SEROPREVALENCE OF BRUCELLOSIS IN PIGS IN SMALLHOLDER FARMS AND LIVESTOCK TRAINING CENTRES IN MPWAPWA DISTRICT OF DODOMA REGION, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Porcine brucellosis is a contagious bacterial zoonotic disease of public health importance worldwide caused by *Brucella suis* .This study aimed to estimate seroprevalence of brucellosis in pigs and potential risk factors for transmission. An epidemiological cross-sectional study was carried out between December 2019 and March 2020 in Mpwapwa district of Dodoma region in Tanzania. A total of 23 villages and 144 pig-keeping households were randomly selected and included in the study. At the household level, two pigs were randomly sampled from herds with less than 10 where in households with more than 10 pigs three pigs were selected for blood sampling. A total of 349 serum samples were collected, (324 from smallholder pig farmers and 25 from the livestock training centres). Samples were transported to the microbiology laboratory at Sokoine University of Agriculture in a cold chain. Rose Bengal Plate Test (RBPT) was used to test for *Brucella* antibodies present in the sera. Out of the 349 pigs tested, 8 (2.3 %) were positive for *Brucella* antibodies, all positive sera were from eight different smallholder pig farms. There was lower seroprevalence in younger pigs (1.7 %) than older pigs (2.3%). In addition, females were more infected (3.8 %) than males (1%). However, the differences were not statistically significant between age and sex ($P>0.05$). Questionnaire survey results showed many pig farmers were not aware that pigs could get infected with brucellosis and transmit to human. Also there was significantly low knowledge on *Brucella* transmission among pig farmers. This study recommends educational campaigns in the study communities concerning with brucellosis transmission as well as further investigations on brucellosis to prevent its implications in public health and livestock production.

DECLARATION

I, Princepius Sebastian, declare to neither Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Princepius Sebastian Date

(MSc. Candidate)

The above declaration is confirmed by supervisor

_____ _____

Prof. Helena A. Ngowi Date

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DEDICATION

This dissertation is dedicated to my parents for laying the foundation of my education career.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
χ^2	Chi square
APHIS	Animal and Plant Health Inspection Service Brucella Mycobacterium Reagents Avenue
BST	Brucellin Skin Test
c-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CFSPH	Centre for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence Interval
CVMBS	College Of Veterinary Medicine and Biomedical Science
e.g	Example
FAO	Food and Agriculture Organization of the United Nation
FPA	Fluorescence Polarization Assay
LITA	Livestock Training Agency
OD	Optical Density
OIE	Office International des Epizooties/World Organization for Animal Health
P value	Probability Value
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
Spp	species
STAT	Standard Tube Agglutination Test
SUA	Sokoine University of Agriculture
USDA	United States of America Department of Agriculture
WHO	World Health Organization
µl	Microliter
^{0}C	Degrees Celcius

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Brucellosis is an infectious contagious disease of domestic and wild animals with the serious zoonotic consequences in humans (Coelho *et al.*, 2015). It is caused by bacteria of the genus *Brucella* (Hadush and Pal, 2015). It was first diagnosed in humans by David Bruce through isolation of the causative organism from fatal cases in 1887 (Mantur, 2007). Historically, brucellosis in pigs was diagnosed for the first time in Indiana by Traum 1914 through isolation bacteria from aborted of porcine fetuses and further isolates were obtained from swine fetuses in 1916 which were used to demonstrate pathogenicity of the bacterial isolates in swine (Olsen and Tatum, 2016).

Brucellosis affects human being and a wide range of animals such as pigs, cattle, sheep, pigs, dogs, water buffalos, horses and recent infections has been observed in marine mammals). The disease is mostly transmitted when human being consumes contaminated raw or partially cooked animal products with *Brucella spp.* or when a human or an animal with skin cuts or injuries comes into contact with blood, placenta, fetuses or uterine secretions of an infected animal (Corbel, 2006). In addition, breeding diseased animals have been found to increase the chance of brucellosis transmission between susceptible animal species (Maes *et al*., 2008; Megid *et al.*, 2010).

The disease is endemic in most Sub-saharan African countries, including Tanzania (Karimuribo *et al.*, 2007), causing substantial morbidity in both humans and their livestock (Carugati *et al*., 2018).

Brucellosis in pig is characterized by abortion in pregnant sows, epididymitis, and orchitis in male animals (Olsen and Tatum, 2016). In humans the disease has an incubation period ranging from three weeks to several months and it is accompanied by several symptoms but commonly are undulating fever, though abortion in pregnant women has been reported (Megid *et al.,* 2010). Brucellois is diagnosed in the laboratory by isolation of the bacteria, serological and molecular methods (Corbel, 2006; Srivastava *et al.,* 2015).

1.2 Problem Statement and study Justification

Brucellosis is among the neglected diseases despite being common in Tanzania and other developing countries, information on prevalence and distribution is scarce although there are fragmented reports on infections in both animals and humans, presumably leading to morbidities and low economies in the livestock sector in terms of production losses and prevention costs (Mirambo *et al.*, 2018).

Furthermore, a study conducted at Mpwapwa livestock Research Institute in Dodoma region, central Tanzania between 2005 and 2010 following abortion storm in cattle, found that 12 people out of 120 livestock keepers were infected with brucellosis. (Shirima *et al.*,2014). This study recommended more epidemiological studies in any other animal species available.

In addition a study of Simon *et al*. (2015) sampled 414 pigs in five pig slaughter slabs in Dar es Salaam city Tanzania, reported two cases of brucellosis in pigs originating from the central zone of Tanzania, which includes Mpwapwa district. Also, some of the pig farmers in Mpwapwa district manage their pigs on free-range and semi-intensive systems especially in rural areas (Munisi *et al.,* 2006). Many cases of abortion, still birth

and retained placenta have been reported to Mpwapwa district veterinary office (weekly report), likewise some of the pigs fed on food left over, fresh animal blood, and rumen liquor collected from slaughter slabs (person communication). Due to these potential risk factors, there were possibilities of brucellosis in pigs in the area.

The disease was rarely reported as brucellosis due to the non-availability of diagnostics capacity in developing countries (Franc *et al.*, 2018). Thus, little was known on brucellosis prevalence in pigs in Tanzania (Mpwapwa inclusive). Many pig farmers prefer to obtain breeding stocks from livestock research and training institutions due to their better genetic potential, although there different source of breeding stock including other small holder farms. There is thus a risk of transmission of brucellosis should these centres be infected by the disease.

Therefore this study aimed at estimating the prevalence of Brucella infection in pigs in smallholder farms and livestock training centers in Mpwapwa of Dododma region, Tanzania.

1.3 Objectives

1.3.1 General objectives

The objective of this study was to estimate seroprevalence of brucellosis in pigs and to determine potential risk factors for transmission in smallholder farms and livestock training centres in Mpwapwa district of Dodoma region, central Tanzania.

1.3.2 Specific objective

i. To estimate seroprevalence of brucellosis in pigs;

ii. To assess potential risk factors for brucellosis transmission**;**

CHAPTER TWO

2.0 LITERATURE REVIEW

Definition of brucellosis in animals and humans has several names such as undulant fever, Mediterranean fever, Malta fever, contagious or infectious abortion, or Bang's disease (Corbel, 2006). Brucellosis is a zoonotic disease mostly transmitted by direct or indirect contact with discharges or materials from infected animals or ingestion of products infected with *Brucella spp* Dadar *et al.* (2019). According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE), brucellosis is considered as one of the most significant and widespread zoonoses across the world (Lopes *et al*., 2010).

2.1 Etiology and Epidemiology

2.2.1 Etiology

The genus *Brucella* comprises a group of gram-negative bacteria that survives almost exclusively in aerobic condition and are facultative intracellular organisms (Tabar 2015; Olsen and Tatum, 2016), its small (0.5 to 0.7 by 0.6 to 1.5 μm), non-motile, non-encapsulated, non-spore forming, rod-shaped (coccobacilli) bacteria that can replicate and persist in host cells and cause the infection (Mathew *et al*., 2015).The common etiology of brucellosis are *B. melitensis* for sheep and goat, *B. abortus* for cattle, *B. suis* for pig and *B. ovis* for sheep (Hasanoglu *et al*., 2014) . Recently, the other four species have been reported to cause brucellosis in humans and animals and they include *B. ceti*, *B. pinnipedialis*, *B. microti* and *B. inopinata* (Hadush and Pal, 2015).

