosis in goats is the commonest zoonotic *Brucella* species encountered in humans. Similar studies elsewhere in African countries have recorded low prevalence of brucellosis in goats (Nigatu *et al*., 2014; Onunkwo *et al*., 2009) and these could be attributed to different animal management systems, awareness of goat keepers about the disease and efficiency of disease control practices. In proportion 71.5% of goats slaughtered at the abattoir were obtained from the livestock market in central zones regions of Dodoma and Singida and 28.5% in Tabora region.

Encountering exposed animals at abattoirs poses a risk to abattoir workers who do not adhere to basic biosafety principles or where such facilities are unavailable. Although the prevalence of human brucellosis in abattoir workers at the Dodoma modern abattoir was very low (1.6%), elsewhere in the country high prevalences have been reported (Mirambo *et al*., 2018; Swai and Schoonman, 2009). The low prevalence of brucellosis in abattoir workers under the current study may be attributed to several factors prevailing at the Dodoma modern abattoir

compared to other facilities where are limited or absent. At this abattoir, personnel comply with biosafety measures by wearing protective gears such as gloves, gumboots, overcoats/aprons and overhead covers. Infrastructure within the abattoir facilitate unidirectional flow of activities, with specific sections for unique activities that prevents cross contamination. Majority of respondents (78%) had post-secondary education coupled with onjob training contrary to the study by Mirambo *et al*. (2018) who reported that only 21% of the respondents had post-secondary education without onjob training. Conversely, brucellosis awareness was not significantly different among the abattoir workers with different level of education. Hence, the awareness may be a combinations of various factors including level of education, duration working in the similar job and onjob training that imparts lifelong practical experience among abattoir workers. Therefore, poor infrastructure, lack of protective gears, low level of education and lack of onjob training may contributes to high seroprevalences reported elsewhere. At the Dodoma abattoir, interviewed participants were 14 times more aware of brucellosis than other zoonoses. The awareness on the occupational risk of brucellosis among the workers may have enhanced compliance to biosafety measures and practices leading to the low prevalence of the disease. These findings concur with similar studies conducted elsewhere, which demonstrated that wearing personal protective gears and level of knowledge are protective factors for acquiring human brucellosis and other zoonoses (Ayoola *et al*., 2017; Islam *et al*., 2013; Madut *et al*., 2018).

When sections within the abattoir were compared in terms of awareness participants from skinning section had low awareness (37.5%) but the human brucellosis case was from cleaners who were well-informed about zoonoses including brucellosis (93%). The observation may be attributted to the fact that skinners are less frequently incontact with viscera and other fluids compared to cleaners who are frequently incontact with condemned parts and effluents. Swai and Schoonman (2009) and Tsegay *et al*. (2017) also argued that cleaners in the abattoir are much more affected since they are most heavily involved in handling and disposing all condemned abattoir materials including aborted foetuses that usually have very high concentrations of the *Brucella* pathogens. All abattoir workers who were interviewed reported not to eat raw meat, offals, drink raw milk and blood because they are aware that eating these animals by-products while raw predisposes humans to brucellosis. This findings is in agreement with other studies conducted elsewhere (Aworh *et al*., 2013; Cash-Goldwasser *et al*., 2018; Lawrencia, 2015) and hence, abattoir exposure could be the main source of infections.

Detection of *Brucella melitensis* in humans working in the abattoir calls for further studies to establish the role of small ruminants in the transmission of the disease. Recent studies conducted in Ngorongoro district reported *B. melitensis* to be predominant in humans although responsible species and routes of transmission need to be further elucidated (Shirima personal communication, 2019). However, the mathematical modelling done by Vianna *et al.* (2016) revealed that small ruminants could be the source of infection in humans.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The present study has revealed that brucellosis is present both in animals destined for slaughter and humans working at the modern abattoir albeit at low prevalence in humans and goats. The low prevalence of the disease in humans was attributed to the implementation of biosafety measures and having modern infrastructure

Brucella melitensis was detected in one of the workers in the abattoir, which implies that small ruminants may be playing a major role in the transmission of the disease to human beings.

Knowledge and awareness of the abattoir workers on brucellosis coupled with onjob training reduces the risks of contracting brucellosis at abattoir settings.

5.2 Recommendations

It is urged that the regulatory organs should ensure that other slaughterhouses improve their infrastructures with clear slaughter line and biosafety measures instituted.

Encountering animals affected with brucellosis in the food chain calls for the need of more education on the disease to farmers and general public as well as instituting control measures at the farm level.

Vaccination of animals at farm level may reduce animal infections and the risk of transmission to humans.

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APPENDICES

Appendix 1: Informed consent form (English version)

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

P. O. Box 447, Arusha, Tanzania.

SCHOOL OF LIFE SCIENCES AND BIOENGINEERING

Consent to participate in a Research Study: "Assessment of brucella infection status in abattoir workers and animals destined for slaughter at Dodoma modern abattoir, Tanzania**."**

INTRODUCTION

You are being asked to take part in a research study because your have been working in Dodoma abattoir for at least six months. Research studies include only people who choose to take part. Please read this consent form carefully and take your time making your decision. As the study representative discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

Dr. Denice Luwumba and Dr. Gabriel Shirima from Nelson Mandela African Institution of Science and Technology and Prof. Lughano Kusiluka from Mzumbe University will conduct the study. The sponsors of this study, the African Development Bank (AfDB) will pay for this research. If you agree to take part in this study, you will be asked to sign this consent form. You will be given a signed and dated copy to keep.

WHAT IS INVOLVED IN THE STUDY?

If you agree to be in this study, you will first be asked to sign this consent form. We would then like to ask you some questions and we would also like to collect some blood to see if you have been exposed to some diseases that people can get from contact with animals.

Individual questionnaire

A study worker will ask you questions. Some basic information about you like your age, education level and marital status will be collected. We will also ask you questions about your home, the things that you eat and drink, some of your daily activities, and what you know about some illnesses of people and animals.

Sample collection and testing

Study workers will collect a blood sample of approximately 10mL from you so that we can test to see if you have been exposed to disease of animals that can also cause illness in people. The samples collected will be tested to look for disease of animals that can cause illness in people.

Data Handling Procedures

The samples and data that we collect about you will be stored at Nelson Mandela African Institution of Science and Technology (NM-AIST). All samples will be stored in a secure place. The samples will be identified by a unique code. Your name will not be on the samples so no one outside of the study team will be able to identify you from the samples. As part of this study we will analyze the data that we collect from you and the results of the tests that we run on your samples to understand more about the factors that influence which people at section and animals are at greater risk of getting diseases that cause fever.

WHAT ARE THE RISKS OF THE STUDY?

There are minimal physical risks associated with this study. Collection of samples may cause temporary discomfort. Potential risks are uncommon but include brief hemorrhage, local bruising and secondary infection. Discussion of personal details could cause feelings of discomfort, sadness or anxiety. There is a potential risk of loss of confidentiality. Every effort will be made to keep your information confidential; however, this can not be guaranteed.

WHAT ARE THE COSTS?

There will be no any cost to you as a result of being in this study

WHAT ABOUT RESEARCH RELATED INJURIES?

