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SEROPREVALENCE AND RISK FACTORS OF *BRUCELLA* INFECTION IN DOGS IN ENUGU AND ANAMBRA STATES.

BY

ANYAOHA, CHIDIEBERE OHAZURIKE

PG/M. Sc/10/52913

A RESEARCH DISSERTATION SUBMITTED TO THE DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, FACULTY OF VETERINARY MEDICINE IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, UNIVERSITY OF NIGERIA, NSUKKA.

FEBRUARY, 2015
DECLARATION

I, Dr. Anyaoha Chidiebere Ohazurike hereby declare that this work was carried out by me and has not been submitted somewhere else.

-------------------------------------------------                                               --------------------------------
                         Dr. Anyaoha Chidiebere Ohazurike                         Date
CERTIFICATION

We certify that Dr. Anyaoha, Chidiebere Ohazurike carried out this research work in the Department of Veterinary Public Health and Preventive Medicine at the University of Nigeria, Nsukka. The work presented herein is original and has not been previously reported or submitted anywhere else.

Prof. J. A. Nwanta
(Supervisor)

Prof. B. M. Anene
(Supervisor)

Prof. A. O. Anaga
(Head of Department)

Prof. C. O. Nwosu
(Dean, Faculty of Vet. Med.)
DEDICATION

To God Almighty, who has protected me and kept me alive till today.

To my lovely parents, Mr. & Mrs C. O. Anyaoha, for their encouragement and support through the course of this work.

To my beloved brothers and sisters, for their inspiration and encouragement.
ACKNOWLEDGEMENT

I wish to express my profound gratitude and appreciation to my Supervisors, Professor J.A. Nwanta and Prof. B.M. Anene for their understanding, masterly guidance and encouragement throughout the execution of the laboratory work and writing of this research project. I also wish to thank them for assisting me in providing additional literature materials and proper correction of the work.

The cooperation and understanding received from the Head of the Department of Veterinary Public Health and Preventive Medicine, Prof. A.O. Anaga is highly appreciated.

I am also particularly grateful to Prof. S.I. Oboegbulem and his wife for their in-depth assistance in the course of this work. I also appreciate his contributions, corrections and his fatherly advice towards hard work and achieving good results and making sure that what is worth doing is worth doing well. His kind personal financial assistance towards the purchase of an extra Immunocomb® Canine Brucellosis Antibodody Test Kit used in this study is highly appreciated.

Thanks, to all the staff of the Department of Veterinary Public Health and Preventive Medicine, starting with the Head of the Department and all the lecturers that tutored me in one way or the other; including: Prof. J. U. Umoh, Dr. R. N. Chizoba and Dr. E. C. Okolocha. I also wish to appreciate the secretary of the Department Mrs. A. C. Uzommadu and her colleague Mrs Uzoamaka Onoja for their friendship and cooperation throughout the duration of my study.
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I would like to thank Dr. Sati Ngulukun for his magnanimity in procuring, shipping and supplying us the *Brucella abortus* SAT and RBPT antigens used in this work.

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A cross-sectional survey was used to assess the seroprevalence of *Brucella canis* and *Brucella abortus* antibodies in dogs in Anambra and Enugu States, Nigeria. The objectives of the study were to determine the seroprevalence of *B. canis* and *B. abortus* in dogs presented to major veterinary clinics, dogs for slaughter in selected areas and apparently healthy owned dogs in visited households in Enugu and Anambra States; to determine the sex, age and breed distribution of *B. canis* and *B. abortus* antibodies in screened dogs in both states and the possible risk factors for *brucella* infections in the study area. Five major veterinary clinics, two each in Enugu and Onitsha metropolis, one in Nsukka Urban; 2 major slaughter points in each state; and households with dogs that have history of infertility or abortion were selected by purposive sampling method. The study population was made up of 3 groups: dogs presented at veterinary clinics, household dogs with history of infertility or abortion and, dogs slaughtered for meat at markets. Visits were made to the purposively selected veterinary clinics, households, and slaughter points, once every other week for six months. A total of 123 dogs made up of 65 clinic dogs, 34 slaughter dogs and 24 household dogs were screened. Profiles of the dogs presented at the clinics and household dogs were also collected. Blood was collected from each dog and processed for serology. For *B. abortus* antibody assay, the serum was subjected to Rose Bengal plate test (RBPT) and Serum agglutination test (SAT). With the SAT, a titer value of 40IU/ml and above was regarded as positive. For *B. canis* antibody identification, Solid Phase Immunoassay technique using Immunocomb® Canine Brucellosis Antibody Test Kit was used. Titer value of 1:200 (IFA titer) and above was regarded as positive. For *B. canis* antibody identification, Solid Phase Immunoassay technique using Immunocomb® Canine Brucellosis Antibody Test Kit was used. Titer value of 1:200 (IFA titer) and above was regarded as positive. Chi-square statistic and odds ratio were used to analyze the data obtained. Out of the 123 dogs, screened, none was positive for *Brucella abortus* antibodies using both the Rose Bengal Plate Test (RBPT) and the Serum Agglutination Test (SAT). Thirty-four (27.7%) of the dogs screened were positive for *B. canis* antibodies using the Solid Phase Immunoassay Technique. Twenty-two out of 65 (18%) dogs presented to veterinary clinics, 8 out of 24 household dogs (6.5%), and 4 out of the 14 slaughter dogs (3.3%) were positive for *B. canis* antibodies. There was a strong association (p<0.05) between infection and sex with the infection being significantly higher (p<0.05) in female than in male dogs. Prevalence was significantly higher (p<0.05) in foreign breeds than in mixed and local breeds. There was no association (p>0.05) between the infection and the antibody titer levels of the
different categories of dogs. Dogs presented at the clinics and household dogs with titer levels 1:600 and above had history of recent abortion or infertility. There was significant association (p<0.05) between the presence of *Brucella canis* antibodies and free roaming of dogs. This study provides the first serological evidence of *B. canis* infection but found no evidence of antibodies to *B. abortus* in dogs in Enugu and Anambra States. This shows that *B. canis* is endemic in both states and can be considered as a new emerging disease, underscoring the need for further studies including isolation of bacteria and DNA extraction. Female dogs, exotic breeds of dogs and free roaming of dogs are at a higher risk of *brucella* infection in the study area. Therefore, preventive and controlling measures are strongly recommended.
SEROPREVALENCE AND RISK FACTORS OF BRUCELLA INFECTION IN DOGS IN ENUGU AND ANAMBRA STATES.

BY

ANYAOHA, CHIDIEBERE OHAZURIKE

PG/M. Sc/10/52913

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF NIGERIA,
NSUKKA.

FEBRUARY, 2015
CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office of International des Epizootics (OIE) as one of the most widespread zoonoses in the world (Schelling et al., 2003). According to OIE, it is the second most important zoonotic disease in the world after rabies. The disease affects cattle, swine, sheep, goats, camel and dogs. It may also infect other ruminants and marine mammals (Corbel, 1988; Radostits et al., 1995; Abubakar et al., 2012). Synonyms of brucellosis include: Undulant fever, Malta fever, Mediterranean fever, Enzootic abortion, Epizootic abortion, Contagious abortion, and Bang’s disease. It is an important Zoonotic disease and causes significant reproductive loss in sexually mature animals (Forbes and Tessaro, 1996; Wadood et al., 2009).

The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk (England et al., 2004). In females the most prominent sign is abortion after 45-55 days of gestation in about 75% of the cases; early embryonic death and resorption, or abortion 10-20 days after mating may occur in some cases (Shin and Carmicheal, 1999). The organism occurs in the fetus, placenta, fetal fluids and vaginal discharges after an abortion or stillbirth. This organism can be found in vaginal discharges for 4 to 6 weeks after abortion; it is also shed in normal vaginal secretion; particularly, during estrus, as well as in milk (Iowa State University, 2012). In males, the main sign is epididymitis of one or both testes and infertility (Shin and Carmicheal, 1999). B. Canis infection are found in semen for up to two months after infection and also found in urine, saliva, nasal and occur in secretions, and feces.
Worldwide, nine species of the genus *Brucella* have been recognized and the genus *Brucella* contains a group of very closely related bacteria. The first member of the group, *Brucella melitensis*, affects primarily sheep and goats. The second member of the group, *B. abortus*, affects primarily cattle *B. suis* affects pigs, *B. Ovis* affects sheep, *B. canis* affects dogs while the other members include *B. neotomae, B.microti, B. ceti* and *B. pinnipedialis* (Corbel, 1988; Sati, 2002; Dauglas, 2006; Foster *et al.*, 2007; Scholtz *et al.*, 2008). Cross transmission of brucellosis can occur between cattle, swine, sheep and goats and other species including dogs, horses, bison, rein deer and camels (FAO, 2003). Dogs can be infected by four of the six species of *Brucella* (*Brucella canis, Brucella abortus, Brucella melitenses and Brucella suis*, excluding *Brucella ovis* and *Brucella neotomae*) (Hollet, 2006). Outbreaks of canine abortions had been reported in 1963, but it was not until 1966 -1967 that *B. canis* was isolated from tissues and vaginal discharges of dogs. (Carmicheal, 1966; Carmicheal *et al.*, 1967, Taul *et al.*, 1967).

Brucellosis was first recognized as a disease affecting human-beings on the Island of Malta in the 19th and early 20th centuries when *Brucella melitensis* was first isolated from the spleen of a resident of the Island of Malta who died from a disease locally known as Malta fever (Brunner and Gilespire, 1966). In Nigeria, an outbreak of brucellosis was first reported in a government cattle farm located in Zaria in 1934. Serum plate agglutination test showed positive reactors of about 15% of the total animals in the farm (Banerjee and Bhalty, 1970).

Brucellosis as a zoonoses poses serious human health hazards worldwide (Hamidy *et al.*, 2002; Maloney and Fraser, 2004; Cadmus *et al*; 2006). Ruminants, pigs, horses, dogs and donkeys play an important role in the transmission of this disease to man (Cadmus *et al.*, 2006). In human, the infection is acquired by consumption of contaminated food of animal origin, and through aerosol (Gul and Khan, 2007). Infection can also result through contact with infected aborted materials
such as aborted fetuses, placenta membranes or fluids and other vaginal exudates (FAO/OIE/WHO, 2006).

Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Maadi et al., 2011). It also causes morbidity and considerable loss of productivity (Pappas et al., 2006). The disease is important from economic point of view; it is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Gul and Khan, 2007). Occupationally, Brucella can gain entry into humans especially veterinarians, animal handlers, butchers, abattoir workers through abraded skins, mucous membranes, conjunctiva, respiratory and gastro intestinal tracts.

1.2 Statement of the Problem

Dogs are the closest domestic animals to humans in Nigeria thus its interactions with human the populace may lead to an increase in the risk of infection (Cadmus et al., 2012).

In dogs, the infection is insidious and many are asymptomatic (Ewalt and Bricker, 2000); with the infected dogs shedding the organisms via urine, vaginal secretions, ejaculates, fetuses and feces and carrier dogs can be a source of infection and transmission to households (Brooks, 2006).

The increase in dog ownership in Nigeria is associated with some risk factors that render them vulnerable to brucellosis. Firstly, many exotic breeds are imported and are not screened before entry into the country (Cadmus et al., 2011). Secondly, some household dogs are fed with fetuses from cows and ruminants from slaughtered cattle with a history of bovine brucellosis from abattoirs (Cadmus et. al, 2010). In addition to these factors, some household dogs roam around
freely, placing them at greater risk of exposure to brucellosis. Also non-screening of mating dogs used for breeding predisposes to the high risk of infection among breeding kennels.

In both clinical and household dogs; as well as in Kennel dogs, abortion was recognized but not considered to be from infections with *Brucella*. Abortion due to trypanosomosis and other causes had also apparently been confused with *Brucella* abortion.

Increased consumption of dog meat has led to a spike in the slaughter of unscreened apparently healthy dogs for meat, hence an increase in risk of infection to the handlers, butchers, etc.

Symptoms in human brucellosis can be highly variable, ranging from non-specific, flu-like symptoms (acute form) to undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems, arthritis, orchitis and epididymitis and may be confused with a wide range of other diseases (Baba *et al.*, 2001). The cost of treatment and work days lost to ill health, combined with the loss of productivity in the animal husbandry, leads to decreased availability of food that adversely affects the health and economic well being of the population (Chauhan *et al.*, 2000; Baba *et al.*, 2001).