2.2.2 Epidemiology

Brucellosis is more prominent in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru and Mexico, recently cases of brucellosis has been reported from central and south west Asia (McGill, P. E. 2003; Tabar, 2015; Franc *et al.*, 2018). However, Western and Northern European countries such as Canada, Japan, Australia and New Zealand are assumed to be free from brucellosis agents (Greening *et al.*, 1995; Grantina *et al*., 2018). On the other hands seroprevalence of brucellosis in developing countries are 5.5% in Tanzania, 10.0% (Uganda), 24.1% (Ethiopia), 4.7% (Nigeria) and 35.7% (Saudi Arabia) (Mirambo *et al.*, 2018). Recently studies of brucellosis in Tanzania reported seroprivalence of 0.7% in pigs (Simon *et al.*, 2015), 5.2% in cattle (Sijapenda *et al.*, 2017) and 1.6% goats, 7.9% buffalo and 0.6 in human (Assenga *et al.*, 2015).

2.3 Brucellosis in Humans

2.3.1 Transmission

Human being acquires brucellosis through direct or indirect contact with B*rucella* organisms when exposed to either infective discharge tissues such as blood, urine, vaginal discharges, aborted fetuses or placentas or from consumption of infested animal byproducts such as meat and milk (foodborne transmission), or breathing in presence of organisms (aerosol) (Corbel, 2006; Franco *et al.*, 2007). Also, human brucellosis can be transmitted between person-to-person through mother-to-offspring however it is not common (Mesner *et al.*, 2007; Hadush *et al.*, 2015). People of all ages and sexes are susceptible to *Brucella* transmission when exposed to any of risk factors and human brucellosis has increased with substantial cases per 100 000 populations from 1997 to 2002 (Kozukeev *et al.*, 2003). Veterinarians, slaughters, farmers, livestock handlers and

laboratory personnel are at high risk of acquaring the infection because of the nature of their work (Luwumba and Kusiluka, 2019).

2.3.2 Clinical Signs

In humans, brucellosis is mainly characterized by undulating fever where the temperature fluctuates from 37ºC before noon and 40ºC in the afternoon; night sweats with a peculiar odor, chills and weakness. Malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness and depression are also common (Corbel 2006). Megid *et al.* (2010) reported that 77.8% of infected patients presented with undulating fever, 21%, with joint pains and 14% with backache. Infection depends to duration of the disease can be categorized into acute, sub acute or chronic (Balı*n et a*l., 2018). when the infection is less than 8 weeks are regarded as acute form, sub-acute form if persist for 8 to 52 weeks and chronic when it takes more than 1 year (Aygen *et al*., 2003; Bislimovska *et al.*, 2010).

2.4 Brucellosis in Animals

Numerous species of domestic and wild animals are infected by brucellosis such as cattle, buffaloes, bison, sheep, goats, pigs, dogs, camels, carbous, elk, and horses (Corbel, 2006; Gul *et al.*, 2007).

2.4.1 Brucellosis in Pigs

Brucellosis in pigs is caused by *B. suis*, specifically there are five biovars, 1 and 3 are more common worldwide, Biovar 2 occurs in Europe, where the hosts are pigs and hares, *B. suis* biovar 4 is enzootic in deer in Siberia, Alaska, and Canada. Although biovar 4 is not pathogenic for pigs, it can cause human brucellosis, *B. suis* biovar 5 causes marine brucellosis (Poeste*r et al*., 2016; Jindal *et al.*, 2016; Grantina-Ievin*a*

et al., 2018). Although, domestic pigs are infected mainly by *B. suis*, but less they may also become infected with *B. abortus* or *B. melitensis* in regions where brucellosis is endemic in cattle or small ruminants (sheep and goat) (Díaz, 2013).

2.4.2 Pathogenesis

Brucella spp invade epithelial cells of the host, allowing infection through mucosal surfaces where undergoes fusion with the lysosome in a controlled manner in the intestine, this site has been identified as a portal of entry for *Brucella spp*, once *Brucella spp*. have entered, they are capable of surviving intracellularly within phagocytic or non-phagocytic host cells and establish the replicative site and survive and finally leave the host cells to promote cell-to-cell (.

2.4.3 Transmission

Pigs become infected with *B suis* either through ingestion of contaminated reproductive materials usually birth and/or abortion products or uterine discharges in feed, water, manure, wool, hay, or sharing contaminated equipment with *Brucella* infections (Ridoutt *et al*., 2014). Natural mating or artificial insemination with infected semen also are reported as a way of brucellosis transmission among of animals (Maes *et al*., 2008; Psoester *et al.*, 2013). In ideal conditions of low temperature, high humidity, and no sunlight, *B suis* can survive several number of days in the environment however persistence has low epidemiological importance rather than direct or close contact (Aune *et al.*, 2012). Besides that an infection, *B suis* can circulate in the bloodstream of infected pigs at a range of 90 days, however some pigs might recover from infection, while others remain permanently infected (Ridoutt *et al*., 2014).

2.4.4 Clinical signs

The rate of abortion is higher in sows or gilts when exposed to *B. suis* via the genital tract at the time of breeding (Megid *et a*l., 2010). However, the retention of placenta, stillbirths, weak offspring, mortality are common, though prolonged furrowing period has been reported as an indication of brucellosis in pigs (Lopes *et al.*, 2010; Hadush and Pal, 2015). Clinical signs of brucellosis infection in pigs differs and depending on the status of the animals such as age,sex however, orchitis, epididymitis, spondylitis of the lumbar, sacral regions, arthritis, paralysis of hind limbs and lameness were also reported as symptom of brucellosis in pigs (Megid *et al*., 2010), though some of infected pigs might not show any clinical sign of infection (Jiang *et al.*, 2019).

2.5 Diagnostic techniques for brucellosis in humans and animals

The diagnosis of *Brucella spp* is confirmed through isolation of the organism from blood, bone marrow, stomach contents, spleen, lungs, placenta, vaginal swabs, semen, infected joints (Kaltungo *et al*., 2014). At necropsy, suggested tissues have included lymph nodes (e.g., those associated with the head, mammary gland, and genital tract (Poester *et al.*, 2016).

Brucella spp may also be isolated in the male reproductive tract (testes, epididymis, vesicular glands, prostate and bulbourethral glands), liver, kidney and any tissues with lesions, such as bones. *Brucella. spp* can be cultured on a variety of nonselective media, or selective media such as Farrell's (Corbel, 2006; Galińska and Zagórski, 2013; Elzbieta *et al.*, 2013).

The *Brucella* infection is detected in laboratory through microscopy culture, slide or tube agglutination, indirect Coombs, Enzyme-linked Immunosorbent assay (ELISA),

Indirect fluorescent antibody (IFA), molecular techniques such as Polymerase Chain Reaction (PCR) (Araj, 2010; Ulu Kilic *et al.,* 2013). The most widely used serological tests are Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and ELISA, numerous PCR assays have also has been developed for rapid identification (Srivastava *et al.*, 2015; Jindal *et a*l., 2016).

2.5.1 Rose Bengal Plate Test (RBPT)

The RBPT test is the screening test to all animal species, positive and questionable results are subsequently tested with the any other confirmatory test (Ridoutt *et al.,* 2014). RBPT is an appropriate test for serological with high excellence for a screening of the serum samples (Alton *et al*., 1988; OIE, 2004). The test is performed on a glass slide with colored bacterial antigen and relies on the principle that IgM antibodies bind with antigen **(**Srivastava and Sigh, 2015). RBPT exhibits high sensitivity but with poor specificity due to cross-reactivity with other pathogens like *Yersinia enterocolitica* or failure to differentiate natural infections from the effects of vaccination (Christopher *et al.,* 2010; Erume *et al*., 2016). The test is valuable in a place where another test is difficult to perform or not readily available (Njeru *et a*l., 2016; Massey *et a*l., 2018).

2.5.2 Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

The c-ELISA tests offer excellent sensitivity and specificity and fairly simple to perform with a minimum equipment requirements are readily available from several commercial sources (Njeru *et a*l., 2016). It has not been fully evaluated and standardized for *Brucella* detection, primary reference for gold standards is currently being developed to be accredited thus little is known about the causes of false positive in this test whenever happens, results confirmation depends on optical density (OD) where > 0.38 at 1:160 and < 0.38 at 1:320 are considered positive while those showing optical density

OD < 0.38 at 1:160 are considered non-reactive and hence negative for anti-*Brucella* antibodies (Zakaria, 2018).

2.5.3 Complement Fixation Test (CFT)

The test has good sensitivity and specificity, but it is a complex method to perform require good laboratory facilities and trained personnel and should be carried out regularly with good attention to assure quality and satisfactory of the results (Yohannes *et al*., 2012). The serum and complement proteins are mixed and incubated, if the test serum contains antibodies to *Brucella,* an antigen-antibody complex is formed (Novita, 2017).