In the event that you are injured as a result of your participation in this research study, you will be referred to your local health care provider. There is no commitment by Nelson Mandela African Institution of Science and Technology, or members of the study team to provide monetary compensation or free medical care in the event of a study related injury.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

There is no any direct benefit from participating but we hope that in the future the information learned from this study will benefit other people in this area and help to prevent human and animal disease.

WILL MY INFORMATION BE KEPT CONFIDENTIAL?

Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security and authorized access. Except when required by law, you will not be identified in the study records disclosed outside of NM AIST. For records disclosed outside NM AIST you will be assigned a unique code number.

The information and data resulting from this study may be presented at scientific meetings or published in scientific journals but your identity will not be revealed. The study results will be retained in your research record forever.

VOLUNTARY PARTICIPATION

You may choose to be in the study, or not to be in the study

WHAT ABOUT COMPENSATION?

We will not be able to provide compensation for any costs associated with your participation in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or if you have complaints, concerns or suggestions about the research, contact Dr Denice Luwumba at 0756 78 40 85. For questions about your rights as a research participant, or to discuss problems, concerns or suggestions related to the research, or to obtain information or offer input about the research, contact the NM AIST Ethics Committee at +255 753 867 382.

SPECIMEN STORAGE

There may be leftover specimens after all testing from the main study is completed. The remaining samples will be stored at the NM AIST Laboratory. All samples will be kept and stored in a secure place. Your samples will be identified by a unique code and your name will not be on the samples. The researchers do not plan to contact you with results from studies done on stored samples.

STATEMENT OF CONSENT

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told that I may contact the NM AIST Ethics Committee at +255753

867 382 if I have questions about my rights as a research subject, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this consent form and agree to be in this study. I have been told that I will be given a signed and dated copy of this consent form."

Name of Subject	Signature	Date

Name of Person Obtaining Consent	Signature	Date

Appendix 2: Informed consent form (Swahili version)

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY
P. O. Box 447, Arusha, T anzania
SCHOOL OF LIFE SCIENCES AND BIOENGINEERING

FOMU YA IDHINI YA RIDHAA

UTANGULIZI

Unaombwa kushiriki katika utafiti kwa kuwa unafanya kazi katika machinjio hii kwa muda wa zaidi ya miezi sita au zaidi. Utafiti huu unahusisha watu pekee ambao wanachagua kushiriki. Tafadhali soma fomu hii ya idhini kwa umakini na uchukue muda kufanya maamuzi yako. Wakati mtafiti akijadiliana nawe fomu hii ya idhini ya ridhaa, tafadhali muulize akuelezee maneno yoyote au taarifa ambazo huzielewi vizuri. Aina ya utafiti, madhara, mambo yasiyopendeza, kusikia vibaya na taarifa zingine muhimu kuhusu utafiti zimeorodheshwa hapa chini.

Wataalamu Daktari Denice Luwumba, Gabriel Shirima kutoka Chuo cha Nelson Mandela cha Sayansi na Teknolojia na Profesa Lughano Kusiluka kutoka Chuo kikuu cha Mzumbe watafanya utafiti huu. Wafadhili wa utafiti ambao ni Benki ya Maendeleo ya Afrika na Chuo Kikuu cha Nelson Mandela cha Sayansi na Teknolojia watagharamia utafiti huu.

Iwapo utakubali kushiriki katika utafiti huu, utatakiwa kuweka saini katika fomu hii ya idhini. Utapatiwa nakala iliyowekwa sahihi kuitunza.

UTAFITI HUU UNAHUSISHA NINI?

Iwapo utakubali kuwa katika utafiti huu,kwanza utaombwa kuweka saini katika hii fomu ya idhini ya ridhaa. Kisha tutapenda kukuuliza baadhi ya maswali na pia kuchukua kiasi cha damu mililita 10 kuona iwapo umeambukizwa na ugonjwa ambao watu wanapata kutokana na kugusana wanyama walioathirika au mazao yake.

<u>Dodoso ya kila mtu</u>

Mtaalam wa utafiti atakuuliza baadhi ya maswali ya msingi kukuhusu wewe kama umri wako, kiwango cha elimu na hali ya ndoa zitakusanywa. Tutakuuliza pia maswali kuhusu nyumbani kwako, vitu unavyokula na kunywa, baadhi ya kazi zako za kila siku na nini unafahamu kuhusu baadhi ya maradhi ya watu na wanyama.

Ukusanyaji wa sampuli na upimaji

Mtaalam wa utafiti atachukua sampuli ya damu kiasi cha mililita 10 kutoka kwako ambayo tunaweza kupima kuona iwapo umeambukizwa na magonjwa ya wanyama ambayo pia yanaweza kusababisha maradhi kwa watu. Tutapima sampuli tutakazokusanya kuangalia baadhi ya magonjwa ya wanyama ambayo yanaweza kusababisha maradhi kwa watu

Taratibu za utunzaji wa taarifa

Sampuli na taarifa tunazochukua kutoka kwako zitahifadhiwa katika Maabara ya Chuo Kikuu cha Sayansi na Teknolojia cha Nelson Mandela. Sampuli zote zitahifadhiwa katika sehemu salama. Sampuli zitatambuliwa kwa namba pekee ya siri. Jina lako halitakuwepo kwenye sampuli hivyo hakuna aliye nje ya utafiti ataweza kukutambua kutoka kwenye sampuli. Kama sehemu ya utafiti huu tutatathimini taarifa tutakazokusanya kutoka kwako na majibu ya vipimo ambavyo tutafanya kutoka kwako kufahamu zaidi kuhusu sababu zinazochangia watu wa kundi fulani na wanyama wanakuwa katika uwezekano zaidi wa kupata magonjwa yanayosababisha homa.

KWANINI UTAFITI HUU UNAFANYIKA?

Madhumuni ya utafiti huu ni kufahamu iwapo wanyama wanaochinjwa katika machinjio hii wana vimelea vya ugonjwa ambao pia unaambukiza na kusababisha homa kwa watu hivyo ni muhimu kufahamu visababishi ili kuweza kutibu kwa ukamilifu.

WATU WANGAPI WATAKUWA KATIKA UTAFITI HUU?

Ni makadirio yetu kuwa takribani watu 100 watajiunga katika utafiti huu. Idadi ya watu watakaojiunga itategemea idadi ya watu katika machinjio hii wataokao kubali kwa hiari yao kushiriki katika utafiti huu.

KUNA MADHARA GANI KWA UTAFITI?

Kuna madhara kidogo ya mwili yanayohusiana na utafiti huu. Ukusanyaji wa sampuli unaweza kusababisha kujisikia vibaya kwa muda mfupi. Uwezekano wa madhara sio kawaida, lakini, unaweza kuhusisha kutoka damu kidogo, kuchubuka kidogo, na maambukizi ya baadae. Majadiliano ya maradhi na taarifa za binafsi yanaweza kusababisha kujisikia vibaya, huzuni au hofu. Kuna uwezekano wa kupoteza usiri. Kila jitihada zitafanyika kuweka taarifa zako kwa usiri; hata hivyo, hii haiwezi kuhakikishwa.

JE KUNA FAIDA KWA KUWA KATIKA UTAFITI HUU?