Although brucellosis is a notifiable disease in Nigeria, the incidence, prevalence and distribution of the disease is difficult to determine as the system of disease surveillance and reporting is fragmentary and inefficient (Ocholi *et al.*, 2004). Serological prevalence rate between 0.20% and 79.70% have been reported in animals and humans in various parts of the country (Esuroso, 1974; Chukwu, 1987b; Ocholi, 1993; Onunkwo *et al.*, 2005; Junaidu *et al*.; 2008); much emphasis is laid on cattle, sheep and goat; the same cannot be said of dogs.

It is also regarded as a disease of public health significance as it is zoonotic in nature, thereby making occupationally risk persons like butchers, abattoir workers, livestock owners and rearers,
veterinarians as well as other humans susceptible (Yagupsky and Baron, 2005; Belizadi and Mogheiseh, 2011). Thus, there is need to know more about the disease.

1.3  **Research Questions**

1. Are *B. canis* and *B. abortus* present in dogs presented in major Veterinary clinics in Enugu and Anambra States?

2. Are *B. canis* and *B. abortus* present in slaughter dogs in areas purposively selected for screening in Enugu and Anambra States?

3. Are *B. canis* and *B. abortus* present in apparently healthy owned dogs not presented to clinics?

4. What is the overall prevalence rate of *Brucella* infection in dogs in the study area?

5. What is the age, sex and breed distribution of *Brucella* infection in dogs in the study area?

6. Are there possible risk factors that may influence infection rate in dogs in the study area?

1.4  **Aim and Objectives of the Study.**

The aim of this study is to conduct a seroprevalence survey of *Brucella canis* and *Brucella abortus* infections in dogs in purposively selected areas of Enugu and Anambra States and to determine the possible risk factors that may influence infection.

The specific objectives are:

1. To determine the seroprevalence of *B. canis* and *B. abortus* in dogs presented to major veterinary clinics in Enugu and Anambra States.
2. To determine the seroprevalence of *B. canis* and *B. abortus* in dogs for slaughter in selected areas in both states.

3. To determine the prevalence of *B. canis* and *B. abortus* in apparently healthy owned dogs in visited households in both states.

4. To determine the overall prevalence rate of *Brucella* infections in the study area.

5. To determine the sex, age and breed distribution of *B. canis* and *B. abortus* in screened dogs in Enugu and Anambra States.

6. To determine the possible risk factors that may influence the infection rates in dogs in the study area.

### 1.5 Significance of the Study

This work will provide information on the current seroprevalence of *Brucella* antibodies in dogs in these States as well as the prevalent species.

The study will also provide information on sex, breed, and age distribution as well as some risk factors that may influence *Brucella* infection in dogs therefore contributing to the epidemiology of *Brucella* infection in dogs in Anambra and Enugu States.

The result will also be used to provide an appropriate recommendation to dog owners, veterinarians, butchers, cooks, public health authorities and government to institute urgent measures towards the control of the disease.
CHAPTER TWO

2.0 LITREATURE REVIEW

2.1 Historical Review

Recent examination of the ancient Egyptian bones, dating to around 750BC, shows evidence of sacroilitis and other osteoarticular lesions, common complications of brucellosis (Pappas and Papadimitrious, 2006). Brucellosis became a problem for the British Garrison in Malta (Malta fever) with substantial morbidity and mortality among the soldiers. Dr David Bruce, a military medic, was sent to try to deal with the problem. He coordinated a team of scientific personnel which succeeded in 1887 in isolating Micrococcus melitenses from the spleen of British soldier as a causative agent from raw goat milk consumed by the military personnel (Bruce, 1887; Bruce, 1893).

In 1897, a Danish veterinarian, Dr. Frederic Bang identified an intracellular microorganism described as Bacillus abortus as the cause of abortion in cattle. The disease was named after him, “Bang’s disease”. Bang’s discovery was of interest only to veterinarians, dairy farmers and meat producers. It had no impact on physicians and none of them made any correlation between Bang’s disease and Malta fever as the organism in Malta fever, was described as “Micrococcus” and Bangs disease as “Bacillus”. Twenty one years later an American microbiologist, Alice Evans reported the close relationship between Bacillus abortus and Micrococcus melitenses (Evans, 1918). Evans also identified Bacillus abortus in cow milk; she suggested a new name for Malta fever and called it ‘Brucellosis’. Brucella suis was isolated in 1914 from aborted swine fetuses (Smith et al., 1972). Brucella ovis was discovered by Buddle and Boyles, (1953) as an organism affecting rams causing sterility. Stoener and Lackeman, (1957) isolated Brucella neotome from desert wood rat (Neotoma lepida) in U.S.A. Carmicheal, (1966) an American
veterinarian from Cornell University first reported *Brucella canis* as the cause of abortion in beagles. *Brucella canis* was first isolated in dogs by Carmicheal and Kenney in 1968. In 1910, a British team led by Dr. David Bruce first isolated and confirmed brucellosis in Africa in the Ankota district of Western Uganda by the isolation of *Brucella melitensis* from goats and man (Smith *et al.*, 1972).

### 2.2 Aetiology

Brucellosis is caused by Gram negative bacteria of the genus *Brucella*, which are facultative intracellular cocobacilli that belong to the α2- proteobacteriacea (Yanagi and Yamasato, 1993). Nine *Brucella* species are currently recognized, seven of them that affect terrestrial animals are: *B. abortus*, *B. melitenses*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae* and *B. microti* (Scholz *et al*; 2008) and two that affect marine mammals are: *B. ceti* and *B. pinnipedalis* (Foster *et al*; 2007). *B. melitenses*, *B. suis* and *B. abortus* are considered the most pathogenic species for humans and have small ruminants, pigs and cattle as preferred hosts respectively (Godfroid *et al*., 2005). *B. ceti* and *B. pinnipedalis*, can also cause human brucellosis (Foster *et al*; 2007). Importantly, *B. canis*, a pathogen of dogs has a comparatively low zoonotic potential, while *B. neotomae* and *B. ovis* that infect desert rats and sheep respectively, are not associated with human disease. Nine biovars are recognized for *B. abortus* (Alton *et al*; 1988), three for *B. melitenses* and five for *B. suis*. The remaining species have not been differentiated into biovars (Seleem *et al*; 2010).

### 2.3 Classification

The genus *Brucellae* belongs to the order Rhizobiales within the class alpha-protobacteria (Gupta, 2005; Williams *et al*; 2007; Whatmore *et al*., 2009). Although *Brucella* represents
intracellular animal pathogens, it shares close relationship with soil organisms. The family *Brucella* consists of the genera *Brucella, Mycoplasma and Octobacterium* (Kaamfer et al., 2006).

2.4 Epidemiology

2.4.1 Distribution of Brucellosis

Brucellosis has been reported from United States (particularly in Southern States), Canada, Central and South America, some European Countries, Tunisia, Nigeria, Madagascar, Malaysia, India, Korea, Japan and China; Brucellosis is probably found throughout most of the world; however, New Zealand and Australia appear to be free of this organism (Iowa State University, 2012).

Dogs are the only species known to be affected by *B. canis*, however antibodies to this organism have been found in other carnivores. Experimental infections can be established in domesticated livestock and chimpanzees; however, these species are considered highly resistant to natural exposure (Iowa State University, 2012). There are reports that dogs can also be infected with other *Brucella* species: *B. melitenses, B. abortus*.

2.4.2 Brucellosis in Nigeria

Investigations and reports have shown that brucellosis is endemic in Nigeria (Halle and Ajogi, 1997). Evidence of, as well as frank out breaks of disease have occurred in cattle (Esuruoso, 1974; Nuru and Dennis, 1975; and Ajogi 1997) human beings (Banarjee and Bhalty, 1970; Alausa 1984) sheep and goats (Falade et al., 1974; Bale et al., 1982; Brisibe et al., 1993) camels
Initially there were doubts as to the presence of canine brucellosis in Nigeria when Falade (1977) found no antibodies in dogs. The first confirmed reports of brucella in dogs were cultural recovery of *Brucella canis* from a case of abortion in an imported female boxer (Oko, *et al.*, 1978). Recent studies suggest increasing trend in the prevalence of the disease (Ocholi *et al.*, 2004); but due to poor disease reporting system in Nigeria, the distribution of the disease is not documented. Subsequently, disease surveillance has not been very effective leading to poor determination of the incidence and the prevalence of Brucellosis in Nigeria.

**2.4.3 Canine Brucellosis**

Brucellosis poses serious human health hazards worldwide (Hamidy *et al.*, 2002, Maloney and Fraser, 2004; Cadmus *et al.*, 2006) while some countries have eliminated or substantially reduced the disease by extensive eradication programmes, it remains endemic in many areas of the world, including Nigeria (Cadmus *et al.*, 2010). The canine brucella was first recognized in 1966 as the cause of abortions and reproductive failures, and it has since been recognized in several countries (Shin and Carmichael 1999). However, with the current rise in dog breeding using imported dog species and the attendant random movement of dogs through trade, and increased slaughter of dogs as meat in the country, the spread of brucellosis among dogs in the country is most likely to increase. This is because puppies or adult dogs from infected kennels may be sold to non-infected kennels (Bertu, 2006).

**2.4.4. Bovine Brucellosis**

*B. abortus* the cause of bovine brucellosis, is transmitted by contact with the placenta, fetus and placental, fetal and vaginal fluids from infected animals. Animals are infectious after either abortion or full-term parturition. *B. abortus* may also be found in the milk, semen, feces and
hygroma fluids. Shedding in milk can be prolonged or lifelong or may be intermittent (Bercovich, 1998). Other natural hosts are horses and humans but are not considered important in the maintenance of the disease. Infection with \( B. \ abortus \), occurs rarely in pigs, sheep and goats (Sati, 2002). Infection occurs in cattle of all ages but persists in sexually matured animals; with females being more susceptible than males (Hungerford, 1975). Pregnant females are more likely to be more infected than non pregnant females and males because of a high concentration of erythriol in gravid uterus which sustains growth of the organism (Sati, 2002). Congenital infection may occur in calves born from infected dams, infection occurring in utero and may remain latent in the calf and the animal may remain serologically negative until first trimester of which it may begin to shed the organism to the environment. Isolation of the organism in dogs has been found in a farm where cattle were serologically positive to brucellosis (Radostis et al., 1995). Infection has been recorded in other animals such as bison, elk, heeves, deer, coyotes and other wild ruminants (Radostis et al., 1995).

2.4.5 Caprine Brucellosis

Nigeria has a large population of domestic ruminants with goats estimated at 25.5 million being the most numerous, followed by sheep and cattle with 14.5 and 12.5 million respectively (FAO/OIE/WHO, 1995). Brucellosis in goat has been reported in various parts of Nigeria (Falade, 1974, Bale, 1980; Ogundipe et al., 1994) all revealing that prevalence rates vary between 2.1-14.5%. Recent studies showed a prevalence of 22.93% in Sokoto State (Junaidu et al., 2008) and Adamu, 2012 showed a prevalence of 5.6% (Adamu et al., 2007).
2.4.6 Brucellosis in Sheep

Brucellosis in small ruminants (sheep and goats) has been reported in Northern Nigeria (Bale et al., 2003). Brucellosis in sheep and goats is usually caused by \textit{B. melitensis}. Infection with \textit{B. abortus} is rare, although the association of \textit{B. abortus} with abortion in sheep has been demonstrated in several countries through isolation of the organism (Luchsinger and Anderson, 1967; Zowghi and Ebadi, 1988; Ocholi et al., 2005). While a broad host range generally exists for \textit{Brucella} species, \textit{Brucella} infection follows a very strict, host-related hierarchy of pathogenicity (Adams, 2002). Thus, goats are the natural hosts of \textit{B. melitensis} and sheep are preferred hosts of the pathogen (Ocholi et al., 2005). Prevalence rates vary throughout and even within the same geographical zones operating different husbandry techniques (Eze, 1977; Bale et al., 2003). Recent studies suggest increasing trend in the prevalence of the disease (Ocholi et al., 2005). A recent sero-epidemiological survey of brucellosis in sheep reveals a prevalence rate of 14.3\% in Bauchi State (Ocholi et al., 2005) and a prevalence of 14.5\% (Bertu et al., 2010) in Plateau State. This further proves the confirmation of the existence of ovine brucellosis in Nigeria.