The diagnostic test holds an indicator system that uses combination of sheep red blood cells, complement-fixing antibody such as immunoglobulin G produced against the sheep red blood cells and an exogenous source of complement usually guinea pig serum, when these elements are mixed in optimum conditions, the anti-sheep antibody binds on the surface of red blood cells (Crawford *et al.*, 1986). It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement, high levels of non-complement fixing antibody isotypes competing for binding to the antigen (Corbel, 2006).

2.5.4 Culture and microscopy

The test involves culturing and isolation of pathogens from infected individuals, it uses mainly sample from foetal membranes, uterine discharges, milk, blood, colostrum, stomach contents, liver and spleen (Corbel, 2006). Retropharyngeal or pre scapular lymph nodes may also be used but supra mammary lymph node is the most suitable specimen (Bishop *et al.,* 1994). Smears are stained using modified Ziehl-Neelsen

(Stamp) or Kosters method. The presence of large aggregates of intracellular, weakly acid-fast organisms with Brucella morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as 26 *Coxiella burnetii* or Chlamydia may superficially resemble Brucella in smears after staining (WHO, 2006).

2.5.5 Polymerase Chain Reaction (PCR)

This is a modern diagnostic technique for molecular biology detection of Brucella organism, its rapid, very specific, highly sensitive for *Brucella spp* DNA detection (Ghassan *et al.,*1996). This test requires specific pairs of primer for direct detection of gene regions of *Brucella spp* (Garcia-Yoldi, 2006). Also require the Bruce-ladder multiplex for molecular identification, typing of *Brucella spp* and enhancing to distinguish between them (Zeki Aras *et al.,* 2015). The technique is too expensive to be used widely and it is more appropriate for differential diagnosis (Yingst *et al.,* 2010).

2.5.6 Serum Agglutination Test (SAT)

The word agglutination originates from the Latin word agglutinate, which means “to glue to.” The antibody or the other molecule then binds the multiple particles and thus joins them and create a large complex thus coalescing of the small particles is suspended sample solution and hence form the precipitate (Marrodan *et al., 2*001). The SAT has been used extensively for brucellosis diagnosis, simple and cheap to perform but it lacks sensitivity and specificity mean, it used in the absence of alternative techniques, the test measures the total quantity of agglutinating antibodies (IgM and IgG) and the quantity of specific IgG is determined by 2-mercaptoethanol (2ME) (Sareyyüpoğlu *et al.*, 2010).

2.5.7 Fluorescence Polarization Assay (FPA)

This test requires special reagents and reading equipment, it has higher sensitivity and specificity, it's using molecular rotational properties, considered a homogenous test due to measuring antibody binding to antigen directly and the rate of rotation of the antigen molecule is reduced when its molecular size is increased by its binding to antibody (Nicola *et al*., 2010). The test has cut-off value between positive and negative reactions which has specific confirmatory and can distinguish vaccinal antibody in most vaccinated animals (Muma, 2007). Also the test has ability to eliminate some cross-reacting antibodies (Nielsen and Yu, 2010).

2.5.8 Brucellin Skin Test (BST)

Is the test used as the screening or confirmatory test in ruminants and swine **(**Nyanhongo *et al.,* 2017). The test has been proved to identify some acute and chronic latent stages of brucellosis and problems associated with false positive reactions in serological due to high sensitive and specificity (Saegerman *et al.,* 1999). It performed in pigs by injecting 0.1 ml of the allergen suspension in intra-dermally at the base of the ear or the base of the tail (OIE 2018). The reaction reactions occurs and are assessed by visual inspection and palpation of the inoculated area after 48 up to 72 hours, the positive reaction is characterised by erythema of non-pigmented skin and an oedematous swelling (Dieste *et al.,* 2015).

2.6 Risk Factors for Prevalence of Brucellosis in Pigs

2.6.1 Knowledge, practices and risk factors

Epidemiological studies reported brucellosis as a major challenge to public health among of livestock farmers in endemic areas (Facciolà *et al.,* 2018). Areas with high prevalence communities had fair knowledge regards to attitudes, perceptions and

practices (Cloete *et al.*, 2019). Lack of knowledge to the mode of transmission, signs/symptoms of susceptible animals leads to wards poor farmers' practices, prevention and control in both human being and animals (Obonyo, 2015).

Societies and individuals who depend on livestock production for their livelihood are at high risks of acquiring brucellosis due to close contact with livestock, and they may be less likely to be diagnosed and hence treated incorrectly (Marcotty *et al*., 2013; WHO, 2006). Though, the cases of brucellosis in human in many endemic countries are under-diagnosed and under-reported this is due to the limited resources and diagnostic tools capacity (Wojno *et al.*, 2016).

2.6.2 Herd size managements

Biosecurity on-farm management is a key point in herd size managements (Maunsell and Donovan 2008). Livestock farmers in endemic areas normally fail to isolate suspected animals with brucellosis which is the major risk factors for transmission of the disease within and between herds (Holt *et al*., 2011). Animals become infected through contact with infected tissues or consumption of pasture or water contaminated with infected aborted materials (Obonyo, 2015). Also, animals brought from different areas either for fattening or breeding purpose without proper disease screening or quarantine may introduce the infection to the farm (Birhanu *et al.*, 2014). Routine screening at the event of every reproductive failure or before the introduction of new animals into the farm is important to detect asymptomatic infected animals hence will reduce the spread of brucellosis (Shome *et al*., 2016).

2.6.3 Pig production systems, housing and feeding

In developing countries, the most reliable means of pig production is Small-scale by

where housing is a simple pen made with locally available materials to modern housing (Mrema *et al.*, 2012). In additional scavenging is the basic traditional system of keeping pigs and commonly in rural areas (Komba, 2008; FAO, 2009). In this free-range system, pigs roam freely around the household and surrounding area, scavenging and feeding in garbage dumps or forests around to the area (Alarcon *et al.*, 2017). Pigs are free-range through out of year and confined during the rainy season or they may be housed at night in a small shelter to protect them against theft and predators, some of pig keepers prefer free range system as it requires minimal inputs and low investment (Leslie *et al.*, 2015; Kimbi *et al*.,2016).

The study by Lopes and Nicolino(2010) reported outbreak of porcine brucellosis *B. suis* biovar 2 in pigs reared under free-range system, outbreaks of pigs brucellosis have been reported after isolation of *B. suis* biovar 2 in many parts of the world where pig herds reared under free-range system (Godfroid *et al.*, 2011). Epidemiological study link the wild pigs infected by *B. suis* as a main source of brucellosis to the endemic (Meng *et al.*, 2009). Also in free-range system, domesticated pigs are thought to acquire brucellosis when they ingest feed or water contaminated with infection fetus, placenta, fetal fluids or vaginal discharges, dead fetuses and fetal membranes (Megid *et al*., 2010).

Moreover, infected pigs can shed *Brucella spp* in environment which remains for a time and hence serve as source of infection (Godfroid, 2017). *Brucella spp* can survival in the environment for several numbers of days in both dryness and freezing temperatures and hence contaminate forage, soil and water which will be utilized by pigs especially those under free range system and semi intensive, though direct sunlight reduces the chance of Brucella bacteria’s survival in the environment (Díaz Aparicio, 2013).

2.6.4 Source of infection during breeding

Whenever infected pigs with brucellosis are brought to the herd (piggery farm), *B. suis* spread quickly and infect more than 50% and frequently up to 70- 80% of the herd, after the organism established in a herd may appear as nonspecific infertility, a slightly reduced furrowing rate and irregular estrus cycles, however, deaths are rarely reported in adult pigs (Olsen and Tatum, 2016). High level of mortality has been reported in piglets and some of the piglets surviving are likely to be seronegative carriers (Megid *et al*., 2010). Copulation have been reported in various studies as the main source of brucellosis infection especially pig reared under free-range and semi-intensive system, likewise contaminated semen also are considered the source of venereal transmission (brucellosis) in domesticated pigs (Olsen and Tatum, 2016).

2.6.5 Brucellosis control

Brucellosis is an infectious disease that has been controlled and eradicated in most developed countries and still endemic in developing countries (Godfroid *et al*., 2004). Proper diagnostic and control have been adopted by veterinary and healthcare services in developed countries where brucellosis has already been controlled (Ridoutt *et al.*, 2014). Prevention and control are largely depend on successful control of the diseased animals either through test and slaughter policy, strict control of animal movement, biosecurity measures, disease surveillance and careful handling of aborted materials and stillbirth (Njeru *et al.*,2016). In addition by prevention of feral pigs from contacting to domestic pigs could reduce the chance for Brucella transmission (Ridoutt *et al.*,, 2014)

2.6.6 Brucella infections due to occupational exposure

In most of the endemic areas where *Brucella* infection is at high level occupational groups have reported acquiring infection through oral, respiratory, or conjunctival

routes, also close contacts with infected animals and ingestion of infected byproducts constitute the of Brucella transmission (Lopes *et al*., 2010). Due to this fact it's important to protect the occupational group from any exposures of Brucella organism (Hadush and Pal, 2015).