Unaweza usipate faida yoyote ya moja kwa moja kutoka katika kushiriki lakini, tunatumaini kuwa katika siku zijazo taarifa tutakazojifunza au kupata kutokana na utafiti huu zitasaidia watu wengine katika eneo hili katika kuzuia magonjwa ya binadamu na wanyama.

JE TAARIFA ZANGU ZITATUNZWA KWA USIRI?

Taarifa za utafiti ambazo zinazokutambulisha wewe zitawekwa katika usiri kama inavyohitajika kisheria. Sheria za usiri za Marekani zinatoa ulinzi kwa usiri, usalama na kufikiwa kwa kupata ruhusa. Isipokuwa inapohitajika kisheria, hautaweza kutambulika katika nakala/rekodi za utafiti zitakazotolewa nje ya NM AIST utapangiwa namba ya kipekee ya kutumika. Taarifa na takwimu zitakazotokana na utafiti huu zinaweza zikatolewa katika mikutano/makongamano ya kisayansi au machapisho katika makala za kisayansi lakini utambulisho wako hautatolewa.

USHIRIKI WA HIARI/HAKI YA KUJITOA

Unaweza kuchagua kuwepo au kutokuwepo katika utafiti huu

KUNA GHARAMA GANI?

Hakutakuwa na gharama za ziada kwako kutokana na kuwa katika utafiti huu.

INAKUWAJE KUHUSU MADHARA YANAYOHUSIANA NA UTAFITI?

Katika matukio ambayo utapata madhara/kuumia kama matokeo ya kushiriki kwako katika utafiti huu, utapatiwa rufaa kwenye kituo cha huduma ya afya unachotumia. Hakuna uwajibakaji kwa Chuo Kikuu cha Nelson Mandela cha Sayansi na Teknolojia au wataalamu wa timu ya utafiti kutoa fidia ya kifedha au huduma ya tiba bila malipo/bure katika tukio la kuumia kuhusiana na utafiti.

INAKUWAJE KUHUSU FIDIA?

Hatutaweza kutoa fidia kwa gharama zozote zinazohusiana na ushiriki wako katika utafiti huu.

NITAWASILIANA NA NANI IWAPO NITAKUWA NA MASWALI AU MATATIZO?

Kwa maswali yanayohusiana na utafiti au iwapo una malalamiko, manung’uniko au mapendekezo kuhusu utafiti, wasiliana na Daktari Denice Luwumba kupitia simu 0756 784 085. Kwa maswali yanayohusu haki zako kama mshiriki wa utafiti, au kujadili matatizo, malalamiko au mapendekezo kuhusiana na utafiti huu au kupata taarifa au kutoa mchango katika utafiti, wasiliana na Kamati ya Maadili ya Chuo kikuu cha Nelson Mandeala kupitia +255 753 867 382

UHIFADHI WA SAMPULI

Kunaweza kuwa na mabaki ya sampuli baada ya vipimo vyote katika utafiti huu kukamilika. Sampuli zitakazobaki zitahifadhiwa Katika maabara ya Chuo kikuu cha Nelson Mandela cha Sayansi na Teknolojia. Sampuli zote zitawekwa na kutunzwa sehemu salama. Sampuli zako zitatambuliwa kwa namba pekee ya siri na jina lako halitakuwa katika sampuli hizo. Watafiti

hawana mpango wa kuwasiliana nawe kwa majibu kutoka katika tafiti zilizofanyika katika sampuli zilizohifadhiwa.

TAMKO LA IDHINI YA RIDHAA

"Madhumuni ya utafiti huu, utaratibu wa kufuatwa, madhara na faida vimeelezwa kwangu. Nimeruhusiwa kuuliza maswali, na maswali yangu yamejibiwa nikaridhika. Nimeambiwa kuwa ninaweza kuwasiliana na Kamati ya Maadili ya NM AIST kwa +255 753 867 382 iwapo nina maswali kuhusu haki zangu kama mshiriki wa utafiti, kujadili matatizo, malalamiko, au mapendekezo yanayohusiana na utafiti, au kupata taarifa au kutoa mchango kuhusu utafiti. Nimesoma fomu hii ya idhini na ninakubali kuwa katika utafiti huu. Nimeambiwa kuwa nitapatiwa nakala ya fomu hii ya idhini iliyowekwa sahihi na tarehe”

Jina la Mshiriki	Saini ya Mshiriki	Tarehe
Jina la Mtu Anayepata Idhini	Saini ya mtu anayepata idhini	Tarehe

Appendix 3: Individual questionnaire (English version)

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

SCHOOL OF LIFE SCIENCE AND BIOENGINEERING
P. O. Box 447, Arusha, Tanzania.

Assessment of brucella infection status in abattoir workers and animals destined for slaughter at Dodoma modern abattoir, Tanzania**.**

CS- INDIVIDUAL QUESTIONNAIRE

Section 1 - Interview details

1.1 Interviewer Name……

1.2 Date of interview *(dd/mm/yyyy)* : ……..

1.3 Interview language : ☐ Kiswahili☐ English ☐ Kigogo

Section 2 - Respondent details

2.1 Sex*:* ☐Male ☐ Female 2.2 Age in years.....

2.3 What is your tribe?
☐Gogo ☐ Mrangi ☐Hehe ☐Sandawe ☐nyaturu ☐nyiramba ☐others

2.4. Highest level of education?
☐No education ☐Primary level (1-7 years) ☐Secondary ordinary level
☐High school level ☐Certificate level ☐Diploma level ☐University/college

2.5 Marital status?
☐Married ☐Single ☐Widowed ☐Divorced

2.6 How long have you worked in this abattoir?
2.5.1 Units: ☐ Years ☐ Months
2.5.2 Number: [_][_][_]

2.7 In which section are you working?
☐ Slaughtering ☐ Skinning ☐Evisceration ☐ Carcass splitting

☐ Meat inspection ☐ Chilling ☐ Meat dispatch ☐ Cleanliness
☐ Hide ☐ Other

Section 3 - Resident location

3.1 Region : Dodoma 3.2 District : Dodoma town 3.3 Village:…….

3.4 Subvillage:……..3.5 Ward ……

4 Work related risks/predisposing factors

4.In your daily routine do you directly come in contact with animals and or animal product?
☐ Yes ☐ No
4.1.If yes specify activity(ies)?
4.1.1 Slaughtering ☐ Yes ☐ No 4.1.2 Skinning ☐ Yes ☐ No
4.1.3 Evisceration ☐ Yes ☐ No 4.1.4 Meat inspection ☐ Yes ☐ No
4.1.5 Cleaning ☐ Yes ☐ No 4.1.6 Packaging ☐ Yes ☐ No
4.1.7 Chilling ☐ Yes ☐ No 4.1.8 Dispatching ☐ Yes ☐ No
4.1.9 Disposal of condemnation materials ☐Yes ☐ No

4.2 Do you wear personal protective equipments during your routine activities?
☐Yes ☐ No
4.2.1 Gloves ☐ Yes ☐ No 4.2.2 Mouth cover ☐ Yes ☐ No
4.2.3 Gum boots ☐ Yes ☐ No 4.2.4 Overcoats ☐ Yes ☐ No