2.4.7 Brucellosis in Horses

Indigenous horses have been used by the army and police force in Nigeria for ceremonial parades, by mounted troops during special occasions to welcome dignitaries, and control crowds. They are accorded special attention due to the immense role they play in polo games, cultural festivals and security. The risk of disease transmission passed by these horses to riders, handlers, and the general public may be significant. Reports on equine brucellosis are rare (Ocholi et al., 2004) despite the endemicity of this disease in Nigeria.
2.4.8 Brucellosis in Camels

Brucellosis is a serious zoonotic disease affecting man and all domestic animals including camels (Radostits et al., 2007). The camel plays vital socio-economic roles and supports the survival of millions of people in the semi dry and arid zones of Asia and Africa. Although camels were considered in the past, and for a fairly long time, as resistant to many disease causing factors (Zaki, 1948; Dalling et al., 1988), it has been proved that they are susceptible to common disease causing pathogens affecting other animal species (Wilson 1984; Abbas and Tilley, 1990; Abbas and Agab 2002). However, camel brucellosis, has not received proper attention from researches and scientists. Brucellosis was reported in camels as early as 1931 (Solonitsunin, 1949); since then the disease has been reported from all camel – keeping countries: Libya with 4% (Azwai et al., 2001), Ethiopia with 5.7% (Teshome et al; 2003) and Nigeria with 11.4% (Junaidu et al., 2006). Camels are not known to be primary hosts of *Brucella*, but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper, 1991). The appearance of brucellosis in camels, depends on the *Brucella* species being prevalent in other animals sharing their habitat, (cross-transmission between species) and on the husbandry system (Musa et al., 2008). Camels play an important role in the epidemiology of brucellosis because the disease may spread through milk (camel milk) especially to those, living in dry and arid zones.

2.4.9 Brucellosis in pigs

Because of differences in cultural and religious beliefs, pig production in Nigeria is limited only to a few states; from the middle belt to the southern parts, therefore, this limits the existence of this disease to this region in pigs. Prevalence rate by Cadmus *et al.*, (2006) in Ibadan, Onunkwo
et al., (2011) in South East Nigeria, implies that the prevalence of brucellosis among swine population in Nigeria is on the increase. Previous studies have also stated that pigs infected with *Brucella* remain so for life and continue to shed the organism (Lucero et al., 2005; OIE, 2009; Godfroid et al., 2010). This indicates that these infected animals will still remain infected and thus pose a threat to humans and other livestock if effective control measures are not adopted (Bello-Onaghise et al., 2012).

### 2.4.10 Brucellosis in Chickens

Chickens are kept in most part of Nigeria due to their nutritional and economic importance (Baba et al., 2001; Junaidu et al., 2006). Chickens have been reported to be susceptible to *brucella* infection (Abdallah et al., 1984) causing a decrease in egg production in infected hens with the recovery of *brucella* from the egg shell, egg yolk and white droppings and internal organs of infected birds (Abdallah et al., 1984). A recent survey carried revealed 0.67% prevalence in Kaduna state (Gugong et al., 2012), with antibodies to *Brucella* demonstrated in the local chickens indicating that there is avian brucellosis in the locality sampled and in Nigeria in general. Also in another survey where *B. melitensis* and *B. abortus* antigen were used, there was a prevalence of 3.0% of *B. melitensis* in the number of birds sampled (Junaidu et al., 2006).

### 2.4.11 Brucellosis in Marine Animals

There is little information on the effects of brucellosis in marine mammals. Prior to the first reports, in 1994, of *brucellae* isolations from stranded harbor seals (*Phoca vitulina*), harbor porpoises (*Phocoena phocoena*) and common dolphins (*Delphinus delphis*) on the coast of Scotland (Ross et al., 1994); and from an aborted fetus of a captive bottlenose dolphin (*Tursiops*
truncates) in California (Ewalt et al; 1994); brucellosis was not only unrecognized, but was not even suspected in marine mammals. However, studies carried out indicated isolation of Brucella from cetaceans and pinnipeds inhabiting seas and oceans of Europe and North America (Ross et al; 1996; Forbes et al; 2000; Cloeckaert et al., 2001) or kept in captivity (Ross et al; 1994). In the recent study concerning experimental infections of Nile Catfish with B. melitenses biovar 3, the bacteria were successfully cultured from visceral organs, suggesting that these fishes are susceptible to Brucellae (Saleem and Moshen 1997). However, in one case, exposure of a laboratory worker to marine mammal brucellae revealed that such bacteria may also be pathogenic to humans (Brew et al., 1999).

2.4.12 Brucellosis in Humans

Brucellosis in humans is hardly diagnosed in hospitals in Nigeria despite suggestions that the magnitude of infections may be greater than appreciated (Njoku 1995; Rajis et al., 2003). This absence may be due to other diseases with similar clinical signs that are endemic and hence often diagnosed. Such diseases are typhoid fever, malaria and pasteurellosis with signs which include acute recurring fever chill, headache, fatigue, night sweat and anorexia (Rajis et al., 2003). The first case of brucellosis in a human being was in 1962 (Collard, 1962) when Brucella antibodies were demonstrated in the sera obtained from healthy persons. In a recent study carried out in Makurdi, North Central Nigeria, a prevalence rate of 7.6% was obtained in the study area, and this agrees with other findings of other workers who reported high seroprevalence of the range of 6% to 28% among hospital patients in Nigeria (Collard 1962; Falade 1978; Asanda and Agbede, 2001; Edu, 2005). Also other studies revealed that Brucella abortus was the main cause (77.2%) of human brucellosis (Ocholi, et al., 1993; Asanda and Agbede, 2001; Junaidu et al; 2004). Most
cases of human brucellosis were occupational hazards occurring amongst workers in the livestock industry. Epidemiological studies have revealed significantly higher prevalence of infection among occupationally exposed person (Bertu, 2006).

2.5. Growth Media and Growth Characteristics of *Brucella* Organism

There is a range of commercially available culture media for growing *Brucella*. The most common basal media in use are: Triptcase soy (BBL®), Bacto Tryptose (Difco®), Triptic soy (Gibco®), Tryptone soya (Oxoid®). The powder media can be used to prepare either broth or agar medium. For culturing blood and other body fluids, it is preferred to use broth or a biphasic medium (Castañeda), mainly because *Brucella* is often present in small numbers. Most *Brucella* strains, particularly *B. abortus* biovar 2 and *B. ovis*, grow better in media containing 5-10% of sterile (equine or bovine) serum free from *Brucella* antibodies (Fernando *et al.*, 2010). During the primary culture, it is very important to add antibacterial and antifungal agents to suppress the growth of contaminants. Such agents are: 100 mg of cycloheximide (fungistat), 25,000 units of bacitracin (active against gram-positive bacteria) and 6,000 units of polymyxin B (active against gram-negative bacteria) (Sati, 2002; Fernando *et al.*, 2010).

After 48-72h of incubation at 37°C, *Brucella* colonies are 0.5 to 1.0 mm in diameter with a convex and circular outline. Smooth strains are transparent and pale yellow, resembling droplets of honey with a shiny surface when observed in transmitted light. Rough colonies are more opaque with a granular surface. Dissociation of *Brucella* can be detected by the emulsification of a colony in 0.1% w/v aqueous acriflavine (Braun and Bonestell, 1947). Smooth colonies produce a yellow uniform suspension whereas rough colonies produce granular agglutinates. Colonial variation can be detected also by examining the plates under oblique light after staining the
colonies with crystal violet (White and Wilson, 1951). Smooth colonies appear translucent and pale yellow and rough colonies are stained with red, purple or blue with opaque and granular appearance. Colonial morphology, staining, slide agglutination with anti-Brucella serum (smooth or rough), urease, catalase and oxidase tests are the basis for a culture to be identified as belonging to the genus Brucella. Once a culture has been identified as Brucella, it is important to classify the species and the biovars. This further classification should be done in specialized or reference laboratories. These tests are cumbersome and include carbon dioxide requirement (CO2), production of hydrogen sulphide (H2S), dye sensitivity (thionin and basic fuchsin), phage lysis, agglutination with A, M or R specific antisera and in some cases it is necessary to use the oxidative metabolic method. This latter test is time consuming and hazardous to laboratory personnel. For these reasons it should be performed only by international reference laboratories.

2.6 Transmission of Brucellosis

Dogs are most commonly infected by contact with vaginal discharges at estrous or after abortions, or by ingesting infected placentas or fetuses. At abortion, the placenta and the discharges can contain up to $10^{10}$ colony forming units (cfu) per mL, and the oral infection dose is $2 \times 10^6$ cfu. Experimentally, $10^4$ cfu has been sufficient for conjunctival infection (Carmichael, 1976; Serikawa and Muraguchi, 1979; Carmichael et al, 1984a). Thus, 1 ml placental tissue or vaginal discharge is equal to approximately 100,000 infectious doses, and the bitches can have a vaginal discharge for up to 6 weeks after an abortion. Dogs can also be infected at mating. It should be observed that chronically infected dogs can be serologically negative and negative on blood culture, although B. canis can be detected from the prostate, epididymis and semen, or in female dogs in vaginal secretion, and thus they can still infect other dogs at mating or via
artificial insemination (Carmichael et al., 1984b; Keid et al., 2007). Environmental infection is possible, especially from areas where dogs often urinate (Serikawa and Muraguchi 1979), or where vaginal discharges are deposited. Dogs living together are at risk of infecting each other bitches (Carmichael and Joubert, 1988). It was suspected that contaminated urine was an important source of infection in these cases, especially from male dogs. The concentration of bacteria in urine and semen is highest from 1 to 4-6 months after infection (Carmichael and Joubert, 1988). *B. canis* can also be spread on fomites. In conditions of high humidity, low temperatures, and no sunlight, *Brucella spp.* can remain viable for several months in water, aborted fetuses, feces, equipment and clothing. *Brucella* species can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing (Iowa State University, 2012).

Transmission of *Brucella abortus* is mainly through the oral route because animals tend to lick aborted fetuses, placentas and vaginal discharges of animals that aborted (Cunningham and Dolan, 1978). Ingestion of contaminated water and feed with *Brucella abortus* have been also reported (Bercovich, 1998). Infection can also occur invitro or when calves and young animals born to a healthy mother are fed colostrums or milk from infected cows (Cathleen and Sheem, 1986).

Transmission in humans is principally by contact with infected materials such as carcasses aborted fetuses, placentas, vaginal discharges, manure, semen and urine (Sati, 2002). Man to man transmission is very rare (Currier 1989). Other routes of infection in man include inhalation of air droplets containing the organism (Currier, 1989). Accidental self inoculation with strain 19 of *Brucella abortus* vaccine is another possible way of contacting the disease (Weidman, 1991).
Because person to person transmission rarely occurs, infected persons do not pose a threat to their surroundings; therefore, eradication of the disease from the natural animal reservoirs leads to a dramatic decrease in the incidence of human infection (Cook, et al., 2002). Only a few cases have been reported of transmission by blood transfusion and bone marrow transplants (Young, 1989). Possible breast milk transmission to infants (Lubani et al., 1988; Meador et al., 1989; LaBrune et al., 1990) and possible venereal transmission from infected laboratory workers to their spouse (Ruben et al., 1991) have also been documented.

2.7 Reservoirs of Brucella canis and Brucella abortus.

Only domestic and wild canids appear to be highly susceptible to B. canis infection. Cats are moderately susceptible. Guinea pigs, mice, rats and non-human primates have been experimentally infected, but the disease is relatively mild in these species (Forbes, 1990). Dogs play a significant role in the epidemiology of bovine brucellosis. Naturally Brucella abortus infection in dogs has been reported and outbreaks of cattle brucellosis in association with infected dogs have been reported (Prior, 1976; Forbes, 1990). Sheep and goats do not easily become infected with Brucella abortus (Alursurp, 1974), but once acquired; they become carriers and continue to excrete the organism (Oko, 1980; Corbel, 1988). Studies also indicate that wild life animals such as buffalos, wild pigs, bears, foxes, rodents, bison and elks are susceptible to Brucella abortus and may serve as reservoirs for cattle (Cook et al., 1988: Davies et al., 1990). Birds can also be a reservoir of Brucella abortus for cattle and humans especially in Nigeria (Bale and Nuru, 1982; Kudi et al., 1997).
2.8 Resistance and survival of *Brucella* organisms in the environment

The survival of *brucellae* organism in the environment plays an important role in the epidemiology of the disease. Temperature, humidity and pH influence the organism ability to survive (Bercovich 1998). Alteration of the environment by people can change the exposure potential for animals and increase the prevalence or even the occurrence of brucellosis in these species of animals. *Brucellae* do not generally multiply outside the host animal, but they may persist in the environment for variable periods of time depending on protection from heat, extremes in pH and drying (Corbel, 1988). *Brucellae*, may persist nearly indefinitely if frozen and protected from the environment in aborted fetuses or placentas. At cool temperature (10-15 °C) the organism may persist for months if kept moist, but at high temperatures (45-50 °C) the organism will die in a few hours. *Brucella* can also survive in bovine feces for at least 1 year, in liquid manure and frozen soil for up to 2 ½ years. Contaminated straw remains infected for more than one month. *Brucellae* is sensitive to a wide range of disinfectants including strong acids and alkali, hypchlorite, iodosphors, 1% lysol and formaldehyde (Corbel, 1988). *Brucellae* show high sensitivity to a wide range of antibiotics and chemotherapeutic agents including penicillins, aminoglycosides, chloramphenicol, tetracycline and aminocydotol. However, most strains of the organism are resistant to nancomycin, bacitracin, polymyxins, metronidazole and antifugal agents (Corbel, 1988).