Good working practice should be emphasized and implement to occupational exposure such as stockmen, shepherds, goatherds, the abattoir workers, butchers, dairymen, artificial inseminators, veterinarians and those involved in of viscera, hides, wool and skin (Corbel, 2006). Persons involved in the maintenance of buildings or equipment in infection place may also be at risk of Brucella infection (Bislimovska *et al.*, 2010). Likewise, laboratory workers who may be exposed to contaminated specimens either during diagnostic procedures or vaccine production (Luo *et a*l., 2019).

2.7 Managements of Brucellosis in Humans and Pigs

2.7.1 Managements in pigs rephrase

As a general rule, treatment of the infected animal is not recommended because of high failure rate and of cost and maintaining infected animals in herds (Solera *et al.*,1997). Currently no feasible drugs for treatment brucellosis in the market (Corbel, 2006). However, use of antibiotics such as penicillin and oxytetracycline tends to reduce the infection (WHO, 2006; Olsen and Tatum, 2016).

2.7.2 Managements in humans

Treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time (Yousefi *et al.*, 2012). Antibiotic treatment should be implemented as early as possible (Corbel, 2006). WHO (2006) recommends drugs for the treatment of brucellosis includes Tetracycline (500 mg every six hours orally)

administered for at least six weeks has long been the standard treatment of human brucellosis, Doxycycline (a long-acting tetracycline analogue) is now the preferred drug because it can be given once or twice daily and is associated with fewer gastrointestinal side effects than tetracycline and are given in a dose of 100 mg every 12 hours orally for six weeks (Rahil *et al.*, 2014).

2.7.3 Vaccine of brucellosis

Currently, there is no vaccine for human and pigs only precautions are emphasized by the WHO 2006 besides that control and eradication programs for infected animals is a high priority and encouraged (Tabar, 2015). Caution should be exercised in the use of anti-inflammatory agents to deal with complications where possible and also specialist advice should be sought in mind (Srivastava *et al.*, 2015; Corbel, 2006; Olsen and Tatum, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Area

The study was carried out in a randomly 23 selected villages of Mpwapwa district. Mpwapwa is one of seven districts of Dodoma region, central Tanzania. It is located at the Southwest about 120 kilometers away from Dodoma city, the district shares borders with Kilosa district (Morogoro region) in the eastern part, Kongwa district on the north, Kilolo (Iringa region) on the south and Chamwino in the western side. The district lies between $06^{0}00$’ and $7^{0}30$’ South of the Equator and between $35^{0}00$’ and $35^{0}45$’ East of Greenwich Meridan.

3.2 Institutional Ethical Permission

This study was approved by SUA as per institution guideline requirements for postgraduate students with reference number SUA/ADM/R.1/8/448 and other research permit was obtained from Mpwapwa district administration office with the reference number HW/MPW/V.10/2VOL11/73. (Appendex …….)

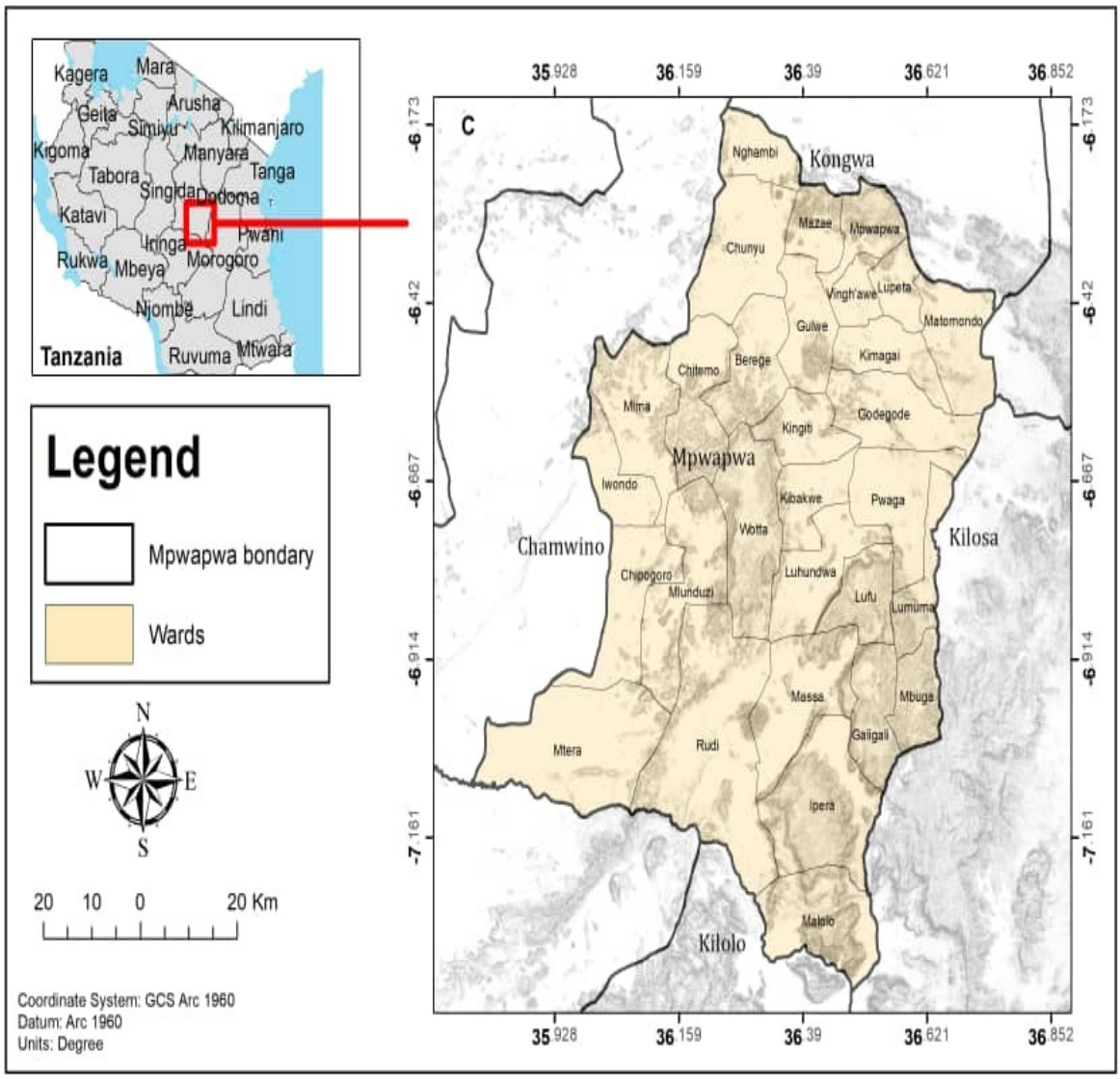

Figure 1: Map of Tanzania zoomed to show administrative boundaries and wards where the study conducted

Source: Mpwapwa district record

3.2 Study Design

A cross-sectional study design was adopted whereby the prevalence of brucellosis in pigs and a potential risk factor for brucellosis prevalence was assessed.

3.3 Sample Size Estimation

The number of pigs sampled was determined by using a formula developed by Fisher *et al*. (1991); Where: n = required sample size, Z = z score (which for 95% confidence level is 1.96), P = known or estimated prevalence of a factor (prevalence of brucellosis in pig). Prevalence was not known, hence P=0.5 was used to obtain the maximum sample size. E = allowable error of estimation (in this study 5% was used).

$$n = \frac{1.96^2 x 0.5\ (1-0.5)}{(0.05)^2} = 384$$

The sample size obtained above was adjusted for the finite population (N) using the formula $n_2 = nN/\ (n + (N-1)$ (Martin *et al.*, 1987), where N = total pig population (estimated was 4000, however Mpwapwa district has 37,015pigs (URT, 2012). Therefore, 384 x 4000/ (384+ (4000-1) =350. Thus, 350 pig were required for this study.

3.4 Field Data Collection

3.4.1 Sampling selection and technique

The multistage sampling design was adopted in this study, whereby twenty-three (23) villages were randomly selected from the clusters established during the study and followed by a random selection of 144 households that were keeping pigs.