4.3 Do you keep domestic animals at home? ☐ Yes ☐ No

4.3.1 If yes which domestic animals
☐ Cattle ☐Goats ☐Sheep ☐Pigs ☐ Others

4.4 Do you asssit your animal(s) during birthing or difficult birthing? ☐ Yes ☐ No

5.0. Meat, blood and offals eating habits

5.1 Do you consume any of the following types of raw meat or offal or raw animal blood?
5.1.1 Raw cow blood? ☐Yes ☐No 5.1.2 Raw goat blood? ☐Yes ☐No
5.1.3 Raw sheep blood? ☐Yes ☐No 5.1.4 Raw meat or offal from cow? ☐Yes ☐No

5.1.5 Raw meat or offal from goat? ☐Yes ☐No 5.1.6 Raw meat or offal from sheep? ☐Yes ☐No

5.1.7 Soup with blood? ☐Yes ☐No 5.1.8 Blood mixed with milk? ☐Yes ☐No

Section 6 Brucellosis and other zoonosis knowledge and awareness

6.1 Do you know of any diseases that people can catch from livestock?
☐Yes ☐ No
If yes, record the name of the first three diseases mentioned
6.1.1: ……

6.2 Do you know how people get diseases from livestock?
☐Yes ☐No
If yes, record the name of the first disease mentioned
6.2.1: ……..

6.3 Have you heard of a disease called brucellosis? ☐Yes ☐No

If no, skip the next question

6.4 Do you know if this disease (brucellosis) affects only animals, only people or both?
☐Only animals
☐Only humans
☐Both

END OF QUESTIONNAIRE

Appendix 4: Individual questionnaire (Swahili version)

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

SCHOOL OF LIFE SCIENCES AND BIOENGINEERING
P.O.Box 447, Arusha,Tanzania.

Assessment of brucella infection status in abattoir workers and animals destined for slaughter at Dodoma modern abattoir, Tanzania

CS- DODOSO LA MTU BINAFSI

Kipengele 1.0- Taarifa za dodoso

1.1 Jina la mdodosaji:……

1.2 Tarehe ya mahojiano(dd/mm/yyyy) : ……..

1.3 Lugha ya mahojiano : ☐ Kiswahili ☐ English ☐ Kigogo

Kipengele 2.0 - Taarifa za mdodoswaji

2.1 Jinsia: ☐ Me ☐ Ke 2.2 Umri:……

2.4 Kabila lako?

☐Mgogo ☐ Mrangi ☐Mhehe ☐Msandawe
☐Wanyaturu ☐wanyiramba ☐Jingine

2.4. Kiwango cha juu kabisa cha elimu yako?

☐ Sijasoma ☐ Msingi (miaka 1-7) ☐ Sekondari kidato cha nne
☐ Sekondari kidato cha sita ☐ Astashahada
☐ Stashahada ☐ Chuo kikuu/chuo

2.5 Hali wa mahusiano ya ndoa?

☐ Nimeolewa/oa ☐ Sijaolewa/oa ☐ Mjane ☐ Nimeachika/acha)

2.6 Ni muda gani umefanya kazi katika machinjio hii?

2.5.1 Units: ☐ Miaka ☐ Miezi

2.5.2 Namba/Idadi: [_][_][_]

2.7 Ni kitengo kipi unafanyia kazi?

☐ Uchinjaji ☐ Uchunaji ☐ Kutoa viungo vya ndani ☐ Kutenganisha nyama
☐ Ukaguaji wa nyama ☐ Chilling ☐ Kupakia nyama
☐ Usafi ☐ Ngozi ☐ Nyinginezo

Kipengele 3.0– Mahali anapoishi

3.1 Mkoa : ..... 3.2 Wilaya :…… 3.3 Mtaa :…… 3.4 Kitongoji :……
3.5 Kata……

Kipengele 4.0 - Visababishi vya Ugonjwa kutokana na kazi ufanyayo

4.Je katika kazi yako huwa unagusa mnyama au mazao yake? ☐ Ndiyo ☐ Hapana
4.1. Kama ndiyo wakati unafanya shughuli ipi?
4.1.1 Uchinjaji ☐ Ndiyo ☐ Hapana 4.1.2 Uchunaji ☐ Ndiyo ☐ Hapana
4.1.3 Kupasua ndani ☐ Ndiyo ☐ Hapana 4.1.4 Kukagua nyama,vitu vya ndani ☐ Ndiyo ☐ Hapana
4.1.5 Usafi ☐ Ndiyo ☐ Hapana 4.1.6 Kufungasha ☐ Ndiyo ☐ Hapana
4.1.7 Upoozaji ☐ Ndiyo ☐ Hapana 4.1.8 Upakiaji ☐ Ndiyo ☐ Hapana
4.1.9 Kutupa vitu visivyotakiwa kuliwa na binadamu ☐ Ndiyo ☐ Hapana
4.2 Je huwa unavaa vifaa au mavazi ya kujikinga unapofanya kazi zako za kila siku? ☐ Ndiyo ☐ Hapana
4.2.1 Glovusi ☐ Ndiyo ☐ Hapana 4.2.2 Kikinga mdomo ☐ Ndiyo ☐ Hapana
4.2.3 Buti ☐ Ndiyo ☐ Hapana 4.2.4 Koti ☐ Ndiyo ☐ Hapana

4.3 Je unafuga wanyama wowote wale nyumbani kwako? ☐ Ndiyo ☐ Hapana
4.3.1 Kama ndiyo ni wanyama wapi?
☐ Ng’ombe ☐ Mbuzi ☐ Kondoo ☐ Nguruwe ☐ Nyinginezo taja

4.4 Je huwa unamsaidia mnyama wakati wa kuzaa au akishindwa kuzaa? ☐ Ndiyo ☐ Hapana

Kipengele 5.0 Ulaji wa nyama, damu na vitu vya ndani

5.1 Je huwa unatumia/kula nyama mbichi au nyama za ndani, au damu mbichi ya mnyama yeyote kati ya hawa?

5.1.1 Damu mbichi ya ng'ombe ☐ Ndiyo ☐ Hapana 5.1.2 Damu mbichi ya mbuzi ☐ Ndiyo ☐ Hapana

5.1.3 Damu mbichi ya kondoo ☐ Ndiyo ☐ Hapana

5.1.4 Nyama mbichi au za ndani kutoka kwa ng'ombe ☐ Ndiyo ☐ Hapana

5.1.5 Nyama mbichi au za ndani kutoka kwa mbuzi ☐ Ndiyo ☐ Hapana

5.1.6 Nyama mbichi au za ndani kutoka kwa kondoo ☐ Ndiyo ☐ Hapana

5.1.7 Kisusio ☐ Ndiyo ☐ Hapana 5.1.8 Damu iliyochanganywa na maziwa ☐ Ndiyo ☐ Hapana

Kipengele 6.0 Ufahamu na uelewa wa Ugonjwa wa Brusela na magonjwa mengine yanayoambukizwa kutoka kwa wanyama

6.1 Je, unajua aina ya magonjwa yoyote ambayo watu wanaweza kupata kutoka kwa mifugo?
☐ Ndiyo ☐ Hapana
Kama ndiyo taja hayo magonjwa 6.1.1: ……

6.2 Je, unajua jinsi gani watu wanaweza kupata magonjwa kutoka kwa mifugo?
☐ Ndiyo ☐ Hapana
Kama ndiyo taja hayo magonjwa 6.2.1: ….