2.9 Zoonotic potential of brucellosis

Five out of the nine known *Brucella* species can affect humans and the most pathogenic and invasive species for human is *B. Melitensis* followed in descending order by *B. suis*, *B. abortus* and *B. canis* (Acha and Szyfres 2003). The zoonotic nature of the mairne *Brucella* (*B. ceti*) has
been documented (McDonald et al., 2006; Sohn et al., 2003). B. melitensis, B suis and B abortus are listed as potential bio-weapons by the centers for Disease Control and Prevention in the USA. This is due to the highly infectious nature of all the three species as they can be easily aerosolized. Moreover, outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of the influenza (Seelem et al., 2009). In places where brucellosis is endemic, humans can get infected via contact with the infected animals for consumption of their products. Some specific occupational groups including farm workers veterinarians, ranchers and meat packaging employees are considered at higher risk (Tabak et al., 2008). B. abortus and B. suis infection usually affect occupational groups while B. melitensis infections occurring more frequently than the other Brucella species in the general population (Acha and Szyfres, 2003; De-Masis et al., 2005) through the consumption of sheep or goat milk containing B. melitensis (De-Masis et al., 2005). In human brucellosis, person to person transmission rarely occurs, infected persons do not pose a threat to their surroundings. Eradication of the disease from the natural reservoirs leads to a dramatic decrease in human infection (De-Masis et al., 2005).

2.10 Pathogenesis

The pathogenic potential of Brucella spp is highly dependent on its ability to enter and survive within host cells. Brucella does not have classic virulence factors such as exotoxins, capsule, or endotoxic, lipopolysacharide (LPS) (Moreno and Moriyon, 2001). The major virulence mechanisms of Brucella are those required for host cell invasion and intracellular survival and replication (Lopez et al., 2002; Arellano et al., 2005; Lapaque et al., 2005). Brucella organisms, enter the body via the mucosal membranes of the oropharynx, genital tract or conjunctiva
(Carmicheal and Kenney, 1970). The bacteria are then phagocytised by macrophages and other phagocytic cells, and transported via the blood to lymph nodes, spleen and genital organs, where they multiply (Carmicheal and Kenney, 1970). Hyperplasia of the lymphoid tissue is seen throughout the body (Spink and Morisset, 1970). Bacteremia occurs 1 to 4 weeks after the infection and persists for at least 6 months, and then reoccurs intermittently for up to 5 years. (Carmicheal et al., 1984)

*Brucella* organism may invade and cause complications in the eye, kidney, and meninges. After an extremely variable duration of bacteremia, dogs infected with *brucella* may spontaneously recover and show decreasing serum agglutination titer. However, utilization of such dogs for breeding is not recommended. When dogs recover, they are reported to be immune to re-infection for 3 months up to 4 years. (Carmicheal 1976; Carmicheal and Greene, 1990).

Bacteremia may persist for varying periods of time depending on the host and *Brucella* species. In goats infected with *B. melitenses*, bacteremia is detectable in 10-20 days and may persist more than 300 days, although the duration is generally less than 2 months. In cattle infected with *B. abortus* the onset of bacteremia is variable – from a few days up to 2 months or more and may last 5 months or more. In swine infected with *B. suis*, bacteremia occurs early and generally lasts 60-90 days but may persist for more than 3 years (Carmicheal and Greene, 1990). Bacteremia with *B. canis* has been shown to be dose dependent. The organism has been detected with a range of 1-4 weeks post-experimental exposure; in some cases the bacteremia was still detectable at 1,120 days (Carmicheal and Greene, 1990).
2.11 Pathology

Lymphadenopathy of the retropharyngeal or inguinal lymph nodes or generalised lymphadenopathy sometimes occurs (Carmichael and Kenney, 1970). Discospondylitis, endocarditis and polygranulomatous dermatitis has been reported (Kerwin et al., 1992; Iowa State University, 2012). Dogs that were experimentally infected with *B. canis* often showed recurrent uveitis for several months after the infection (Carmichael, 1976) and there are several case reports describing ocular signs, such as endophthalmitis and anterior uveitis, in dogs infected with *B. canis* (Vinayak et al., 2004, Ledbetter et al., 2009). Male dogs are reported to have enlarged scrotums often accompanied by surface dermatitis with possible discomfort at the time of ejaculation. Lymphadenopathy and splenomegally are observed in both male and female dogs. Often the male dog will have scrotal swelling; however, if chronically infected, testicular atrophy is usually observed. Osteomyelitis related to hip prostheses has been described in two dogs (Smeak et al., 1987). Chronic meningitis and non-suppurative encephalitis has been associated with bacteria belonging to the genus *Brucella* (Carmichael and Kenney, 1970). In the testicles, tubuli are fibrosed (Moore and Kakuk, 1967). Lesions in infected lymph nodes are generally serous or suppurative lymphadenitis with accumulations of macrophages and neutrophils. *Brucella abortus* and *B. melitenses* generally do not cause gross abscesses, whereas *B. suis* frequently result in abscess formation (Ray, 1979; Blasco, 1990). The presence of erythritol in the uterus of cattle, goats and sheep, and swine favours the growth of *Brucella*. Proliferation of *brucellae* results in necrosis of the maternal and fetal placental membranes, resulting in the death and eventual expulsion of the fetus. Abortion in sow can occur any time during pregnancy, and fetal death can occur without subsequent expulsion of the fetus; hence dead and live pigs can be born in the same litter (Ray, 1979; Alton, 1990; Blasco, 1990).
2.12 Immunity

2.12.1 Humoral Immunity and immunoglobulin production during infection

Natural hosts defenses against *Brucella* play an important role in protecting animals against infection. Humoral immune response in animals are similar in most species affected by *Brucella*. Antibodies produced in immune response to *Brucella* infection include IgA, IgM, IgG1 and IgG2 (Hadju, 1971; Bel and Lescelles, 1973; Butter *et al.*, 1981). IgM are produced first following infection with virulent strains of *Brucella* organism or parenteral vaccination with *Brucella abortus* strain 19 (Corbel, 1988). They are usually in the first and second week of exposure. IgG1, IgG2 and IgGA thereby follow. Appearance of immunoglobulins in the serum of various animal species probably follows patterns similar to those observed in cattle following vaccination and natural infection. In vaccinated cattle the IgG begins to recede soon after reaching a peak and, depending on dose and age of the animal when vaccinated, may not be present in measurable quantities after a few months.

2.12.2 Cell-mediated immunity

*Brucella* are facultative intracellular parasites and can survive and multiply within polymorphonuclear and mononuclear phagocytes as well as other cell types (Radostits *et al.*, 1995). When there is infection with *Brucella* organism, the polymorphous and mononuclear cells internalize the bacteria and destroy them due to intracellular bactericidal activity (Corbel, 1988; Sati, 2002). This process of activation of mononuclear and polynuclear cells for phagocytosis is stimulated by interleukins released by T-lymphocytes responding specifically to the antigens (Corbel, 1984).
2.13 Diagnosis of *Brucella* Infections

### 2.13.1 Clinical signs

Clinical signs are associated principally with the reproductive tract. In females, the most prominent clinical sign is late-pregnancy abortion, after 45-55 days of gestation in about 75% of the cases, (Shin and Carmicheal, 1999), and this was the first sign described to be related to infection with *Brucella canis* when the bacterium was first recognized in 1966 (Carmichael, 1967). Aborted pups are partially autolysed and show signs of a generalized bacterial infection, such as subcutaneous edema, abdominal haemorrhages, and degenerative lesions in liver, kidneys, spleen and intestines. The bitch continues to excrete a brownish or green-gray discharge for 1-6 weeks after the abortion (Carmichael and Kenney, 1968). Sometimes early embryonic death and resorption occurs or abortion 10-20 days after mating (Shin and Carmicheal, 1999). Weak pups may die within a few hours, but other times may survive up to a month. Seemingly normal pups can also be born, and develop the disease later (Carmichael and Kenney, 1970).

Before puberty, a generalized lymphadenopathy is the most common clinical sign. Brucellosis does not affect the estrous cycle, and bitches that have aborted can give birth to normal litters in subsequent pregnancy, or have intermittent reproductive disturbances (Carmichael and Kenney, 1970).

In male dogs, epididymitis and prostatitis are the most common clinical signs. In the acute stage, the epididymis increases in size and is painful. Frequent licking may lead to scrotal edema and dermatitis. In the chronic phase the epididymis may become small and hard, and the testicle atrophic. Chronically infected dogs are often oligo- or azoospermic, and infertile. Sperm defects that can be seen include tail defects, loose heads and distal droplets (Carmichael, 1976). The testicular damage leads to production of auto-antibodies towards the sperm cells. These
antibodies can be detected in serum and seminal plasma from three months after the infection (Serikawa et al., 1984). From 4 months after infection the sperm cells autoagglutinate and probably contribute to male infertility (Serikawa et al., 1984).

Most often there are no general clinical signs, and infected dogs do not usually have a fever. Sometimes the dogs have a dull coat, or show decreased exercise tolerance, but this is not common (Wanke, 2004).

2.13.2 Direct Smear Examination

The organism can be demonstrated through stained smears prepared from fetal membranes, fetal stomach contents, vaginal swabs, semen, vaginal discharges, fetal tissues including the lungs, liver, and spleen, colostrums or milk samples, blood, samples of tissues collected during post mortem examination like supramammary lymphnodes, illac lymphodes and retropharyngeal lymphnodes, etc. The most common methods in use are the modified Ziehl-Neelsen and the mollified Köster staining methods (Jenny and Berman, 1962; Corbel, 1973; Morgan et al., 1978).

2.13.3 Identification and Typing

Brucellae are cocobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. However, they are resistant to decolorization by weak acids (Poester et al., 2010). After 48-72 hours of incubation, at 37 °C, Brucella colonies are 0.5 to 1.0 mm in diameter with a convex and circular outline. Smooth strains are transparent and pale yellow, resembling droplets of honey with a shiny surface when observed in transmitted light, while rough colonies are more opaque with granular surface (Braun and Bonesteal, 1947). Colonial morphology, staining, slide agglutination with anti-Brucella serum (smooth or rough), urease, catalase and oxidase tests are
the basis for a culture to be identified as belonging to the genus *Brucella*. After identification of a culture as *brucella*, further classification can be done in specialized reference laboratories. These tests are cumbersome and include carbon dioxide ($\text{CO}_2$) requirement, production of hydrogen sulphide ($\text{H}_2\text{S}$), dye sensitivity (thionin and basic fuchsin), phage lysis, agglutination with A, M or R specific antisera and in some cases it is necessary to use the oxidative metabolic method (Poester *et al.*, 2010).

Another approach is the typing of *Brucella* strains for epidemiological investigations, including taxonomic studies as well as tracing back strains to their origins. The strategy for the development of these tests is based on the observation that most organisms (prokaryotic and Eucaryotic) contain strings of tandem repeat sequences classified as microsatellites and minisatellites distributed throughout their genomes that may affect protein expression (Poester *et al.*, 2010).

### 2.13.4 Animal Inoculation

Another method for diagnosing *Brucella* is by animal inoculation. Even though *B. canis* is only known to be important in dogs, antibodies to this organism have been reported occasionally in wild canids including foxes and coyotes, as well as in racoon. Experimental infections have been established in chimpanzees, mice, rabbits and guinea pigs. Guinea pigs are more susceptible than Sheep, swine and cattle were reported to be highly resistant to experimental infection by oral and conjunctival inoculation; however, two field infections of *B. canis* have been reported in cattle (Iowa State University 2012). After oral inoculation, three out of 14 experimentally infected cats developed bacteremia, but agglutinating antibodies were not detected (Iowa State University, 2012).
2.13.5 Serological Diagnosis

A definitive diagnosis of *Brucella* infection requires simultaneous application of different laboratory techniques due to the lack of a single, highly reliable diagnostic method (Gyuraneozi *et al*.; 2011). Rose Bengal plate test (RBPT) and serum Agglutination test (SAT) are commonly used. Both tests detect certain levels of antibody produced against *Brucella* organism present in serum, semen and milk in a chronic disease. The serum may contain varying levels of antibodies in form of IgM, IgG1, IgG2 and IgGA at different stages of infection (Onunkwo, 2005). Several factors including long and variable incubation period, vaccination status and type of exposure during infection may influence the quantity of antibody produced making serological diagnosis difficult (Smith *et al*., 1972).