Table 2: Number of pigs and household sampled in in study area

Wards	Number of villages	Pigs sampled	Households

Mpwapwa mjini	3	38	19
Mazae	3	39	17
Idilo	2	33	18
Pwaga	2	27	11
Chunyu	2	28	9
Gulwe	2	31	12
Massa	2	22	9
Lupeta	2	23	10
Ng’ambi	1	18	9
Ving’hawe	3	43	17
Msagali	1	22	13
Total	23	324	144

3.5 Criteria for Inclusion and Exclusion during Sampling of Pigs

Number of pigs were randomly selected according to size of farm where by herds with less than 10 pigs two pigs were sampled and those with more than 10 pigs three pigs were considered, this was done due to the facts that some of the households pig farmers selected had few pigs, (up to less than two). Pregnant sows nearest to furrow, piglets with less than two months of age as well as weak pigs were excluded to avoid stress in response to handling and bleeding. In addition, 35 pigs were randomly selected from animal research unit and training centers nearby the study area. Pigs from LITA Mpwapwa campus 19 and Visele Live-Crop Skills Training Centre 6. For the questionnaire survey, one respondent from each household (pig farmer) was requested to fill in the information after introducing to him/her by reading the consent form which contained all necessary information regards his/her participation to the study. Household heads (father/mother) were most preferable but in cases of their absence, any member of the family who could deliver well the required information was interviewed. In addition, prior permission was obtained from village and district administrative leaders.

3.6 Animal Preparation for Blood Sampling

Whole blood samples were collected from live pigs by adhering to animal welfare precautions. Three people were involved two for restraining and one for blood sample

collection. In addition, before entering any new piggery farm the team members disinfected themselves and used new hand gloves and other protective gears to avoid transmission of infection between farms.

3.7 Blood Sampling, Serum Preparation, Preservation and Analysis

3.7.1 Blood sample collection

First, a pig was restrained in dorsal recumbency and the blood collection site (anterior vena cava) was cleaned and disinfected with methylated spirit as described by Klein *et al.*, 2012 and Dyce *et al*. (1996). About 4-5ml of the blood was collected into a plain vacutainer tube, labeled and packed into a cool box. The samples were transported to LITA Mpwapwa -Microbiology laboratory for extraction of serum. Field sample collection took 4 - 6 hours per day.

3.7.2 Serum preparation and storage

The sera were extracted through centrifugation at 3000 rotation per minute for 10 minutes. The resulting supernatant was collected into a labeled sterile cryovial tube and stored in a deep freezer at -20°C, subsequently sera were transported while frozen under ice condition to the microbiology Laboratory at the College of Veterinary Medicine and Biomedical Science, Sokoine University of Agriculture for detection antibodies against natural *Brucella* infection.

3.7.3 Laboratory analysis

The frozen serum samples in (cryovial tubes) were left at room temperature for 30 minutes to defreeze before analysis started.

3.7.4 Reagents and Serological tests

Brucella species Rose Bengal Reagent antigen earlier imported from USA (USDA APHIS) and kept at 4^0C refrigeration during analysis. *Brucella* antigen for positive and negative control sera was obtained from microbiology laboratory in the CVMBS at SUA.

3.7.5 Rose bengal plate test

Rose Bengal Plate Test (RBPT) was used according to Alton *et al.* (1988). Briefly, a drop of the test serum (30 µl) was taken by using a clean and sterile micropipette tip and placed onto test plate beside an equal (30 µl) drop of RBPT antigen. The solution was well mixed by using a sterile applicator stick (new per each sample). The mixture was shaken manually gradually for 4 minutes before examination. The presence of distinct pink granules was recorded as a positive case while samples without granules were recorded as negative cases.

3.8 Questionnaire Survey

A structured questionnaire (Appendix 1) was used to collect information on farmers' awareness regarding brucellosis (one respondent per household). The respondents who were not able to read and write face to face interview was conducted to collect useful information. The questionnaire was translated to kiswahili language, pretested and adjusted accordingly. In addition, veterinary services providers in the area of study were provided with checklist questions to gather addition information. Furthermore, environments where pigs reared were examined visually to identify any observable potential risk factor that could lead to brucellosis transmission.

3.9 Data Analysis

Data generated from the questionnaire survey and laboratory investigations were coded and entered into Microsoft Office Excel 2016 and analyzed in the statistical package stata version 12.0. Descriptive statistics were computed to determine the overall district prevalence of brucellosis in pigs which were calculated as the total number of seropositive samples obtained divided by the total number of samples tested. Similarly, factors leading to brucellosis in pigs were evaluated by using response delivered by respondents. Logistic regression model was used to analyze for statistically significant differences where disease status (laboratory results) was the dependent variable and age (categorized as youngs and adults) and sex were independent variables. P-value of less than 0.05 was regarded as a statistically significant (Appendex….).

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-Demographic Information of Pig Farmers and Characteristics

A total of 144 households (pig farmers) were interviewed regarding the knowledge of brucellosis in pigs and potential factors leading to prevalence of pig brucellosis in the study areas. Males showed higher participation than female, however the difference was not statistically significant. Most households were practicing mixed farming included crop production and livestock farming such as poultry, sheep and goat, cattle and few owns donkey. Land cultivation was the major economic activity especially in rural areas. There were few households that were practicing pig farming only. Most pig farmers had been in pig farming for more than 5 years and animal husbandry practices such as feeding, cleanness and other daily routine related practices were mainly performed by any member of the households, with only a few farmers (6%) employing causal labours. The rest of the information regarding social-demographic characteristics and farm characterization is summarized in the Table 1.

Table 3: Selected social-demographic characteristics of pig-keeping households

Factor	Frequency	Percentage%
Gender participants		
Male	100(144)	69.4

Female	44(144)	30.5
Age group		
5-25	21(144)	14.5
525-50	69(144)	47.9
>50	54(144)	37.5
Highest education level in household		
Non formal education	14(144)	9.7
Primary level	73(144)	50.6
Secondary level	30(144)	20.8
Tertiary level/college education	27(144)	18.7
Farm characterization Number of pigs owned by each household		
1-5	47(144)	32.6
5-10	58(144)	40.2
>10	39(144)	27.0
Breed of pig		
Local/cross breed	346(349)	99.1
Exotic	3(349)	0.8
Sex of pig		
Male	192(349)	55.5
Female	157(349)	44.9
Age of pigs		
Adult > 6months	291(349)	83.38
Young <6months	58(349)	16.6

4.2 Laboratory Results

4.2.1 Prevalence of brucellosis in pigs

The seroprevalence of brucellosis in pigs in smallholder farms and livestock training centres in Mpwapwa district of Dodoma region Tanzania, between December 2019 and March 2020 based on RPBT test were summarized in Table 2.

Table 4: Seroprevalence of brucellosis in pigs in smallholder farms and livestock training centres in Mpwapwa district of Dodoma region, Tanzania

Variable	**Number pig serum screened**	**Number positive (%)**	***P-value***

Age			**0.733**
Adults >6months	302	7(2.3)	
Young <6months	57	1(1.7)	
Sex			**0.236**
Male	191	2(1)	
Female	158	6(3.8)	
Study area			
Mpwapwa District -pig farmers	324	8(2.5)	
Livestock Training Centres			
LITA Mpwapwa	19	0	
Visele Live- Crop Skills	6	0	
Overall	349	2.3	

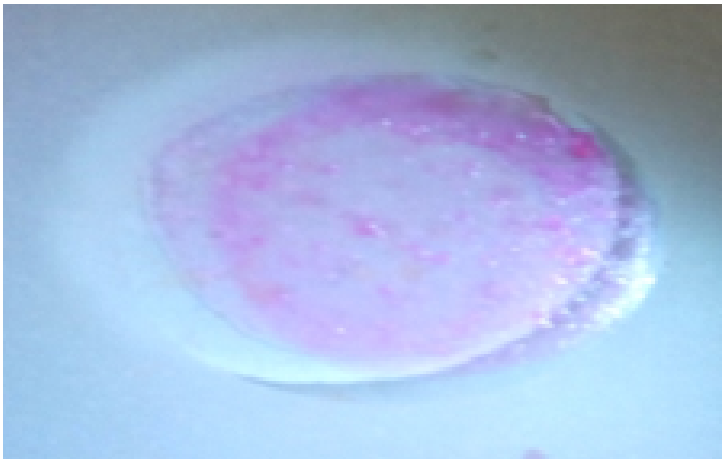

Figure 2: Rose Bengal Plate Test showing agglutination of positive pig serum samples during a study to estimate seroprevalence of brucellosis in pigs

4.2.2 Knowledge and awareness regarding to brucellosis in pigs among pig farmers

Brucellosis has been given different names in the study areas; ***kuhopora, kulafa inda.*** However, ***kutupa mimba*** in Kiswahili language which means abortion was the most commonly known to pig farmers in the study areas.