6.3 Je, umewahi kusikia ugonjwa unaoitwa brucellosis/brusela/ugonjwa wa kutupa mimba?
☐ Ndiyo ☐ Hapana
Kama Hapana ruka swali linalofuata
6.4 Je unafahamu kuwa ugonjwa huu (brucellosis/brusela) unaathiri wanyama peke yake, watu tu, au wote?
☐ Wanyama tu
☐ Binadamu tu
☐ Wote

Asante

Mwisho wa dodoso.

RESEARCH OUTPUTS

Output 1: Paper on Occupational hazards associated with human bucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania

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Full Length Research Paper

Occupational hazards associated with human brucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania

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Brucellosis is one of the most widespread zoonotic diseases posing a serious obstacle to public health, food safety and security and, socio-economic development in most African countries. A cross-sectional study was conducted to establish practices that may pose occupational risks of transmission of brucellosis to people working in abattoirs in Tanzania. A total of 452 serum samples; 190, 200 and 62 from cattle, goats and human, respectively were collected in animals and workers at Dodoma abattoir, Tanzania. The samples were screened for brucellosis using Rose Bengal Plate test (RBPT) and indirect enzyme-linked immunosorbent assay (iELISA). A semi-structured questionnaire was used to collect data for assessing the knowledge, awareness and practices related to brucellosis exposure. Data were analyzed to determine the association of brucellosis seropositivity with the knowledge, awareness and practices of the workers. The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3, 1.5 and 1.6%, respectively based on Rose Bengal Plate Test. The seroprevalence was 4.7% in cattle, 1.6% in humans and none in goats when samples were tested by indirect enzyme-linked immunosorbent assay. The results of this study show that, there is a potential occupational risk of acquisition of brucellosis for abattoir workers and hence, the need for awareness campaigns and taking appropriate precautions to minimize the zoonotic risks is greatly required.

Key words: Brucellosis, abattoir, occupational risk, Rose Bengal plate test, indirect enzyme-linked immunosorbent assay.

INTRODUCTION

Brucellosis is one of the most widespread zoonotic diseases posing a serious obstacle to public health, food safety and security and, socio-economic development in most African countries (Mcdermott et al., 2013; Mcdermott and Arimi, 2002). Brucellosis affects many animal species, including such as cattle, sheep, and goats.

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Most human infections are acquired through direct contact with infected animals, placentas, foetal membranes, vaginal discharges or aborted foetuses, inhalation of infectious materials or via indirect transmission through untreated milk and milk products, undercooked meat or blood (Liu, 2015; Makita et al., 2008; Galińska and Zagórski, 2013). Brucellosis has wide-ranging impacts that include animal losses due to abortion, loss in milk production, death of infected animals and debilitating human illness causing reduced working capacity (Jergefa et al., 2009; Mcdermott et al., 2013).

Brucellosis in humans, also known as undulant fever, presents with intermittent fever, sweating, joint and low back pains, headache, fatigue, weight loss and general weakness persisting for a long time (Dean et al., 2012; Madut et al., 2018). These clinical signs resemble other febrile illnesses such as malaria, typhoid, rheumatic fever and viral diseases hence, the disease is often misdiagnosed and under-reported in most countries in sub-Saharan Africa (Bouley et al., 2012; Crump et al., 2013). Brucellosis is an occupational disease to farmers, herders, veterinarians, slaughterhouse workers, butchers and laboratory personnel who commonly get into contact with the animals or animal by-products in the course of their work (Gardner, 2005; Mirambo et al., 2018; Schneider et al., 2013). However, abattoir workers may be the more risky group compared to other occupations because they are overexposed to carcasses, viscera, body fluids and organs of infected animals (Mirambo et al., 2018; Mukhtar, 2010; Swai and Schoonman, 2009), and this is compounded by their proneness to knife cut wounds (Banjo et al., 2013). Consequently, abattoir workers are at greatest risk to get brucellosis through open wounds on bare hands, splashing of infected fluids in the conjunctiva and inhalation of aerosols in the slaughtering area (Aworh et al., 2013; Goncalves et al., 2006). The seroprevalence of human brucellosis in Tanzania has been reported to range from 14.1 to 28.2% (Chota et al., 2016; Mngumi et al., 2016) in pastoral and agropastoral settings.

In Tanzania, brucellosis in slaughterhouse personnel has been previously reported to be high, ranging from 19.5 to 42.1% in several slaughterhouses (Mirambo et al., 2018; Swai and Schoonman, 2009). The high prevalence may be attributed to lack of awareness on the disease among the workers and non-use of protective gears. It is well-known that a well-designed abattoir having separate sections from stunning to packaging with minimum exposure and coupled with strict adherence to biosafety measures and practices can reduce the chances of the occupational exposure to brucellosis (Zakaria et al., 2018). The objective of this study, therefore, was to determine the risk of occupational exposure to brucellosis among workers in Dodoma modern abattoir in Tanzania. The results of the study would be used to inform public health authorities on the magnitude of the problem so that they can design appropriate interventions for reducing the risk.

MATERIALS AND METHODS

Study area

The study was conducted at Dodoma abattoir, one of among the most modern abattoirs in Dodoma Region, Tanzania. Dodoma Region is located between latitudes 6° 57'S and 3° 82'S and between 36° 26' and 35° 26'E and, bordered by four regions, namely; Manyara, Iringa, Singida and Morogoro (Population and Housing Census of Tanzania [PHCT], 2013). Dodoma is the current capital city of the United Republic of Tanzania and is growing very fast in terms of both human population and economic activities resulting in increased demand for food supplies including meat.

Dodoma abattoir is the largest and most modern one in the central zone, currently the only in the country slaughtering animals for export. The animals slaughtered at the abattoir include cattle, goats and in a very few occasions sheep. Meat from goats is mainly exported to Comoro, Iraq, Vietnam, Oman, Qatar, Kuwait and United Arabs Emirates while beef is consumed locally. Dodoma abattoir has well established infrastructures from stunning area to packaging area which facilitates unidirectional flow of carcass. Animals slaughtered at Dodoma abattoir originate mainly from neighbouring regions of Iringa, Singida and Manyara as well as some from Lake and Western zone regions of Kigoma, Tabora, Mwanza, Shinyanga and Geita where brucellosis has been reported to be prevalent in livestock (Chitupila et al., 2015).

Study design

A cross-sectional study design was adopted. It was carried out from March to August 2018 to determine the occupational hazards and practices associated with risk of exposure of abattoir workers to brucellosis and assess their knowledge on the disease and other zoonotic diseases.

Determination of sample size

The sample size of animals was determined using the formula,

$N=1.96^2P\ (1-P)/d^2$ (Naing et al., 2006).

Where; N=Sample size, P=Expected prevalence, d=Precision level.

By using the expected prevalence (P) of 12%, precision level (d) of 0.05 and confidence level of 95%. Thus, 324 samples were to be collected from all animal species.