2.13.5.1 Rose Bengal Plate Test (RBPT)

This test is also called Agglutination test; and was introduced in 1957 by Rose and Roekpe to differentiate specific *Brucella* agglutinins from non-specific factors (Alton *et al*.; 1975b, Sati, 2002). The antigen used in RBPT method consists of *Brucella* cells stained with Rose Bengal and suspended in buffer at Ph 3.65±0.05. RBPT is a screening test and samples that test positive are further subjected to other tests such as Serum Agglutination Test, Complement Fixation Test, Enzyme Linked Immunorobent Assay (ELISA) (Ocholi, 1990). The RBPT detects antibodies of IgG1, IgG2 and IgM which are produced during early infections (Ocholi, 1990; Onunkwo, 2005). Different researches have different ratings for RBPT and SAT. Some researchers gave credence to RBPT while others rate SAT high more than RBPT (Morgan *et al*., 1969; Kazi *et al*., 2005).
2.13.5.2 Serum Agglutination Test (SAT)

Serum agglutination test referred to as the Standard Tube *Brucella* Agglutination Test (STT) is commonly used for diagnosis of acute brucellosis (Bercovich, 1998). However 2-mercaptoethanol (2ME) and Compliment Fixation Test (CFTs) are used for chronic brucellosis where active infection continues even though agglutination titers return to low levels (Acha and Szyfers, 2003). Serum Agglutination Test measures agglutination antibodies against IgM, IgG2, IgA. Morgan (1968), recorded that the SAT may be negative in the early infection and chronic infections. This limitation results from the test not detecting IgG1, a relevant antibody type involved in early infection. Some of the limitations of SAT include:

1. The test detects non specific agglutinins and cross reacting antibodies as well as specific antibodies arising from *Brucella* infection and vaccination.

2. In the incubative stage of the disease, the test may be the last of all the serological tests to detect diagnostically significant titers.

3. In the chronic stage, the agglutinin titers tend to wane, often becoming negative when those of some other tests remain positive.

2.13.5.3 Rapid Slide Agglutination Test (RSAT)

This test is commonly used, although a drawback to this method is the risk of false positive samples. By treating the sample with 2 mercapto –ethanol (2-ME) the number of false positive samples decreases (Mateude – Antonio *et al*., 1994; Keid *et al*.,2007) because IgM is dissociated and IgM cross-reacts with other bacteria more commonly than IgG (Deutsch and Morton, 1957)
2.13.5.4 Agar Gel Immunodiffusion Test (AGID)

The Agar Gel Immunodiffusion (AGID) Test has been described as suitable if a chance infection is suspected, because more chronic infections are positive using an AGID than using other Tests (Carmicheal et al., 1984). However, the AGID is not used by many laboratories today because of its low sensitivity and because it requires trained personnel and special media (Hollelt, 2006).

2.13.5.5 Agglutination Tests

Agglutination test generally cannot be used efficiently for the diagnosis of infection with Brucella canis and Brucella ovis which are rough species of Brucella. (Poester et al., 2010). As the whole cell antigens autoagglutinate, precipitin tests using soluble antigens are used instead (Poester et al., 2010).

2.13.5.6 Other Serological Tests

An array of other serological test for the diagnosis of brucellosis in dogs and other animal species including man are available. Such tests including their development, test, procedure, use and limitations have been described (Alton et al., 1975 a &b; Morgan et al; 1978; Seifert 1996; Poester et al; 2010). Some of these tests include;

1. Compliment Fixation Test
2. Enzyme Liked Immunosorbent Assay (ELISA)
3. 2 – Mercap- Ethanol Test (2-MET).
4. Radio Immunodiffussion Test (RID)
5. Radio Immunassay Test (RIA)
6. Fluorescent Antibody Test (FAT)

7. Antiglobulin Test (AGT) or Coomb’s test

8. Heat Inactivation Test (HIT)

9. Primary Binding Assay (PBs)

10. Fluorescence Polarization Assay (FPA)

11. Milk Ring Test (MRT).

No serological test has been shown to be reliable in routine diagnosis of swine brucellosis. While in the diagnosis of ovine and caprine brucellosis the Rose Bengal plate agglutination, CF and indirect ELISA tests are usually recommended for screening flocks and individual animals (Robinson, 2003)

2.14 Treatment of Brucellosis in Animals and Humans

Treatment of canine brucellosis in dogs is generally not recommended because affected dogs may continue to be a source of infection for other dogs and people in contact with them (Johnson and Walker, 1992). Treatment is not recommended for dogs in breeding facilities because antibiotics have limited effect and therapy is often unrewarding. Extensive use of antibiotics will reduce bacteremia but cannot reliably eliminate the intracellular organism from the body. Relapses are common, spaying or neutering the animal can reduce but not eliminate the transmission risk (Wanke, 2004). Even in uncomplicated cases, treatment of canine brucellosis is difficult, and relapses and treatment failures are common, especially in males (even neutered) (Scheftel, 2003). Current recommendations for treatment of spayed and neutered pet dogs consist of 4 to 8 week course of doxycycline (5 to 10mg/kg PO bid) together with streptomycin (5 to 10mg/kg IM bid) on the first and fourth weeks. Gentamycin, 2 to 4 mg/kg Sc or IM bid, may be
substituted for streptomycin if the drug is unavailable. (Carmicheal and Green, 1990). Antibiotic therapy is described but no good long term studies have demonstrated complete remission with antibiotic therapy (Hollett, 2006). The only reliable method of complete elimination of canine brucellosis involves isolation, testing and euthanasia of positive animals (Crow, 2012).

The World Health Organisation (WHO) issued recommendations for the treatment of human brucellosis in 1986 (FAO/WHO, 1986), suggesting the use of doxycycline, 100mg twice daily for six weeks combined with rifampicin, 600-900mg daily for 2-3 weeks.

2.15 Public Health Importance

Worldwide prevalence of brucellosis in human populations has been studied and reviewed. The Mediterranean Basin, South and Central America, Eastern Europe, Asia, Africa, the Caribbean and Middle East are considered as high risk countries (Abubakar et al., 2012). From public health view point, brucellosis is considered to be an occupational disease that mainly affects farm labourers, slaughter house workers, butchers, shepherds, laboratory workers and veterinarians (Yagupsky and Baron, 2005; Behzadi and Mogheiseh, 2011).

The slaughter house workers are more prone to acquire infection as compared to other occupations because they are exposed to carcasses and viscera of infected animals and get infected through cuts and wounds and splashing of infected blood and other fluid in the conjunctiva (Ramos et al., 2008).

Transmission typically occurs through contact with infected animals, materials with skin abrasions, inhalation of aerosols or ingestion of contaminated or unpasteurized food products (Christopher et al., 2010). Symptoms in humans can be highly variable, ranging from non-specific, flu-like symptoms (acute form) including intermittent fever, chills, night sweat,
headache, malaise, diarrhea with stomach cramps which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems and other problems like arthritis, orchitis and epididymitis (Alusurp, 1974). According to Schnurrenberger et al., (1975), larger proportion of veterinary graduates show serological evidence to *Brucella* infection than undergraduates and this was attributed to the regular coming in contact the practicing veterinarians have with infected animals than student veterinarians.

A history of exposure to dogs is usually needed to raise suspicion of infection with canine brucellosis in humans (Rumley and Chapman, 1986). The infection may be under diagnosed due to its rather unspecific symptoms. In Nigeria, high *Brucella* antibody titers have been reported among occupationally- exposed persons (Adekolu, 1989; Adesiyun and Oni, 1990). Positive blood cultures confirm the diagnosis, but as the patients have been treated with antibiotics in many cases, the risk of a false negative culture is increased (Polt et al., 1982).

2.16 Economic Importance of Brucellosis

Brucellosis causes economic losses from abortion, temporary infertility, chronic metritis, decreased milk production by dairy cows, death from acute metritis, sterility, increased cost of animal replacement and low sale values of infected animals (Rikin, 1988b; Ajogi et al., 1998; Sati, 2002). The dog breeding industry has grown significantly since *B. canis* was first recognized in 1966 and because of this, *B. canis*, has become a major source of economic loss in both large and small dog breeding facilities as well as households as a single outbreak can lead to the euthanasia of hundreds of dogs (Brower et al., 2007).
Human losses include illness, physical incapacitation, loss of man power, economic losses due to medical cost (Onunkwo, 2005). Although in Nigeria, no economic losses in terms of monetary value as a result of canine brucellosis in dog have been estimated, economic loss due to bovine brucellosis in Nigeria was estimated between N148.8m and N224m US Dollars excluding losses in human productivity (Esuruoso 1979; Ocholi et al., 2004).

2.17 Control, Prevention and Eradication of Brucellosis

Currently control of canine brucellosis within a kennel typically relies on prevention of infection and euthanasia of infected dogs (Carmicheal and Greene, 2006). In kennels that do not elect to treat infected dogs, repeated post-treatment testing is necessary before the dog can be considered to be free of infection. Treatment is usually reserved for pet animals early in the course of infection, typically involving castration or ovariohysterectomy in combination with antibiotic, therapy (Carmicheal and Greene, 2006). Dogs from kennels in which B. canis has been diagnosed should not be used for breeding. Dogs from endemic areas should be kept isolated until tested free of B. canis to avoid further spread of the disease. This is recommended for natural mating, artificial insemination with fresh, chilled or frozen semen, and when introducing new dogs into the kennels. For kennels in endemic areas, it is often recommended that breeding animals are tested annually, and that all new dogs are tested before being introduced into the kennel. Serologic tests can be negative up to 4 weeks after infection and at least 12 weeks must pass to be sure of detecting antibodies in an infected animal (Carmicheal et al., 1984).
2.17.1 Vaccines

*Brucella* infections in animals have an important economic impact especially in developing countries as they cause abortion in the pregnant animals, reduce milk production and cause infertility. In regions with high prevalence of the disease, the only way of controlling and eradicating these zoonoses is by vaccination of all susceptible hosts and elimination of infected animals (Briones *et al*., 2001). The most commonly used vaccines against bovine brucellosis are *B. abortus* Strain 19 and the recently USDA approved Strain RB51; the later unlike Strain 19 does not interfere with serological diagnosis (Moriyon *et al*., 2004). The use of *B. abortus* Strain 19 vaccine leads to the production of antibodies whose persistence depends mainly on the age of the animals at the time of vaccination. *B. melitensis* Strain Rev1 vaccine although highly infectious to human, is considered as the best vaccine available for the control of ovine and caprine brucellosis, especially when administered at the standard dose by conjunctival route. Vaccination alone will not eradicate *Brucella* as the immunity produced by *Brucella* vaccines are not absolute and can be circumvented by increasing the level of infection (Morgan, 1968).

Live human vaccines *B. abortus* Strain 19-BA and strain 104M are being used only in the former Soviet Union and China, respectively (Acha and Szyfres, 2003). The development of a live vaccine for the control and eradication of canine brucellosis is of great importance. Nevertheless, currently, there is no information that describes an appropriate candidate that can be used as a vaccine against *B. canis* (Palomares –Resendiiz *et al*., 2012).

2.17.2 Control and Prevention of Brucellosis in Humans

In many developing countries where brucellosis infection is prevalent the animal/human relationship is close and high percentages of people are involved in animal agriculture as an
occupation. In developed countries where effective control and eradication programs have been instituted, very low incidence of the disease in animals, leads to a corresponding low incidence of the disease in man (MZCP, 1998). Consistent with exposure potential, farm workers and animal health personnel should take precautions when working with animals that abort or give birth and should refrain from drinking raw milk unless its source is known to be uninected with Brucella or other pathogens excreted in milk. Laboratory workers should use safety cabinets when handling brucella cultures or tissues. Persons handling specimens for laboratory submission should wear gloves and submit samples in leak-proof and crush-proof containers. Vaccinating people in high risk agricultural occupation areas has been attempted in Russia and China (FAO/WHO, 2006).
CHAPTER 3
MATERIALS AND METHODS

3.1. Study design
The study is a cross-sectional survey using purposive sampling technique to screen dogs for Brucella antibodies.