Of the 144 households interviewed, 76 (52.8%) had heard brucellosis disease from different sources and mostly were neighbors (53%) who practiced livestock farming, veterinary service providers (13%) and other (10%) of the respondents got information from community gathering places, media such as radio, television and written books. Knowledge of pig farmers regarding to brucellosis as captured during this study to estimate seroprevalence in pigs in Mpwapwa district as shown in Table 3.

Table 5: Knowledge of pig farmers regarding to brucellosis in Mpwapwa district

Variable	Number of respondents	Percentage (%)who knows	Percentage (%) who didn't knows
Mode of transmission from pigs to humans	144	18.8	81.2
Common clinical signs in	144		
i. Humans		1.4	98.6
ii. pigs		11.2	88.8
Managements of pigs with brucellosis	144	43.1	56.9
Human protection against brucellosis	144	31.3	68.7

4.2.3 Common clinical signs of brucellosis reported in the study areas

Pig farmers were interviewed whether had ever experienced any clinical sign of brucellosis in pigs since they started pig farming. The results are summarized in Figure 4 below.

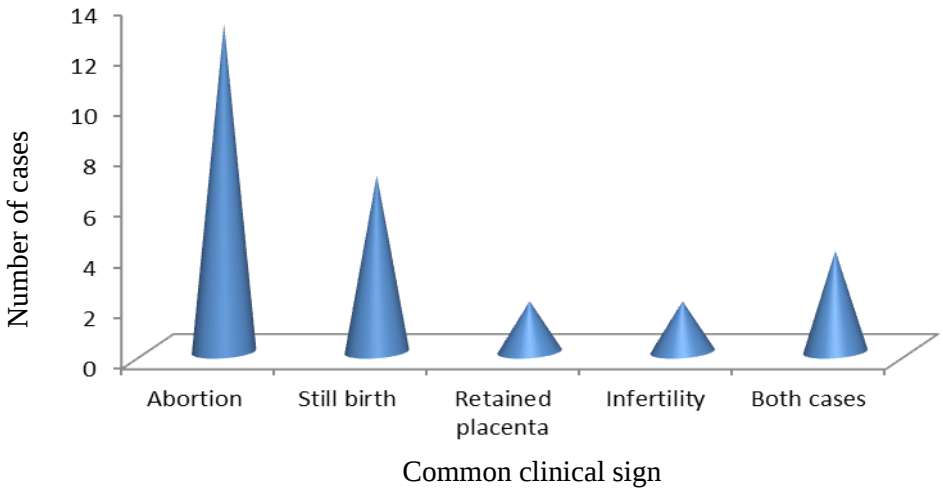

Figure 3: Common clinical signs of brucellosis in pigs experienced by pig farmers in Mpwapwa district.

4.3 Potential Risk Factors Leading to Prevalence of Brucellosis in Mpwapwa District

The following were some of the factors that could lead to brucellosis transmission in pigs in Mpwapwa district.

4.3.1 Pig feeding and rearing system

The common feeds in the study area was concentrates, natural forage and food left over. Though, 6 (4%) households (pig farming) were feeding their pigs a mixture of rumen liquor and animal blood collected from slaughter slabs around to them (Fig 6). One of the pig farmers interviewed regards to such feeding practices responds to use such feed for long period of time especially when there is scarcity of animal feed or when the animal feed is at a high price and no any adverse nutrition effects he has been observed.

Figure 4: A pig farmer preparing feed ready to feed his pigs as captured during this study. A- rumen li, B ……., C……. and C……..

Similarly, 136 (94%) respondents (households) surveyed were practicing intensive rearing system while 8 (6%) practicing either free-range or semi-intensive system throughout the year. However, some respondents said that during crop cultivation (rain season) they practice intensive system of production but after harvesting pigs are left to free-range. This study was carried out during rain season when pigs were under confinement however some of the pigs were found roaming outside as captured in Fig. 7.

(A) **(B)**

Figure 5: (A) Pig scavenging nearest to waste disposal materials at Ving'hawe village and (B) Pigs roaming outside after escaping from its pen at Mji mpya Mpwapwa township

4.3.2 Source of parent stock and breeding

A total of 120 (83%) respondents reported to obtain pig parent stock from neighbours who practice pig farming without considering the breeding history and 24 (17%) obtains breeding parent stock from livestock research and training centres where breeding history is exactly known. In addition, 106 (73%) respondents reported to be in sharing

boars for breeding, however, 38 (27%) respondents reported to raise their boars for breeding purpose, but in case of fearing inbreeding effects is when they share boars from neighbor who practiced pig farming.

4.3.3 Methods for disposing of aborted materials in the study area

Common methods used in the study areas for disposing of aborted fetus as reported by farmers include; burying 127 (88%), incinerating 2(1%), feeding to dogs 8(6%), and throwing in bushes 7(5%), similarly some dead animal bodies were found in the study areas as captured in Fig. 8.

Figure 6: Dead animal body found in the study areas at Ndenje village, main road to Kibakwe township.

A: Decayed cadaver and **B:** dead pig deposted near the culverts as captured during the study visit . Some of the households (pig farming) in Ilolo village dispose aborted foetus in the baobab tree which had developed natural holes inside with a depth approximately 5-10 meters and sufficient circumference as shown in Fig. 8.

Figure 7: Baobab tree with natural holes inside used as decomposition pits by some of households pig farmers for disposing aborted materials or any dead animals in the study area.

CHAPTER FIVE

5.0 DISCUSSION

Overview

The seropostive cases of brucellosis in pigs were found in smallholder farms while in livestock training centres no positive cases were detected. The observed lack of cases in these centres might be linked to small number of pigs sampled compared to those in the smallholder farms. Thus, more research is needed in this aspect as the previous study of Shirima *et al.*(2014) reported the *Brucella* infection in livestock research centre of Mpwapwa Dodoma, Tanzania.

Out of 349 pig sera samples screened using RBPT, only 8 (2.3%) were found positive in this study, which were slightly higher than those reported in Dar es salaam, Tanzania by Simon *et al*. (2015). The variation seroprevalence of brucellosis observed in this study

was probably due to difference of diagnostic test used, husbandry practices, biovar of an organism, breed susceptibility, geographical location, environmental factors and management systems practiced by the farmers (Rahman *et al.*, 2012; Franc *et al.* 2018). In this study it was observed that some rural areas of Mpwapwa district pigs were reared in free range system especially in dry season this could have resulted into high infection agents in the study area, however some of pigs in other areas are likely to develop disease resistant due strong antibodies immunities acquired from the environment though this hypothesis could be difficult to prove since there no controlled study done in pigs regards to disease susceptibility in the environment.

Earlier studies have showed that *Brucella suis* is moderately influenced by environmental factors such as climate, the microorganism can survive in the environment in both dryness and freezing temperatures for several years (Aune *et al.*, 2012). This study reported brucellosis to small area of Mpwapwa district which represents small part of the country hence higher prevalence is likely to be higher in other parts of the country.

According to Franc *et al*. (2018) seroprevalence of pigs brucellosis infection in Sub Saharan African countries ranged from (1-12%) and such countries reported low seroprevalence included Nigeria, Onunkwo *et al.*, 2011), Tanzania, Simon *et al.* (2015), Ethiopia, Kebeta *et al*. (2015) and Uganda Erume *et al*. (2016). These findings suggested poor veterinary infrastructures, poor animal husbandry and low knowledge among pig farmers. The similar factors also were experienced in this study (Table 3) who found low knowledge regards to mode of transmission and management of *Brucella* infection, hence prevalence was likely to be under or over estimated due limited diagnostic tools used for this study.

The seroprevalence reported in this study is rather low, when compared with what reported in other animal species such as cattle, sheep and goats in Tanzania (Shirima, 2005), Assenga *et al*., 2015; Chitupila *et al.*, 2015; Karimuribo *et al.*, 2007; Swai *et al.*, 2010 and Sijapenda *et al.,* 2017). This might be due to management practices practiced by livestock farmers and animal species susceptibility with the infection (Wu *et al*., 2012); Leslie *et al.,* 2015). The low prevalence observed in this study could have influenced by the system of production (intensive, semi- intensive and free range), in this study some of pig farmers in rural areas manage their pigs either free range or backyard system depends on the season of agriculture. During rain season is where pigs were under controlled but in dry season were subjected to free range system of production, but with the regards to other animal species were mainly subjected to free grazing system through out of production, such practices could influence high prevalence due to feeding food with Brucella contamination during grazing.