Sampling procedure

A systematic random sampling method was used to select cattle and goats. By using a sampling fraction of 20% every fifth animal was sampled (Cadmus et al., 2006). Before sample collection, all the animals to be slaughtered in that same day were arranged in the crush and the first animal was randomly selected followed by every fifth animal counted from the first selected in a row (Cadmus et al., 2006). Abattoir workers above 18 years who had worked in the facility for at least one year were included in the study upon their consent to participate in the study.

Collection of blood samples

A total of 390 animals destined for slaughter comprising 200 goats

and 190 cattle were bled for brucellosis screening. Furthermore, 62 human blood samples were collected from the abattoir workers who agreed and consented to participate in the study. The inclusion criteria of individuals to participate included age of at least 18 years and working in the abattoir for at least one year. However, prior to bleeding animals, verbal consent was sought from livestock traders. Prior to bleeding, individual animal information such as breed, age, sex and geographical origin were recorded. Sterile plain vacutainer tubes were used to collect approximately 5 ml of blood from the jugular vein in animals and brachial vein in humans. All blood samples were stored in a cool box with ice packs and transported to the Tanzania Veterinary Laboratory Agency station in Dodoma, where they were left at room temperature for six hours to allow separation of clear serum and blood clots.

A semi-structured questionnaire was used to assess the knowledge of abattoir workers about brucellosis as well as their attitude and practices with regard to the potential of occupational exposure to the disease. Social and demographic factors such as sex, age, marital status, education level, period of time worked at the abattoir, types of activities within and outside the abattoir were gathered. The questionnaire was administered in Swahili language because most of the workers were conversant with the same.

Laboratory analysis

Blood samples were centrifuged at 3000 g for 20 min for serum separation. Serum samples were collected in cryovials, well labelled and stored at -20°C until analysis.

Rose Bengal Plate Test (RBPT)

Four hundred and fifty-two serum samples were screened for anti-brucella antibodies using Rose Bengal buffered antigen (ID Vet, France). The test detects both the IgM and IgG. The test procedure was performed as recommended by (OIE, 2016). Briefly, 25 µl of buffered antigen and 25 µl of test serum were pipetted on the white tile plate and then mixed thoroughly. The white tile plate was rocked for four minutes while observing the degree of agglutination. The sample was classified as positive if agglutination was observed and negative if there was no agglutination. Sample with some degree of agglutinations whether weak or strong was considered positive (Figure 1).

Indirect Enzyme-Linked Immunosorbent Assay (SVANOVIR® Brucella-Ab I-ELISA) was used to further test positive samples from the Rose Bengal plate test. Indirect ELISA measures the binding of secondary antibodies to a primary antibody isotype bound onto the *Brucella* lipopolysaccharides antigen. The test detects only the Ig G. The test was performed according to the manufacturer's instructions. Briefly, 90 µl of sample dilution buffer was added to each well to be used for serum samples and controls followed by adding 10 µl of positive control serum and 10 µl negative control serum, respectively to selected wells coated with *Brucella abortus* antigen. For confirmation purposes, the control sera were run in duplicates.

In the remaining wells coated with *B. abortus* antigen, 10 µl of serum samples was added, shaken thoroughly, sealed and incubated at 37°C for one hour. The plate was rinsed with PBS-tween buffer by filling up the wells at each rinse, emptied and taps hard to remove all remains of fluid. After rinsing, 100 µl of Horseradish peroxidase(HRP) conjugate was added to each well, sealed the plate and incubated at 37°C for one hour, followed by adding 100 µl of substrate solution to each well and incubation for 10 min at room temperature. The reaction was stopped by adding 50 µl of stop solution to each well mixed thoroughly and the optical density of the controls and samples were measured at 450 nm in a microplate photometer within 15 min. The optical density (OD) values were read in a microplate reader (ELISA reader, Multiskan RC version 6.0, Thermo Labsystems, Helsinki). Strong positive (considered as 100% positivity) and negative standards were used. The results were expressed as per cent positivity (PP) of the sample tested in relation to the strong positive control. The criterion for considering a sample positive or negative was based on percent positivity calculated as follows:

$$\text{Percent Positivity (PP)} = \frac{\text{Mean ODD of tested sample}}{\text{Mean ODD of Positive control}} \times 100$$

A sample was considered positive or negative using the cut-off values recommended by the manufacturer. Samples with equal or above 60% positivity were considered positive.

Data analysis

Data were stored and cleaned in Microsoft Excel spreadsheet and analyzed using R software version 3.4.3 ("Kite-Eating Tree" Copyright (C) 2017 The R Foundation for Statistical Computing platform). Descriptive statistics, particularly frequencies were computed for proportions of *Brucella* positive animals and human. Categorical dichotomous variables were computed and compared using Chi-square test at a critical probability of $\alpha \leq 0.05$. Odds ratio, 95% confidence interval, Chi-square and Fisher's exact tests were computed to determine the degree of association between *Brucella* seropositivity with knowledge, awareness and practices of abattoir workers.

Ethical consideration

The ethical clearance for conducting this study was granted by the Institutional Review Board of Medical Research Coordinating Committee of the National Institute for Medical Research; reference number NIMR/HQ/R8a /Vol. IX /1627. Permission to conduct the study at the abattoir was sought and granted by the General Manager of the Tanzania Meat Company (TMC). Permission to conduct the study in Dodoma municipality was sought and granted by District Livestock Officer for animal studies and District Medical Officers for human studies, respectively. Additionally, the verbal consent was sought from the livestock traders after explaining to them the aim and benefit of the study. The research was conducted in compliance with the Animal Welfare Act 2008 and using guidelines and protocols stipulating how human subjects are used in research.

RESULTS

The seroprevalence of brucellosis in abattoir workers and animals slaughtered at Dodoma abattoir

The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3, 1.5 and 1.6% based on Rose Bengal plate test, respectively. The positive sera by Rose Bengal plate test were further tested by enzyme-linked immunosorbent assay and only nine (4.7%) cattle and one (1.6%) human samples were positive. All samples from goats were seronegative (Table 1).

Demographic characteristics of participants

A total of 62 (51.7%) out of the 120 abattoir workers volunteered for blood sample collection and of these, 49

Table 1. The seroprevalence of brucellosis in animals and abattoir workers at Dodoma abattoir.

Sample source	Rose Bengal plate test (RBPT)		Enzyme-linked immunosorbent assay (ELISA)	
	No (Positive)	Prevalence (%)	No (Positive)	Prevalence (%)
Cattle	190 (14)	7.3	190 (9)	4.7
Goats	200 (3)	1.5	200 (0)	0.0
Human	62 (1)	1.6	62 (1)	1.6
Total	**452 (18)**	**3.98**	**452 (10)**	**2.1**

Table 2. Demographic characteristics of workers at Dodoma abattoir.