3.2. Area of study
This study was carried out in Enugu and Anambra States of Nigeria.

Enugu State is located between latitude 5°55N and 7°55N and longitude 6°53E and 7°55E. It covers a total land area of about 802, 295km² and has a population of 2.5million with a population density of 240 persons per square kilometer (NPC, 2006). It is bounded in the South by Abia and Imo States, in the East by Ebonyi State, in the North-East by Benue State, in the North-west by Kogi State and in the West by Anambra State. Enugu State is made up of 3 Senatorial Zones and 17 Local Government Areas. The senatorial zones are: Enugu-East, Enugu-West and Enugu-North. Tropical forests and savannah predominates the area ecologically. The wet season lasts from April to October while the dry season lasts from October to early April. The indigenous people of Enugu State are predominantly Igbo-speaking and are involved in two major farm activities, crop and livestock. Dogs are kept as part of the culture of the people for breeding, hunting and for security. Dog meat is eaten by a section of the population.

Anambra State is located between latitude 6°20N and longitude 7°00E. It covers a total land area of about 4, 844km². According to National Population Commission (2006), it has a population of about 3, 902, 051 with a population density of about 840 persons per square kilometer. It is bounded by Delta State to the West, Enugu State to the East, Imo State to the West and Kogi State to the North. The state is made up of three Senatorial Zones and 21 Local Government Areas. The zones comprise of Anambra- North, Anambra- Central and Anambra-South. The indigenous people of Anambra State are predominantly Igbo-speaking and are involved mainly in trading, with the State having two seasons (dry and wet) and a tropical rain forest. Like in Enugu State, dog keeping and dog eating is a common feature of some of the indigenous population.
3.3 Map of Enugu State

Source: Local Government Areas http://www.google.com.ng/url?
3.4 Map of Anambra State

3.5 Study duration
This study lasted for 24 weeks (February – July, 2013).

3.6 Study population
The study was purposively targeted towards 3 groups: (a) Dogs presented at veterinary clinics (b) Household dogs with history of infertility or abortion and (c) Dogs at slaughter points in the markets.

3.7 Sample Size Determination
The required sample size was determined using the following formula:

\[ n = \frac{Z^2 pq}{d^2} \]  
(Thrusfield, 2005)

Where

- \( n \) = Desired sample size
- \( Z^2 = 1.96 \) (normal distribution) from table
- \( p \) = Prevalence rate from the average of previous studies
- \( d \) = Desired absolute precision of ± 5% with 95% Confidence Interval
- \( q = 1 – p \)

In this study, according to Adedoyin (2012), a prevalence rate of 7.94% was used for sample size determination. Using the formula above, a sample size of 112 was calculated. However, based on the number of Canine Brucellosis Antibody Test Kit® bought which was 11 packs (each pack screens 12 dogs), 123 dogs were screened in this study.

3.8 Sampling technique
Five major veterinary clinics, two each in Enugu and Onitsha metropolis, one in Nsukka Urban; 2 major slaughter points in each State; and households with dogs that have history of infertility or abortion were selected by purposive sampling method. Visits were made to the purposively selected veterinary clinics, households, and slaughter points, once every other week for six months.
A total of 123 dogs made up of 65 clinic dogs, 34 slaughtered dogs and 24 household dogs were screened. Profile of the dogs presented at the clinics and household dogs were also collected. Information requested included: sex, age, and breed, history of infertility / abortion in female dogs, and some possible risk factors / management practices for *Brucella* infection in dogs.

3.9 Sample collection.

Collection of blood

Aseptically, 5mls of blood sample was collected from the cephalic vein of each animal, after a proper restraint, using sterile syringe and needles. The blood was kept in a slanting position for about 30minutes to allow for proper clotting. It was then centrifuged at 3000rpm for 5minutes. The sera were afterward harvested one after the other using separate syringes into labeled bijoux bottles and stored at - 20°C until they were analyzed.

3.10 Sample analysis

3.10.1 Serological tests

(A) For *B. abortus* identification, two tests were used:

i) Rose Bengal Plate Test (RBPT): Antigen was procured from Central Veterinary Lab., New Haw, Weybridge Surrey, England.

ii) Serum Agglutination Test (SAT)

SAT specific for *B. abortus* antigen was further diluted as described by the manufacturer (Onderstepoort Biological Product (LTD) Republic of South Africa). One ml of antigen was dissolved in 99ml phenol saline.

(B) For *B. canis* identification: ImmunoComb® Canine Brucellosis Antibody Test Kit specific for *B. canis* antigen (Biogal Galed Labo., Kibbatz Galed 19240, Israel) was used following the manufacturer’s instructions.
3.10.2 Test procedure.

3.10.2.1 Procedure for Rose Bengal Plate test (RBPT)

The RBPT was carried out as described by Morgan et al (1978) and Alton et al (1975). Using a pipette, 1 drop (0.03ml) of the test serum was placed on one spot of the slide using another pipette. An equal volume of RBPT B. canis antigen was placed close to the test serum on the slide. Using an applicator stick, the antigen and the test serum were mixed thoroughly, the slide was then hand-rocked for about 4 minutes after which the slide was examined for agglutination under a good source of light. Formation of pink granules (agglutination) was recorded as positive while absence of pink granules (agglutination) was recorded as negative. Interpretation of the test was as follows

++++100% agglutination (background is clear)
+++75% agglutination (background slightly turbid)
++50% agglutination (background moderately turbid)
+25% agglutination (background very turbid)
-No agglutination (remain homogenous)

3.10.2.2 Procedure for Serum Agglutination Tests (SAT)

The Serum Agglutination Test was carried out as described by Morgan et al., (1978) and Alton et al., (1975). Five sterile test tubes were arranged in rows in a test tube rack labeled 1-5. A volume 0f 0.8ml of phenol saline was added into the first tube and 0.5ml of the phenol saline was added into the 2nd to the 5th test tube. Then 0.2ml of the test serum sample was placed into the first tube, mixed thoroughly without frothing and 0.5ml of the mixture in the first test tube was transferred to the next test tube and mixed. Serial dilution was carried on to the last test tube (tube 5) and 0.5ml of the mixture from the 5th test tube was discarded. One ml of the concentrated solution was added to 99ml of phenol saline to form a 1% solution and 0.5ml of the reconstituted Serum Agglutination Test. B. abortus antigen will then be added to each of the 5 test tubes using a dropping pipette. The serum dilutions in the 5 tubes became 1:10, 1:20, 1:40, 1:80 and 1:160 representing agglutination of >12.5 IU/ml, >25 IU/ml, >50 IU/ml, >100 IU/ml, and >200 IU/ml
respectively (Morgan et al., 1978; Sati, 2002). The tubes were incubated at a temperature of 37°C for 20-24 hours. The degree of agglutination and clearance was read under a black background against a light source. Titers of 1:40 (50 IU/ml) and above were taken as diagnostic for *B. abortus* (Morgan et al., 1978; Sati, 2002). The results of this test were interpreted as follows:

++++100% agglutination (background is clear)
+++75% agglutination (background slightly turbid)
++50% agglutination (background moderately turbid)
+25% agglutination (background very turbid)
- No agglutination (remained homogenous)

### 3.10.2.3 Procedure for the ImmunoComb® Canine Brucellosis Antibody Test Kit Specific for *B. canis* antigen (Biogal Galed Laboratories., Kibbatz Galed 19240, Isreal) used was based on the solid phase immunoassay principle:

The test was carried out by dropping 5ul of each serum sample into each sample wells of the multi-compartment developing plate (wells A) after opening the aluminium cover with the tweezers. Then, the Immunocomb on which purified *B. canis* antigen is attached was removed from its protective wrapping and fixed into well A so that antibodies from the samples if present, will bind to the antigen on the comb. The conjugate was incubated for 5 minutes. After incubation in well A, the developing plate was removed from the incubator and the rows of well B were opened and the comb put into well B and incubated for 2 minutes so that unbound antibodies were washed off. The Immunocomb was then transferred into wells of row C, row D, row E, and row F and incubated for 5 minutes, 2 minutes, 2 minutes and 5 minutes respectively. This was to allow for the colour reaction process to develop. Upon completion of colour development in row F, the comb was moved back to row E for 2 minutes for colour fixation to take place after which the comb was removed and allowed to dry. Upon drying, the Immunocomb was aligned and compared with the Immunocomb scale, and shades of grey colour that matches the positive reference spot, were regarded as positive. **Titre levels of 1:200 and above was regarded as positive.**
3.11 Data Analysis

Using Statistical Package for Social Sciences (SPSS) 17.0, Chi-square ($\chi^2$) statistic, odds ratio and 95% confidence interval were used to determine if there were significant association between *Brucella* antibody prevalence and sex, age, breed, history of infertility/abortion in female dogs, and some possible risk factors/management practices for *Brucella* infection in dogs.
CHAPTER FOUR

RESULTS

4.1 Distribution of dogs based on States and sources where samples were collected.

Over the period of the study, a total of 123 dogs were screened. Table 4.1 summarizes the sources/ categories of the dogs screened. Sixty-eight dogs were screened in Enugu State while 55 dogs were screened in Anambra State. Veterinary clinics contributed 65 of the total dogs screened while 34 and 24 dogs were screened from Slaughter points and households respectively.
Table 4.1: Distribution of dogs based on States and sources where samples were collected.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Number of dogs</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anambra State</td>
<td>Enugu State</td>
<td>Total</td>
</tr>
<tr>
<td>Veterinary Clinics</td>
<td>37</td>
<td>28</td>
<td>65</td>
</tr>
<tr>
<td>Slaughter House/ Market</td>
<td>6</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>Household</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>55</strong></td>
<td><strong>68</strong></td>
<td><strong>123</strong></td>
</tr>
</tbody>
</table>
4.2: Prevalence of *Brucella abortus* antibodies based on the sources of the dogs screened.

Of the 123 sera samples screened for *Brucella abortus* antibodies, none 0(0%) was positive using both the Rose Bengal Plate Test (RBPT) and the Serum Agglutination Test (SAT) (Table 4.2).
Table 4.2: Prevalence of *Brucella abortus* antibodies based on the sources of the dogs screened.

<table>
<thead>
<tr>
<th>Source</th>
<th>Number screened</th>
<th>Number positive</th>
<th>RBPT</th>
<th>SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vet. Clinics (Clinical dogs)</td>
<td>65</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Slaughter points</td>
<td>34</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Households</td>
<td>24</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>123</strong></td>
<td><strong>0 (0.00)</strong></td>
<td><strong>0 (0.00)</strong></td>
<td><strong>0 (0.00)</strong></td>
</tr>
</tbody>
</table>

Legend: RBPT=Rose Bengal Plate Test  
SAT= Serum Agglutination Test.
4.3: Prevalence of *Brucella canis* antibodies based on the sources of the dogs.

Out of 123 dogs from the different sources screened, 34 were positive for *B. canis* antibodies using the Immunocomb® Canine *Brucella* Antibody Test Kit. This gave an overall seroprevalence rate of 27.7 percent. Out of 65 dogs screened in the Veterinary Clinics (Clinical dogs) in Anambra and Enugu States, 22 (18%) were positive for *B. canis* (Table 4.3). Four (3.3%) out of 34 Slaughter dogs screened were positive. Of the 24 dogs screened from Households, 8 (6.5%) were positive. There was no association between infection rate and sources/categories of the dogs screened ($\chi^2 = 5.925, P>0.05$).
Table 4.3: Prevalence of *Brucella canis* antibodies based on the sources of the dogs screened.

<table>
<thead>
<tr>
<th>Source</th>
<th>Number screened</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunocomb® B. canis Antibody Test Kit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vet. Clinics (Clinical dogs)</td>
<td>65</td>
<td>22 (18)</td>
</tr>
<tr>
<td>Slaughter points</td>
<td>34</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>Households</td>
<td>24</td>
<td>8 (6.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>123</td>
<td>34 (27.7)</td>
</tr>
</tbody>
</table>

\(\chi^2 = 5.925, \ P > 0.05\)
4.4: Antibody titer levels of *B. canis* positive dogs screened in Anambra and Enugu States.

Out of the 34 *B. canis* positive dogs, 16 (47.1%) had a titer level of 1:200 (IFA Titer); 10 (29.4%) had titer level of 1:400 (IFA Titer), 4 (11.8%) had a titer level of 1:600 (IFA Titer); while the remaining 4 (11.8%) had a titer level of 1:800 (IFA Titer) (Figure 1)
Figure 1: Antibody titer levels of *B. canis* positive dogs screened in Anambra and Enugu States.
4.5: Antibody titer levels of *Brucella canis* positive dogs based on the sources of dogs.