The lower seroprevalence was observed in young pigs compared to adults. Similarly, female pigs had higher seroprevalence than male pigs. There is no controlled and intensive study that has been conducted to find out the influence of age and sex of pigs to brucellosis prevalence. However, findings from earlier studies reported more cases in adults compared to young pigs (Megid *et al.,* 2010; Njoga and Eze, 2011; Wang *et al*., 2012; Woldemeskel, 2013). In contrast some of studies have reported higher seroprevalence in young pigs than adults (Kibete *et al.,* 2015 and Ngbede *et al.,* 2013). Higher prevalence of brucellosis in adult pigs thought to be associated with maturity, as sows advancing age, the *Brucella* organism may propagate and become dormant, in additional young pigs are protected by maternal immunity which provides antibodies

against *Brucella* antigen, thus resulting to cross reactivity however the immunity may disappear as piglets grow (Kazi *et al*., 2005; Rahman *et al*., 2012).

Higher seroprevalence of brucellosis in pigs were observed in female than male in this study, this findings are similar to the observation reported by Rahman *et al*. (2012 and Kibete *et al.* (2015) who found a high prevalence in female than male. However, there different to the findings by Ngbede *et al*. (2013) who reported relatively higher seroprevalence of brucellosis in males than female pigs in Nigeria. The higher rate of infection in female were associated with the reproductive tract which acts as a potential reservoir and predilection site for *Brucella* organism (Megid *et al.*, 2010; Kebeta *et al*., 2015). Also, in traditional husbandry practices female pigs are maintained for prolonged time than male hence female is likely to be more exposed to Brucella infection. However, there was no statistical difference ($P>0.05$) between age and sex between for positive reactors and non reactors in this study.

Studies have demonstrated that in endemic areas, Brucella infection in pigs have been associated with several factors such as breeding of infected pigs especially through sharing boars or introducing infected pigs in the herds (Corbel 2006; Megid *et al.*, 2010; Shome *et al.*, 2016; Maes *et al.*, 2008; Psoester *et al.*, 2013). In this study, sharing of boars for breeding was common. Moreover, most farmers were obtaining their pig breeding stocks from their neighbors. Both practices could enhance transmission of brucellosis in the study areas.

Elsewhere studies have shown that brucellosis infection rate increases with free-ranging type of pig management (Lopes and Nicolino, 2010; Leslie *et al.,* 2015; Nwanta *et al.*, 2011 and Ridoutt *et al.*, 2014). In this study, free ranging pig management was the

commonest system in smallholder farming and almost the sole practice during dry season (post-crop harvesting), this allows pigs to scavenge and get exposed to infections, brucellosis inclusive.

Lack of knowledge and awareness regarding to transmission of brucellosis among pig farmers were a feature in most of the respondents (Fig 6) and likely to contribute the prevalence brucellosis in the study areas. Studies elsewhere have found out that low extension service leads to poor knowledge and awareness among pig farmers (Holt *et al*. (2011), Obonyo *et al.*,(2015), Buhari *et a*l. (2015), Nabirye *et a*l. (2017) and Cloete *et a*l. (2019). This is in agreement with the findings from this study which showed low knowledge between respondents who knows the mode of transmission and those who didn't know (Table three). Therefore, there is great value of brucellosis prevalence in pigs and likely to be associated with lack of knowledge regarding to brucellosis among of pig farmers in the study area. Therefore, these factors leads to poor protection of animals and themselves against Brucella infection especially those exposed to risk factors (Al-Shamahy *et al.*, 2000; Aworh *et al.*, 2013).

Farmers' day meetings and community meetings with veterinary services providers in the study areas were the most options suggested by respondents to be used as community education tool in order to safeguard their animals and themselves against Brucella infection. This idea is similar to what was suggested by previous authors (Godfroid, 2017; Ntirandekura *et al*., 2018; Cloete *et a*l., 2019).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study showed an overall porcine brucellosis prevalence of 2.3% based on RBPT in Mpwapwa district. Although the prevalence is apparently low, it can lead to adverse impacts on the public health due to zoonotic potential of the bacterium. Smallholder pig farmers were affected by a number of factors but mainly was low knowledge regards to brucellosis transmission and was due to low extension service received from the responsible authorities.

6.2 Recommendations

i. Further studies on the molecular epidemiology of porcine brucellosis should be done to confirm and identify specific biotypes/strains of *Brucella* spp in pigs in Mpwapwa district.

ii. Provision of educational campaigns aimed to create awareness on disease transmission, impacts and control measures among of livestock farmers.

iii. Authorities responsible for disease surveillance, monitoring and control should emphasize ‘test and slaughter policy’ this will render free animals with *Brucella* infections.

iv. This study reported Brucella infection in pigs in small parts of the country and further studies in other parts of the country are essentially required to map the disease throughout the country in order to safeguard public health and prevent economic losses.

6.3 Limitations of the study

i. This study used screening serological test (RBT). Due to limitation of this test, it could be that the reported prevalence under or over estimated the real situation. However, this study indicated presence of circulating *Brucella spp* in pigs in the study area. There was a need for complementary test such as FPA or PCR but could not be done because of the budget.

ii. Some of selected village were not reachable due to high rainfall which resulted into floods and hence breaking road communication between villages.

iii. Some pig farmers were not present at the time of sampling due to the reason that was of agriculture activities (land cultivation and crop production).

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Aygen, M. D. and Doganay, M. (2003). *Human brucellosis : an overview*. *International Journal of Infectious Diseases* 7: 173-182.**APPENDICES**

Appendix 2: Questionnaire

Questionnaire for cross-sectional survey to assess the knowledge of pig farmers on pigs brucellosis and identify risk factors leading to brucellosis prevalence among pig farmers in Mpwapwa district.

Instructions

Fill blanks or circle the letter of the correct answer

SECTION A-Socio-demographic information of the respondent.

1) District name……Date…… Questionnaire No…….

2) Ward name ……

3) Village name ……..……

4) Participant's name ……Sex (a) male (b) female

(i) Marital Status (a) single (b) married (c) engaged (d) in relationship (e) divorced

Any other specify……

5) Mobile phone number ……

6) Age of participant (a) 10-25 years (b) 26-45 years (c) Above 45 years

7) Education level: (a) Illiterate (b) Primary (c) Secondary (d) college education/tertially

SECTION B – Farm characterization

8) How many pigs do you have on your farm? (a)1-5 (b)6-10 (c) >10

9) When did you start pig farming? (a) 6months -1 year ago (b) 2 years-4year ago (c) more than 5years ago

10. Who takes care of your pigs and your other animals?

(a) father (b) mother (c) children 5-18 years (d) any member of the family

Any other (specify) ……...

SECTION C- Knowledge on Brucellosis

11. Have you ever heard about a disease known as brucellosis?

(a) Yes

(b) No

If Yes, where did you hear……..?

(a)Veterinary services providers

(b)Community gathering and/or talk

(c)Neighbors and/or friends and/or family

(d) Radio and/or television

(e) Reading from books

(b)Any other source of information specifies ……

12. Can pig be affected by brucellosis?

(a) Yes

(b) No

(c) I don’t know

If yes write clinical signs to question number 15a

13. Can pig transmit brucellosis to the human beings?

(a) Yes

(b)No

(d) I don’t know

(i) If Yes, mention the modes of transmission for brucellosis to the human being

……

……

14. Have you ever been affected by brucellosis? (a)Yes (b) No (c) I don’t know

(i) If Yes, how did you know that you were affected by

brucellosis? .....

.....

15. Which signs indicate brucellosis in:

(a) Animals? ……..

(b) Human? .....

16. Which measure do you take when an animal is infected with

brucellosis? .....

.....

17. What measure do you take when a person in the family is infected with brucellosis?

……

……

18. (i)Have you ever seen any of the following cases in your pigs?