Variable	Levels	Frequency (N=49)	Percentage
Age	20-30	22	44.9
	31-40	19	38.8
	41-50	6	12.2
	51-60	2	4.1
Sex	Male	27	55.1
	Female	22	44.9
Level of education	Primary	20	40.8
	Secondary	23	46.9
	Certificate	1	2.0
	Diploma	4	8.2
	University	1	2.0
Marital status	Married	33	67.3
	Single	15	30.6
	Widow	1	2.0
Section at work	Carcass splitting	1	2.0
	Cleanliness	13	26.5
	Evisceration	7	14.3
	Meat inspection	6	12.2
	Skinning	16	32.7
	Slaughtering	1	2.0
Duration of work	1-3 years	9	18.4
	4 – 6 years	30	61.2
	7-9 years	9	18.4
	10-12 years	2	4.1
	13-15 years	3	6.1

(79%) also participated in semi-structured questionnaire survey. The remaining participants did not participate in the questionnaire survey because they were retrenched before the study was completed. The participants' age ranged from 21 to 59 years with the overall average age of 31 ± 8.087) years and the majority, 44.9% (n =22) were in the 20–30 years age group. Most of the participants were males 55.1% (n = 27) and 59.2% (n = 29) had post-primary education. The participants duration of work at the abattoir ranged from 1 to 14 years' duration of work at the abattoir ranged from 1 to 14 years with an average age of 5 ± 2.79) years (Table 2).

Table 3. Awareness of abattoir workers on brucellosis and other zoonotic diseases.

Variable	Level	No	Brucellosis (Yes) No (%)	χ^2 (df)	*P*-value	Zoonosis (Yes) No (%)	χ^2 (df)	*P*-value
Sex	Female	22	22 (100.0)			16 (72.73)		
	Male	27	15 (55.56)	10.56 (1)	**0.001**	14 (51.85)	1.43 (1)	0.231
Age	20-30	22	16 (72.73)			11(50.0)		
	31-40	19	13 (68.42)			11(57.89)		
	41-50	6	6 (100.0)			6 (100.0)		
	51-60	2	2 (100.0)	28.7 (23)	0.181	2 (100.0)		
Education	Primary	20	13 (65.0)			11 (55.0)		
	Secondary	23	18 (78.26)			13 (56.52)		
	Tertiary	6	6 (100.0)	3.23 (4)	0.519	6 (100.0)	4.34 (4)	0.362
Occupation	Carcass splitting	1	1 (100.0)			1 (100.0)		
	Cleanliness	13	12 (92.31)	23.7 (9)	**0.0048**	8 (61.54)	11.33(9)	0.254
	Evisceration	7	7 (100.0)			5 (71.43)		
	Meat inspection	6	6 (100.0)			6 (100.0)		
	Skinning	16	6 (37.5)			7 (43.75)		
	Slaughtering	1	1 (100.0)			1 (100.0)		
Duration of work (years)	≤3	9	4 (44.44)	18.8(10)	**0.05**	4 (44.44)	13.77(10)	0.179
	4-6	29	20 (68.97)			14 (48.28)		
	7-9	9	8 (88.89)			7 (77.78)		
	10-12	2	2 (100.0)			2 (100.0)		
	13-15	3	3 (100.0)			3 (100.0)		

Awareness of abattoir workers on brucellosis and other zoonotic diseases

In assessing the awareness level of abattoir workers on brucellosis and other zoonotic diseases, it was found that there is a significant difference in awareness on brucellosis among sex (p=0.001), occupation (p=0.0048), duration of work (p=0.05) groups while there was no significant difference in the education (p=0.519) and age categories (p=0.181). The participants were 14 times aware of brucellosis than other zoonotic diseases (OR 14, 95%CI 2.47,160.37, p=0.00046) (Table 3). However, there was no statistically significant difference in the knowledge of participants on other zoonotic diseases among gender, age, education, occupation and duration on the job categories (p > 0.05). Furthermore, 76% (n=37) of abattoir workers had knowledge of brucellosis and 61% (n=30) other zoonoses

DISCUSSION

Brucellosis is a zoonotic disease which affects both animals and humans who come in contact with infected animals and their products such as blood, milk, meat or foetus and placental fluids as well as other contaminated materials. Therefore, abattoir workers are at the greatest risk of acquiring brucellosis because they frequently come in contact with the animal and by-products during their routine activities.

The seroprevalence of 4.7% in slaughtered cattle in this study is an indication that brucellosis is prevalent in areas where the slaughtered animals originated. This poses a risk to abattoir workers who do not comply with basic biosafety principles in slaughter facilities. This prevalence recorded in this study is lower than those reported by other workers such as 12% in Tanga (Swai and Schoonman, 2012) and 21% in Karagwe (Kiputa et al., 2008). This finding may probably suggest that slaughter animals may have originated from areas with low infection rates.

Although the prevalence of brucellosis in humans was low (1.6%), it still indicates that workers at the abattoir were at risk of acquiring brucellosis in the course of their work, that is handling live animals and carcases. The observed prevalence was also low when compared to findings recorded in other studies in Tanzania (Mirambo et al., 2018; Swai and Schoonman, 2009) and elsewhere (Agada et al., 2018; Aworh et al., 2013; Cadmus et al., 2006; Mukhtar, 2010; Nabukenya et al., 2013; Osoro et

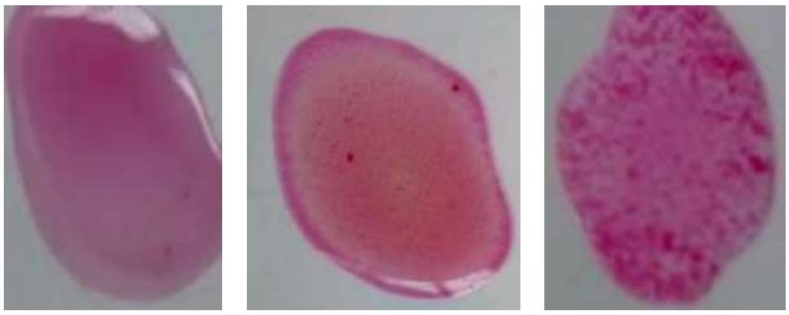

Figure 1. Rose Bengal plate test showing agglutinations. From left No agglutination, Moderate agglutinations, Strong agglutunations.

al., 2015). The difference may be attributed to wearing of protective gears, awareness through training and separation of various processes in the abattoir to enhance proper handling and cleanness. It was observed that all workers in the abattoir wear gloves, gumboots, overcoats and overhead covers, and a few also wore mouth covers and goggles. This was further supported by the fact that 59% of the workforce had post-primary education and 76% were aware of brucellosis. The interviewed participants were 14 times more aware of brucellosis than other zoonoses. The awareness on the occupational risk of brucellosis among the abattoir workers may have enhanced compliance with biosafety measures and practices leading to low prevalence found in this study. These findings concur with similar studies conducted elsewhere, which demonstrated that wearing personal protective gears and level of knowledge are protective factors for acquiring human brucellosis and other zoonoses (Ayoola et al., 2017; Islam et al., 2013; Madut et al., 2018).

In this study, it was observed that there was a statistical significant difference ($p<0.05$) in the knowledge of brucellosis among workers working in different sections. Sections having individuals with low awareness may be more prone to infection if the frequency of contact is high with susceptible infectious materials. The human seropositive case reported in this study was from the group of cleaners. Swai and Schoonman (2009) and Tsegay et al. (2017) argued that cleaners in the abattoir being the least educated group may lack knowledge on how brucellosis is transmitted. On the other hand, since they are most heavily involved in handling and disposing all condemned abattoir materials including aborted foetuses that usually have very high concentrations of *Brucella* pathogens; they are at a much higher risk of infection when compared to other groups.