The antibody titer levels of *B. canis* positive dogs based on sources/ categories of screened dogs are shown in Table 4.5. Ten (29.4%) of 16 positive dogs with antibody titer 1:200 (IFA Titer) are dogs from veterinary clinics; 4 (11.8%) are slaughter dogs and 2 (5.9%) are household dogs. Seven (20.6%) of 10 positive dogs with titer 1:400 (IFA Titer) are dogs from veterinary clinics; 3 (8.8%) are slaughter dogs with none from household dogs. Five (14.7%) of 8 dogs with titer 1:600 (IFA Titer) and above are dogs from veterinary clinics; 2 (5.8%) were household dogs and only one (2.9%) is a slaughter dog. Chi- Square analysis showed no association between antibody titer levels and sources of the dogs ($\chi^2 = 3.767; p>0.05$). Two household dogs and 2 of the 5 dogs from veterinary clinics with titer 1:600 (IFA Titer) and above had history of recent abortion or infertility.
Table 4.5: Antibody titer levels of *Brucella canis* positive dogs based on the sources of dogs

<table>
<thead>
<tr>
<th>Titer level</th>
<th>Total</th>
<th>Dogs from Vet. Clinics (%)</th>
<th>Slaughter dogs (%)</th>
<th>Household dogs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:200</td>
<td>16</td>
<td>10 (29.4)</td>
<td>4 (11.8)</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>1:400</td>
<td>10</td>
<td>7 (20.6)</td>
<td>3 (8.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1:600</td>
<td>4</td>
<td>3 (8.8)</td>
<td>0 (0)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>1:800</td>
<td>4</td>
<td>2 (5.9)</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34</strong></td>
<td><strong>22 (64.7)</strong></td>
<td><strong>8 (23.5)</strong></td>
<td><strong>4 (11.8)</strong></td>
</tr>
</tbody>
</table>

($\chi^2 = 3.767; p>0.05$)
4.6: Sex distribution of *Brucella canis* antibody positive dogs.

The female dogs had a seroprevalence of 22 percent (Table 4.6) while the male dogs had a seroprevalence of 6%. There was a strong association (p<0.05) between the infection of *Brucella canis* and sex of the dogs screened (Table 4.6).
Table 4.6: Sex distribution of *Brucella canis* antibodies of dogs sampled in South-East Nigeria.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number sampled</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>45 (36.6)</td>
<td>7 (5.9)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>78 (63.4)</td>
<td>27 (21.9)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>123 (100.0)</td>
<td>34 (27.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> \( \chi^2 = 5.174, p<0.023, \text{df} = 1 \)

<sup>b</sup>
4.7: Antibody titer levels of *B. canis* positive dogs according to sex.

From the 27 positive female dogs, 13(38.2%), 8(23.5%), 3(8.8%) and another 3(8.8%) had antibody titer levels of 1:200(IFATiter), 1:400(IFATiter), 1:600(IFATiter) and 1:800(IFATiter) respectively. In males out of the 7 positive dogs, 3(8.8%), 2(5.9%), 1(2.9%) and 1(2.9%) had titer levels of 1:200(IFATiter), 1:400(IFATiter), 1:600(IFATiter) and 1:800(IFATiter) respectively. Chi-square analysis showed that there is no association (P>0.05) between titer level and sex (Table 4.7).
Table 4.7: Antibody Titer Levels Of *B. Canis* Positive Dogs According To Sex.

<table>
<thead>
<tr>
<th>Titer level</th>
<th>Total (%)</th>
<th>Female (%)</th>
<th>Male (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:200</td>
<td>16</td>
<td>13 (38.2)</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>1:400</td>
<td>10</td>
<td>8 (23.5)</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>1:600</td>
<td>4</td>
<td>3 (8.8)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>1:800</td>
<td>4</td>
<td>3 (8.8)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34 (100)</strong></td>
<td><strong>27 (79.4)</strong></td>
<td><strong>7 (20.6)</strong></td>
</tr>
</tbody>
</table>

(P > 0.05; $\chi^2 = 0.130$)
4.8: Age Distribution of *Brucella Canis* Antibody Positive Dogs (Clinical And Household Dogs).

Dogs below 1 year old had a seroprevalence of 3.4%, dogs 1-<3 years of age had seroprevalence of 10.1%, while those 3-<5 years and 5 years and above, had seroprevalence of 15.7% and 4.5% respectively. Thus 23 out of 66 dogs aged between 1 year and below 5 years were positive, giving a seroprevalence of 34 percent. There was no association (p>0.05) between *Brucella canis* infection and age in the dogs screened (Table 4.8).
Table 4.8: Age Distribution Of *Brucella Canis* Antibody Positive Dogs (Clinical And Household Dogs).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number sampled</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>11</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>1-&lt;3 years</td>
<td>32</td>
<td>9 (10.1)</td>
</tr>
<tr>
<td>3-&lt;5 years</td>
<td>34</td>
<td>14 (15.7)</td>
</tr>
<tr>
<td>5 &amp; above</td>
<td>12</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>89</strong></td>
<td><strong>30 (33.7)</strong></td>
</tr>
</tbody>
</table>

($\chi^2 = 1.500, P>0.05$)
4.9: Breed Distribution of *Brucella Canis* Antibody Positive Dogs.

Twenty-six out of 76 exotic breed of dogs were positive for *B. canis* antibody; this gives a seroprevalence of 21.1% (Table 4.9). Mixed and local breeds each had seroprevalence of 3.3%. Chi-square analysis revealed there was a strong association (P<0.05) between infection and the breeds of dogs, with the infection being higher in exotic breeds of dogs.
Table 4.9: Breed Distribution Of *Brucella Canis* Antibody Positive Dogs.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number sampled</th>
<th>Number Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exotic*</td>
<td>76</td>
<td>26 (21.1)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mixed</td>
<td>13</td>
<td>4 (3.3)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mongrel</td>
<td>34</td>
<td>4 (3.3)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>123</strong></td>
<td><strong>34 (27.6)</strong></td>
</tr>
</tbody>
</table>

\(X^2 = 0.04; \ p<0.05\)

- * Total no of dogs sampled= Rottweiler 28 (36.8%); Mastiff 20 (26.3%); Caucasian 13 (17.1%); Alsatian 13 (17.1%); Boer bull 1 (1.3%); Persian 1 (1.3%)
CHAPTER FIVE
DISCUSSION

*Brucella abortus* antibody was not detected in any of the 123 dogs screened; this means that out of the 34 *Brucella* positive dogs, no antibody for *B. abortus* was found using both the Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). The zero prevalence suggests that *B. abortus*, though important in livestock, is not of major importance in the epidemiology of canine brucellosis in the study area. The apparent absence of *B. abortus* antibody may be attributed to the type of management system practiced in the South-East. Dogs are either housed or caged and though some dogs may roam freely in the neighborhood, they do not usually come in contact with ruminants like, cattle, sheep, and goats. The dog profiles also showed that most clients feed their dogs cooked household foods only. Therefore, the likelihood of being infected with *B. abortus* is rare; as most infections of *B. abortus* in dogs is by dogs coming in close contact with aborted tissues of infected farm animals through the consumption of these infected materials like aborted fetuses and feces, placental, fetal and vaginal fluids. The findings of this study on *B. abortus* is in contrast to the works of Cadmus *et al.*, (2011) and Adedoyin *et al.*, (2012) who reported seroprevalence of 5.46% and 2.48% respectively in household dogs in Ibadan, South-West Nigeria.

The result of the present study suggests a high seroprevalence (27.7%) of *Brucella canis* in dogs presented to veterinary clinics, apparently healthy slaughter dogs and those in the screened households. None of the dogs screened in the study area were vaccinated, as vaccination of dogs against brucellosis is not routinely carried out in Nigeria. Also, there is no information of any vaccine against *B. canis* (Palomares –Resendiiz *et al.*, 2012). Therefore, demonstration of antibodies to *brucella* in dogs in the study area is suggestive of natural exposure to the organism.
It may also indicate lack of brucellosis control programme in animals. The seroprevalence of *B. canis* in this study was relatively high when compared to other studies in Nigeria. This may be attributed to the higher sensitivity of the diagnostic technique (Biogals Immunocomb® Canine *Brucella* Antibody Test Kit) used which is 99% sensitive and specific to the detection of *Brucella canis* antibodies. Cadmus *et al.*, (2011) and Adedoyin *et al.*, (2012) reported a seroprevalence of 0.27% in household dogs and 3.11% in household and hunting dogs respectively, using *B. canis* Rapid Slide Agglutination Test (RSAT). However, the present result was lower than the prevalence of 59.43% reported by Cadmus *et al.*, (2012) in dogs used for hunting in an indigenous community in Ibadan, South-Western Nigeria. The hunting dogs may have high exposure potential to *brucella*.

Female dogs had a higher seroprevalence (22%) than male dogs (6.0%). A major contributing factor to higher rates in females, could be that a single male dog, if infected and is used in mating with different females, can transmit the infection through infected semen (Cadmus *et al.*, 2011). Also it may be due to the fact in this region, most dog owners in the area sampled prefer to keep more female dogs than males (as they pay male dog owners for their male dogs to mate the female dogs) for the purpose of additional income through the sale of their puppies. This increases the chances of more females getting infected during mating. However, Radostits *et al.*, (2007), have shown that erythritol, a polyhydric acid found in higher concentration in the placentas and fetal fluids of females than in seminal vesicles and testis of males can be responsible for females being more susceptible than males. This result is in agreement with the findings of Cadmus *et al.*, (2011) who reported a prevalence of 6.17% in females and 4.9% in
males. But in another study a slightly higher rate in males (29.6%) than in females (26.7%) was recorded by Adesiyun et al., (1986).

The decrease in the positive samples as titer level increased maybe due to the fact that infection in dogs with higher titer levels, may have entered into the chronic phase thereby making these infected dogs not to be productive thus leading to the disposing or selling off of these non producing females as complained by the owners while those with lower titer levels, are more in number because the infection may not have started manifesting its characteristics in them.

Dogs presented at the clinics had the highest number of positive samples in the different titer levels more than the other categories of dogs sampled; and this may be attributed to the fact that more samples were gotten from clinical cases as most dogs brought to the Clinics came as a result of either illness, routine check up or vaccination.

The different titer levels were also higher in females more than in males and this may be attributed to the fact that female dogs where sampled more in the study. Also, Radostits et al., (2007), have shown that erythritol, a polyhydric acid found in higher concentration in the placentas and fetal fluids of females than in seminal vesicles and testis of males can be responsible for females being more susceptible than males. In addition to this, due to the asymptomatic nature of dogs infected with *B. canis*, most male dogs used for breeding are unscreened, and carry the infection for a long period of time, shedding it in the environment through urine and semen; this may result in bitches being mated severally by the same infected male dog as a result of repeated unsuccessful breeding, thereby increasing the infection load in females.
Prevalence was lower among the young animals screened in this study compared to the older ones. Usually young animals are protected by maternal immunity and thus are less susceptible to infections. This shows that the infection increases with age. The high prevalence seen in older animals is demonstrative of the chronic nature of brucellosis as it has been shown to increase with age, and most affected animals carry the infection throughout their lives (Radiostits et al., 2007). The reason for the increase in prevalence with respect to increase in age may be attributed to the fact that the bacteria localizes mainly in the reproductive tracts, especially in pregnant animals. There is also evidence that the mammary gland may even be more favoured for localization than the reproductive tract (Abubakar et al., 2012). Age-wise prevalence studied by Aulakh et al., (2008) and Abubakar et al., (2010) showed that the incidence is high in sexually mature animals. Therefore, increase in age, increases exposing probability to infection in dogs. However, the result does not agree with the study carried out by Cadmus et al., (2011), where he reported more prevalence in dogs less than one year old than in adult dogs.

There is a strong association between the infection rate and breeds of dogs screened; with the infection occurring more in the exotic breeds of dog than the mixed and local (mongrel) breeds. This may be related to the fact that exotic breeds of dogs are better cared for by the owners in the two states, and therefore constituted the dominant breed (61.8%) in the population sampled (76/123) because it gives much more income from the sale of its puppies than the other breeds thus, the chances of the disease occurring is more in these breeds. Behzadi and Mogheiseh, (2011), argued that detection of canine brucellosis in exotic dogs may indicate a new source of infection from abroad as these dogs may be imported from countries and regions where the disease is endemic and due to lack of screening of dogs before mating, may be a source of
infection to uninfected dogs. The higher prevalence among the exotic breeds is in agreement with the findings of Behzadi and Mogheseh (2011), were they got a prevalence of 19.35% in exotic breeds of dogs. It is also in agreement with the findings of Cadmus et al., (2011) who got 50.55% in Alsatian breeds of dogs.