(a) Abortion

(b)Stillbirth

(c) Retention placenta

(d) Infertility

(ii) if no any case has been observed to your piggery farm go to the next question

19. Do you think brucellosis can be controlled?

(a) Yes

(b) No

(c) I don’t know

20. What are the precautions do you take to control/prevent brucellosis in your family and pigs? (**Put a tick for appropriate answer**)

(a)Handling of the aborted fetus by wearing gloves

(b) Washing of hands with soap immediately after assisting pig during furrowing

(c) Disposing aborted materials in pit/hole and cover them

(d) Prohibit anyone with cut/scratches from assisting furrowing or handle aborted materials

(e)Proper Cleaning the environment and disinfection where pig lives

(f)Proper cooking pork

If there is any means specify

……

……

SECTION D

Factors leading for porcine brucellosis among of pig farmers

21.what is the system are you using for pig production
(a) Intensive
(b) Semi-intensive
(c) Free-range system
(d) Both either of the system listed above
Any means of production specify……

22. What do you feed your pigs?
(a) Concentrates
(b) Food left over
(c) Rumen liquor mixed with fresh animal blood
Any other means of feeding specify ……
23. (i)Where do you get the parent breed stock?
(a) Neighbors
(b) Livestock breeding centers (institution)

(ii) Do you consider breeding history of animals before brought to your farm?
(a)Yes
(b) No
If yes, what key issues do you ask the animal vendors…….

…….
(ii)Do you impose newly pigs into quarantine before introducing in the farm?
(a)Yes
(b)No
If yes, how many days……..
24. How do you breed your pigs when shows sign of heat?

(a) Hire boar from neighbors

(b) Using Artificial insemination

(c) Sent to breeding centers

Any other means specify…….

……..

25. Do you consider the breeding history of the boars before breeding your sows/gilt?

(a)Yes

(b)No

If yes, what do you consider?.....

…….

26. How do you dispose of materials following abortion or stillbirth?

(a) Dug the soil and Burry

(b)Incinerating

(c)Throwing in bushes

(d)Feeding dogs

Any other means specify ……...

Appendix 3: Checklist questions for veterinary service providers at Mpwapwa district

Qn1. Are you aware of brucellosis in pigs or have you ever come across any case of brucellosis in pigs to your working areas?

(a) Yes

(b)No

If yes, do you provide veterinary services whenever needed?

(a)Yes

(b)No

If not, why……..

……..

Qn2. How do you diagnose brucellosis in pigs before treatment?

(a)Based on observable clinical sign

(b)Seek for veterinary diagnostic lab results

Any other specify ……..……

……..…….

Qn3.What do you do if you are called to attend brucellosis or any cases with similar clinical signs to brucellosis in

pigs?.....

……..

Qn4. How do you manage the brucellosis cases in your area……..……

……..…

Qn5. How do you dispose aborted materials following abortion?…….…….

……..

6. Do you provide extension service regards to brucellosis in pigs to your pig farmers (a)

(a)Yes

(b) No

If not why…….……..

…….

7. What do you think the government and other livestock stakeholders should do to eliminate/ control brucellosis in pigs whenever confirmed in your working areas

……...…..

…….

END

THANK YOU FOR YOUR CO-OPERATION GOD BLESS YOU

Appendix 4: Spreadsheets design layout used to asses pig farmers and animals

Name of the farmer	Household No.	No. of pigs	Breed	Age		Sex		Location
				Adult	Young	Male	Female	

Age records of the animals were based on farmers memory record thus adult pigs those aged more than 6months and young pigs with less 6months.

Appendix 5: Layout during laboratory analysis

Positive control	1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18	19
20	21	22	23	24	25	26	27	28	29
30	31	32	33	34	35	36	37	38	39

The layout above shows how sera sample were placed on the on-Rose Bengal test Plate during lab analysis.

Appendix 5:

```
Notes:

. import excel "G:\Book2 (2).xls", sheet("new") firstrow

. logistic labresults age sex

Logistic regression                               Number of obs   =        349
                                                  LR chi2(2)      =       1.68
                                                  Prob > chi2     =     0.4314
Log likelihood = -37.271978                       Pseudo R2       =     0.0221
```

labresults	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
age	1.44604	1.564606	0.34	0.733	.1734544	12.05522
sex	.375986	.3100662	-1.19	0.236	.0746805	1.892937
_cons	.0444322	.1004823	-1.38	0.169	.0005281	3.738268

Appendix 6: Template Consent form for participants

Good morning / afternoon, Mr / Mrs .....…… I **Princepius Sebastian,** I am coming from Sokoine University of agriculture (SUA) Morogoro Tanzania**,** currently I'm pursuing a master of Science in **Public Health and Food Safety**. I am conducting a prevalence study on brucellosis in pigs among of pig farmers at Mpwapwa district.

I request your permission to take blood sample from your pigs and also please help me fill in the information in this questionnaire. Feel free to give the answers you know, your answers and your name will remain as confidential and you're not forced to answer the question that you don't know and you can stop this interview whenever it happens an emergency and then back after the emergency ends. This questionnaire will be completed in less than 30 minutes

Do you agree? **Yes / No**

Do you have any questions before signing this conset form? **Yes / No**

Respondent's signature …… Date ……..……

contact: princeseba69@ gmail.com /Mobile phone number: 0713916955/0756523561

Appendix 7:

SOKOINE UNIVERSITY OF AGRICULTURE
DIRECTORATE OF POSTGRADUATE STUDIES, RESEARCH, TECHNOLOGY TRANSFER AND CONSULTANCY
P.O. Box 3151, MOROGORO, Tanzania,
Tel: +255 23 264 0013, 023 264006-9, E-mail Address: drpgs@sua.ac.tz

Our Ref:	MPH/D/2018/2018/0004/11	**Our Date**	25th September, 2019

Mr. Princepius Sebastian,
Department of Veterinary Medicine and Public Health,
SUA – Morogoro.

U.f.s Head,
Department of Veterinary Medicine and Public Health,
SUA – Morogoro.

Fowarded 09.10.2019

U.f.s Principal,
College of Veterinary Medicine and Biomedical Sciences,
SUA – Morogoro.

Forwarded 9/10/2019.

Dear Mr. Sebastian,

RE: APPROVAL OF YOUR MSc. (PUBLIC HEALTH AND FOOD SAFETY) RESEARCH PROPOSAL

Please refer to the above captioned subject.

This is to inform you that, the Chairman of Senate Postgraduate Studies Committee (SPGSC) has noted the approval made by the Board of College of Veterinary Medicine and Biomedical Sciences for your MSc. research proposal. This means, you are now permitted to conduct research as per your approved research proposal.

In addition to the permission granted, please be reminded that, you are required to duly fill in progress report for the six month period ended June, 2019 and submit the same.

Wishing you all the best in your research work.

Yours sincerely,

Director
Postgraduate studies, Research,
Technology Transfer and Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro
TANZANIA

P. L. Mresa,
For: DIRECTOR.

CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA

SOKOINE UNIVERSITY OF AGRICULTURE
OFFICE OF THE VICE-CHANCELLOR
P.O. Box 3000 CHUO KIKUU, MOROGORO, TANZANIA
Phone: 255-023-2640006/7/8/9, Direct VC: 2640015; Fax: 2640021;
Email: vc@sua.ac.tz;

Our Ref. SUA/ADM/R.1/8/448 **Date:** 10th October, 2019

The Regional Administrative Secretary,
Dodoma Region,
P.O. Box 914,
DODOMA.

Re: UNIVERSITY STAFF, STUDENTS AND RESEARCHERS CLEARANCE

The Sokoine University of Agriculture was established by University Act No. 7 of 2005 and SUA Charter, 2007 which became operational on 1st January 2007 repealing Act No. 6 of 1984. One of the mission objectives of the University is to generate and apply knowledge through research. For this reason the staff and researchers undertake research activities from time to time.

To facilitate the research function, the Vice Chancellor of the Sokoine University of Agriculture (SUA) is empowered to issue research clearance to staff, students, research associate and researchers of SUA on behalf of the Tanzania Commission for Science and Technology.

The purpose of this letter is to introduce to you **Mr. Princepius Sebastian** a bonafide **MSc. (Public Health and Food Safety)** student with Registration number **MPH/D/2018/0004** of SUA. By this letter **Mr. Princepius Sebastian** has been granted clearance to conduct research in the country. The title of the research in question is "**Determination of Prevalence of Brucellosis in Pigs kept By Smallholders Farmers in Mpwapwa District and Nearby Livestock Training Centre".**

The period for which this permission has been granted is from **October, 2019** to **June, 2020**. The research will be conducted in **Mpwapwa District and Livestock Training Agency (LITA) Mpwapwa, Visele Livecrop.**

Should some of these areas/institutions/offices be restricted, you are requested to kindly advice the researcher(s) on alternative areas/institutions/ offices which could be visited. In case you may require further information on the researcher please contact me.

We thank you in advance for your cooperation and facilitation of this research activity.

Yours sincerely,

Prof. Peter R. Gillah
FOR: VICE-CHANCELLOR

Copy to: Student – **Mr. Princepius Sebastian**

VICE CHANCELLOR
SOKOINE UNIVERSITY OF AGRICULTURE
P. O. Box 3000
MOROGORO, TANZANIA