Although the abattoir workers had some knowledge on brucellosis but it was observed that some of them had the tendency of opening the reproductive tract of slaughtered animals and aborted foetuses within the facility and sold them to vendors outside the abattoir premises. This practice may contribute to the contamination of the carcasses and environments resulting into increased risks of transmission of human brucellosis. Other studies have reported that consumption of gravid uteri increases the risks of humans to contract brucellosis (Adesokan et al., 2016). The reproductive tract is the predilection site for *Brucella* and it has to be removed from the animal without opening it in order to minimize the chances of dissemination of *Brucella* organisms thereby contaminating the meat and the surrounding environment. This increases the risk of transmission of the pathogen to the abattoir workers, meat vendors and the public in general. Therefore, training of all abattoir personnel on the proper practices, including proper disposal of reproductive organs should be emphasised. Despite the fact that the prevalence of human brucellosis was low in this study, the importance of human life underscores the need to undertake more systematic studies using the one-health approach in order to establish the magnitude of the problem country-wide as the basis for recommending practical control measures for the disease.

In conclusion, the present study has revealed that brucellosis is present both in animals slaughtered at the abattoir and humans working in the same facility. In view of this, there is a need for adopting biosafety measures such as wearing protective gear as well as educating the workers on the occupational risk of the disease in order to reduce its transmission. It is also recommended that integrated approaches be used in controlling the disease at the farm level in order to minimize transmission to abattoir workers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLIMENTS

S1.docx: Questionnaire
S2:Rose Bengal plate test

Output 2: Poster presentation

ASSESSMENT OF BRUCELLA INFECTION STATUS IN ABATTOIR WORKERS AND ANIMALS DESTINED FOR SLAUGHTER AT DODOMA MODERN ABATTOIR, TANZANIA

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Introduction

Brucellosis is an endemic bacterial zoonotic infection and a serious obstacle to public health, socio-economic development, food safety and security in most low and middle-income countries (Corbel, 2006; Dean *et al*., 2012; Mcdermott *et al*., 2013). It affects most marginalized poor livestock keeping communities, therefore, its impacts is most seen in rural areas where people's livelihood depend heavily on livestock production or sales of dairy products. It always poses risk of human infection due to close contact between livestock and humans and consumption of unsafe animal products (Baddour, 2012). The impact of disease is very big as evidenced by a study conducted in India which estimated the cost of brucellosis on livestock production to be USD 3.4 billion (Singh *et al*., 2015). Brucellosis is classified as one of the eight neglected endemic zoonotic diseases which contribute to the continuation of poverty in developing countries (Mableson *et al*., 2014).

Objectives

(i) To determine the prevalence of brucellosis in abattoir workers and animals brought for slaughter at Dodoma modern abattoir.
(ii) To conduct molecular characterization of *Brucella* species.
(iii) To assess the risk factors associated with *Brucella* exposure in human at the modern abattoir

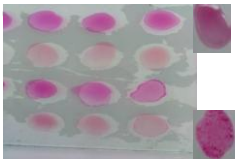
Materials and Methods

The study was conducted at Dodoma modern abattoir in Dodoma municipality which is located between latitudes 6.00° and 6.30° South, and Longitude 35.30° and 36.02° East

A cross-sectional study was conducted to determine the prevalence of brucellosis in abattoir workers and animals destined for slaughter; molecular characterization of *Brucella* species; and assess risk factors, knowledge, awareness and practices associated with transmission of human brucellosis at an abattoir setting.

A total of 452 blood samples; 190, 200 and 62 from cattle, goats and humans, respectively were collected in animals and workers at Dodoma modern abattoir, Tanzania.

Rose Bengal Plate Test



Real time PCR

Multiplex polymerase chain reaction

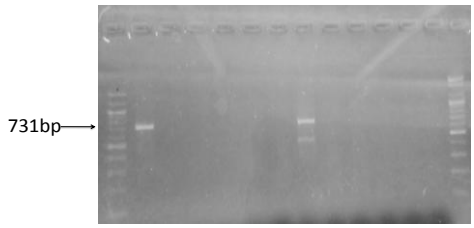


Figure 2:Identification of DNA amplified fragments by agarose gel electrophoresis. Lanes: M -100bp ladder marker; 1- Human sample; 2, 3, 4, 5-Goat samples; 6 -Negative control; 7 -Positive control *B. melitensis*;8,9,10,11,12 - Cattle samples

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Results and Discussion

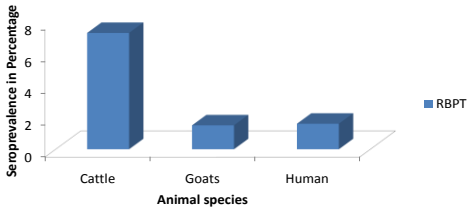

Figure 1: Seroprevalence of Brucellosis in abattoir workers and animals destined for slaughter

Table 2: Proportion of abattoir workers with knowledge and awareness of brucellosis and other zoonoses

Variables	Levels	Frequencies N=49	Percentage
Knowledge on zoonoses	Yes	30	61.2
	No	19	38.8
Zoonotic diseases mentioned	Anthrax	3	6.1
	Brucellosis	21	42.9
	Rift Valley fever	2	4.1
	Tuberculosis	1	2
	Helminthosis	3	6.1
	Do not know	19	38.8
Knowledge on disease transmission	Yes	29	59.2
	No	20	40.8
Means of transmission	Drinking raw milk	1	2
	Eating infected meat	8	16.3
	Eating raw meat	1	2
	Removing retained placenta without wearing gloves	1	2
	Through wounds	1	2
	Touching aborted foetus without gloves	6	12.2
	Touching infected meat	5	10.2
	Touching blood	5	10.2
	Touching animals	1	2
	Do not know	20	40.8
Heard about brucellosis	Yes	37	75.5
	No	12	24.5
Affects only human	Yes	1	2
	No	48	98
Affects only animals	Yes	2	4.1
	No	47	95.9
Affects both animals and human	Yes	34	69.4
	No	15	30.6

The low prevalence of brucellosis in abattoir workers under the current study may be attributed to several factors prevailing at the Dodoma modern abattoir compared to other facilities where are limited or absent. At this abattoir, personnel comply with biosafety measures by wearing protective gears such as gloves, gumboots, overcoats/aprons and overhead covers. Infrastructure within the abattoir facilitate unidirectional flow of activities, with specific sections for unique activities that prevents cross contamination. Majority of respondents (78%) had post-secondary education coupled with onjob training contrary to the study by Mirambo *et al*. (2018) who reported that only 21% of the respondents had post-secondary education without onjob training. Conversely, brucellosis awareness was not significantly different among the abattoir workers with different level of education. Hence, the awareness may be a combinations of various factors including level of education, duration working in the similar job and onjob training that imparts lifelong practical experience among abattoir workers. Therefore, poor infrastructure, lack of protective gears, low level of education and lack of onjob training may contributes to high seroprevalences reported elsewhere

Conclusion

The low prevalence of human brucellosis at the abattoir may be attributed to the institution of biosafety measures and training of the workers. Therefore need of more education on the disease to farmers, abattoir workers and general public as well as instituting control measures at the farm level and biosafety measures at abattoir setting

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