There is a strong association (p<0.05) between *B. canis* infection and some risk factors such as sex, breed and dogs moving freely in the neighbourhood. This was further supported by the multivariate logistic regression (Appendix IV).

Some risk factors such as: dogs more than one year and above, dogs being used for breeding, dogs sharing rooms with the members of the household, female dogs with history of infertility and abortion, and male dogs used for mating, all gave a considerable higher positive number and increases the chances of *brucella* infection in dogs in the study area even though not significant (Appendix IV).
CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion
The zero seroprevalence of \textit{B. abortus} antibodies in dogs in this study does not totally mean the non-existence of the disease in the localities studied but may infer that \textit{B. abortus} in dogs does rarely occur in this region, except when the dogs are in close contact with infected farm animals; through the consumption of their infected materials like aborted fetuses and feces, placental, fetal and vaginal fluids from infected animals as proven by previous researchers.

Also, the present study has shown a \textit{Brucella canis} antibodies seroprevalence of 27.6 \% in the study area. This means that the general public is at risk and this calls for serious interventions considering the zoonotic implications of the disease as infected dogs can be a source of infection not only to animals but also to humans especially those that are in close contact with these animals due to the nature of their work / occupation.

Some factors such as sex, breed, age, dogs moving freely in the neighbourhood, dogs being used for breeding and male dogs used for mating increases the chances of \textit{brucella} infection in dogs in the study area and therefore should be considered in the epidemiology of canine brucellosis in the study area.

The Immunocomb®Canine Brucellosis Antibody Test Kit used for this study has proven to be a reliable test method (99\% sensitive and specific) in the diagnosis of \textit{Brucella canis} in dogs.
6.2 Recommendations

i. Management practices in keeping dogs is a risk factor for transmission of *Brucella canis* infection in the study area, thus dogs should be restricted within cages in a fenced residence.

ii. Dog breeds is an associated risk factor to seroprevalence of brucellosis, exotic breeds of dogs imported into Nigeria should be screened against the infection.

iii. Control measures should be instituted by dog owners and kennel attendants through constant cleaning of the environment and kennels in the study area to stamp out or eradicate the infections among dogs and to avoid spread to other uninfected dogs and possible transmission to humans because of its zoonotic implication.

iv. Hygienic measures such as proper disposal of aborted fetuses, placenta and other contaminated materials and disinfection of kennels, premises should be strictly observed by the dog owners especially those that keep dogs as pet and allowed them into their living rooms so as to avoid being infected with infected dog secretions especially in children.

v. Butchers, animal health workers, meat handlers and laboratory workers, including veterinarians should endeavor to wear protective clothings, hand gloves and face masks to avoid direct contact and inhalation of contaminated aerosols in the clinics and kennels.

vi. States and Federal Ministries of Health should create a Veterinary Public Health Unit that will be handling zoonotic diseases as is found in other developed countries, for the prevention, surveillance and control of zoonoses in general and brucellosis in particular.
REFERENCES


Fernando P.P.; Klaus N.; Luis E. S and Wei L. Y (2010). Diagnosis of Brucellosis. The Open Veterinary Science Journal, 4: 46-60


Gyuranecz Miklo´ s, Levente Szeredi, Zsuzsanna Ro´ nai, Be´ la De´ nes, La´ szlo´ Dencso´ , A´ da´ m Da´ n, Nimro´ d Pa´ lmai, Zso´ fia Hauser, Erzse´ bet Lami, La´ szlo´ Makrai, Ka´ roly


APPENDIX I
SOURCES WHERE SAMPLES WHERE PURPOSIVELY COLLECTED

A) Clinic dogs
I) Anambra State. (a) Apex Veterinary Clinic Onitsha . . . . . . 24
   (b) Omega Veterinary Clinic Onitsha . . . . . 13
   Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 37

II) Enugu State. (a) VTH Nsukka . . . . . . . . . . . . . . 7
   (b) Enugu State Vet. Clinic Uwani . . . . . 9
   (c) Animal Vision World Clinic Enugu . . . . 12
   Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 28
   Sub-Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . 65

B) Slaughter dogs.
I) Anambra State  (a) Igboukwu Market . . . . . . . . . . . . . . 6
II) Enugu State   (b) Orba Market . . . . . . . . . . . . . . . . 28
   Sub-Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . 34

C) Household dogs
I) Anambra State (Onitsha metropolis) . . . . . . . . . . . . . . 12
II) Enugu State (Enugu metropolis) . . . . . . . . . . . . . . . . 12
   Sub-Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . 24

Grand Total . . . . . . . . . . . . . . . . . . . . . . . . . . . . 123
APPENDIX II

ANIMAL PROFILE

Client details

1. Location of clinic (city/town) ________________________________

2. Location of owner (city/town) ________________________________

Animal profile, (please tick where appropriate)

3. No of dog(s) in the household ________ (a) male [   ] (b) female [   ]

   If more than one, please complete the following for each dog.

4. Breed (a) Exotic [   ]
   (b) Mixed [   ]
   (c) Local [   ]

5. Sex: (a) Male [   ]  (b) Female [   ]

6. Age (a) less than 1 year [   ]
   (b) 1 to 3 years [   ]
   (c) 3 to 5 years [   ]
   (d) Above 5 years [   ]

7. Any other domestic animal(s) in the household (tick which ones apply)
   (a) Goat [   ]  (b) Sheep [   ]  (c) cat [   ]  (d) Others, please indicate ________________

8. (a) Do your dog most of the time, move freely in the neighborhood [   ]
(b) Do your dog roam freely (i) at night [ ] (ii) all the time [ ]

9. Feeding habits of Dog: (a) scavenging all the time [ ]
   (b) Both household feed and scavenging [ ]
   (c) Household feed only [ ]

10. Purpose of keeping dogs (tick where applicable):
    (a) Companion for children and other members of the family [ ]
    (b) Guard/breeding [ ]
    (c) Hunting [ ]

11. Do your dog share rooms with members of the household:
    (a) Yes [ ]
    (b) No [ ]

**For female dog:**

12. (i) Any history of abortion? (a) Yes [ ] (b) No [ ]

    (ii) If yes, number of times (a) Once [ ] (b) Twice [ ] (c) More [ ]

13. If more please state ________________________________

**For male dog:**

14. Has the male dog ever been used for mating: (a) Yes [ ] (b) No [ ]
APPENDIX III

SAMPLE SIZE DETERMINATION

Formula:

\[ n = \frac{Z^2 P q}{d^2} \]

\( n \) = the desired sample size (when population is > 10,000)

\( z \) = the standard normal deviation, usually set at 1.96 (or more simply at 2.0) which corresponds to the 95% confidence interval.

\( P \) = the proportion in the target population estimated to have a particular characteristics,

\( q = 1.0 - p. \)

\( d \) = degree of accuracy desired, usually set at 0.05 or occasionally at 0.02.

\[
\begin{array}{c}
\frac{(1.96)^2(0.0794)(0.92)}{(0.05)^2} \\
n = 112
\end{array}
\]
APPENDIX IV: Odds ratio and (95% C.I) from Multivariate Logistic Regressions comparing seropositive and seronegative dogs for *Brucella canis* antibodies in Anambra and Enugu States in Southeast, Nigeria (n = 123; np = 34)

### Antibody of *Brucella canis*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total no Sampled</th>
<th>Total no positive (%)</th>
<th>Positive OR</th>
<th>95% confidence interval</th>
<th>Negative OR</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>7(15.6)</td>
<td>0.449</td>
<td>0.213</td>
<td>0.947</td>
<td>1.292</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>27(34.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Moving freely In the neighbourhood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>22(62.9)</td>
<td>4.243</td>
<td>2.132</td>
<td>8.446</td>
<td>0.436</td>
</tr>
<tr>
<td>No</td>
<td>54</td>
<td>8(14.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Feeding habits of dog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scavenging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>27</td>
<td>11(40.7)</td>
<td>1.325</td>
<td>0.738</td>
<td>2.396</td>
<td>0.854</td>
</tr>
<tr>
<td>HF only</td>
<td>62</td>
<td>19(30.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Purpose of keeping dogs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Companion/security</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>breeding</td>
<td>87</td>
<td>30(34.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.655</td>
</tr>
<tr>
<td>Hunting</td>
<td>2</td>
<td>0(0.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Dog share rooms with Household</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>6(27.3)</td>
<td>0.761</td>
<td>0.358</td>
<td>1.618</td>
<td>1.133</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>24(72.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Abortion history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>16(34.8)</td>
<td>0.957</td>
<td>0.485</td>
<td>1.888</td>
<td>1.025</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>8(36.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Male dog used For mating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>5(31.3)</td>
<td>1.563</td>
<td>0.234</td>
<td>10.423</td>
<td>0.859</td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>1(20.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.05, n = total no of sampled dogs, np = total no of positive dogs, HF= Household food.*
APPENDIX V: Risk Factors associated with the presence of *Brucella canis* antibodies in dogs presented at the Clinics and Household dogs sampled in Southeast Nigeria (Total number sampled =123, number positive = 34). (*= p<0.05)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total no sample</th>
<th>Total no positive (%)</th>
<th>Chi-square value</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>7 (15.6)</td>
<td>5.183</td>
<td>0.023*</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>27 (34.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 1 year</td>
<td>11</td>
<td>3(27.3)</td>
<td>1.5</td>
<td>0.682</td>
</tr>
<tr>
<td>1 - &lt; 3 years</td>
<td>32</td>
<td>9(28.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - &lt; 5 years</td>
<td>34</td>
<td>14(41.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 and above</td>
<td>12</td>
<td>4(33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exotic</td>
<td>76</td>
<td>26(32.4)</td>
<td>5.988</td>
<td>0.050*</td>
</tr>
<tr>
<td>Mixed Mongrel</td>
<td>13</td>
<td>4(30.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>34</td>
<td>4(11.8)</td>
<td></td>
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</tr>
<tr>
<td><strong>Other animals in the household</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>1</td>
<td>1(100.0)</td>
<td>5.264</td>
<td>0.072</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>4(66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>82</td>
<td>25(30.5)</td>
<td></td>
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<tr>
<td><strong>Animal move freely in the neighbourhood</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>22(62.9)</td>
<td>21.934</td>
<td>0.000*</td>
</tr>
<tr>
<td>No</td>
<td>54</td>
<td>8(14.8)</td>
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</tr>
<tr>
<td><strong>Feeding habits of dog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Scavenging all the time</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Both scavenging and household</td>
<td>27</td>
<td>11(40.7)</td>
<td>0.858</td>
<td>0.354</td>
</tr>
<tr>
<td>Household food only</td>
<td>62</td>
<td>19(30.6)</td>
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<tr>
<td><strong>Purpose of keeping dogs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Companion for children/security</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Breeding</td>
<td>87</td>
<td>30(34.5)</td>
<td>1.04</td>
<td>0.308</td>
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<tr>
<td>Hunting</td>
<td>2</td>
<td>0(0.0)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Dog sharing rooms with the household</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>6(27.3)</td>
<td>0.542</td>
<td>0.462</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>0(35.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>History of infertility/abortion in females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>16(34.8)</td>
<td>0.016</td>
<td>0.898</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>8(36.4)</td>
<td></td>
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</tr>
<tr>
<td><strong>Male dog used for mating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>5(31.3)</td>
<td>0.236</td>
<td>0.627</td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>1(20.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX VI

COMPONENTS OF THE CANINE BRUCELLOSIS ANTIBODY TEST KIT USED.

(a) A multi-compartment development plate (b) Twelve capillary tubes (c) One disposable tweezers (d) Immuno-comb
APPENDIX VII
HARVESTED SERUM SAMPLES READY TO BE ANALYZED
APPENDIX VIII

IMMUNO-COMB SHOWING POSITIVE SERUM SAMPLES
APPENDIX IX
DOG MARKET/SLAUGHTER POINT AT IGBOUKWU MARKET.
APPENDIX X

DOG MARKET/SLAUGHTER POINT AT ORIE ORBA MARKET.
APPENDIX XI
DOGS READY TO BE SLAUGHTERED FOR MEAT AT ORIE ORBA MARKET