PREVALENCE AND RISK FACTORS OF *LEPTOSPIRA* AND *BRUCELLA* INFECTION IN STABLED HORSES IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA

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DECLARATION

I hereby declare that the work in this thesis titled "**PREVALENCE AND RISK FACTORS OF *LEPTOSPIRA* AND *BRUCELLA INFECTION* IN STABLED HORSES IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA**" has been performed by me in the Department of Public Health and Preventive Medicine under the supervision of Professor J. U. Umoh and Professor L. B. Tekdek. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any university.

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Name of student	Signature	Date

CERTIFICATION

This thesis titled "**PREVALENCE AND RISK FACTORS OF *LEPTOSPIRA* AND *BRUCELLA* INFECTION IN STABLED HORSES IN KADUNA METROPOLIS, KADUNA STATE,NIGERIA**" by NWOKIKE EDITH CHINYERE meets the regulations governing the award of the degree of Master of Science, in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my God, Almighty first, then my parents, Mrs Mercy Nwokike and Late Mr Obadiah Nwokike of blessed memory. You have taught and reinforced in me how brave the fight for life is and how to live life fully, honestly, faithfully with an open mind. This dedication is but small and humble in front of your courageous battles.

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All thanks and praises go to the sovereignty God, the most high for sparing my life up to the time of completing this work.

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ABSTRACT

Equine leptospirosis and brucellosis are important zoonotic bacteria infections of horses causing a range of clinical conditions including abortion and reduced fertility. This study was aimed to determine antibodies to *Leptospira* and *Brucella species* in horses and to assess the risk factors associated with the infections of these bacteria in horses. A cross-sectional study was set up to sample 284 horses in seven stables within Kaduna Metropolis. Sera from the horses were screened for antibodies to *Leptospira* species using indirect enzyme linked immunosorbent assay and those of *Brucella species using* competitive enzyme linked immunosorbent assay methods. Structured questionnaire that sought information on risk factors associated with infections with the bacteria were prepared and administered to 77 horse handlers, horse owners, veterinarians and stable managers in the various stables. Associations of risk factors with the presence of antibodies were assessed using chi square analysis, odds ratio and by setting up 95% confidence interval on the odds ratio. About 27.2% of the horses were seropositive for *Leptospira* antibodies and 53.5% were seropositive for *Brucella* antibodies. Sex -specific rate for antibodies to *Leptospira* species were 34.0% for males and 23.43% for females and antibodies to *Brucella* species were 57.0% and 51.0% for male and females respectively. There were no significant ($p > 0.05$) associations between sex of horses and seroprevalences of either *Leptospira* or *Bruella* antibodies. The highest age-specific seroprevalences for both *Leptospira* species and *Brucella* species were in horses of 16 years and above. There were no significant ($p > 0.05$) associations between age and seroprevalence of both infections. This study revealed that not having open source of drinking water for horses (OR = 0.04, 95% CI 0.01- 0.12), having no wounds on the body of the horses ($p<0.05$, $\chi^2 = 8.59$, OR = 0.29, 95% CI 0.10 - 0.79), being fed with home-made only ($p<0.05$,

χ^2 = 16.69, OR = 0.07, 95% CI 0.03 - 0.17), and not having veterinary clinic in the stable ($p< 0.05$, χ^2 = 6.59, OR = 0.13, 95% CI 0.05 - 0.35) were significantly protective for a horse being seropositive to *Leptospira* species. This implies that having open source of drinking water, presence of wound on the horse body, being feed with both home and commercial-made feed, having veterinary clinic in the stable were risk factors for *Leptospira* species. Also, this study revealed that horse being fed with home-made feed ($p<0.05$, χ^2 = 68.18, OR = 0.08, 95% CI 0.02 - 0.43) and having veterinary clinic in the stable ($p< 0.05$, χ^2 59.04, OR = 0.13, 95% CI 0.04 - 0.40) were protective to being seropositive for *Brucella* species while not having open source drinking water ($p<0.05$, χ^2 = 34.67, crude OR = 5.18, 95% CI 2.92 - 9.17) and absence of other animals species in the stable ($p<0.05$, χ^2 = 47.57, crude OR = 6.73, 95% CI 3.80 - 11.95) were significant associated with presence of antibodies to *Brucella* species. This also implies that being fed with home-made feed, having veterinary clinic in the stable, not having open source drinking water and not having other animals species in the stables were risk factors to *Brucella* species infection. Proper stable management practices would decrease the risk factors and thus reduce the risk of infection of horses with the bacteria.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
BST	Brucellosis skin test
CDC	Center for Disease Control
c- ELISA	Competitive ELISA
CFT	Complement Fixation Test
CMI	Cell Mediated Immunity
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ERU	Equine Reccurrent Uveitis
FAFLP	Fluorescent Fragment length polymorphism
FAT	Fluorescent Antibody Test
HIT	Heat Inactivation test
i-ELISA	Indirect ELISA
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
KDSG	Kaduna State Government
LPS	Lipopolysaccharide
MAT	Microscopic Agglutination Test
MFIN	Multifocal Interstitial Nephritis

MLST	Multilocus Sequence Typing
MRT	Milk Ring Test
NDA	Nigerian Defense Academy
NPF	Nigerian Police Force
OE	Outer Envelope
OIE	Office of International Epizootic
PC	Protoplasmic cylinder
PCR	Polymerase Chain Reaction
PF	Periplasmic Flagella
PFGE	Pulse Field Gel Electrophoresis
RAPD	Random Amplified Fragment Polymorphic DNA Fingerprinting
RBPT	Rose Bengal Plate Test
REA	Restriction Endonuclease
RELP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal Ribonucleic Acid
SAT	Serum Agglutination Test
2-MET	2 – Marcapto – ethanol test
UK	United Kingdom
USA	United States of America
VNTR	Variable Number of Tandem Repeats
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Leptospirosis and brucellosis are significant zoonotic infectious diseases caused by bacteria of genus *Leptospira* and *Brucella* respectively. These two pathogenic bacteria infect a wide spectrum of hosts, including cattle, sheep, goat, camel, buffalo, dog, swine and horse around the world (Cutler *et al*., 2005; Cheema *et al*., 2007). The diseases pose not only public health risk but also have impact on the reproductive efficiency of livestock (Ocholi *et al*., 2005).

The genus *Leptospira* is within the family *Leptospiraceae,* order spirochaetales (Faine *et al.*, 1999; Magalhaes *et al*., 2010). The genus *Leptospira* is divided into twenty (20) species (Brenner et al., 1999), which are either pathogenic or saprophytic in nature. Presently, there are fourteen (14) pathogenic species; *Leptospira Alexanderi, Leptospira alstoni, Leptospira borgpetersenii, Leptospira inadai, Leptospira interrogans, Leptospira fainei, Leptospira kirschneri, Leptospira licerasiae. Leptospira noguchi, Leptospira santarosai, Leptospira weilii, Leptospira broomii, Leptospira kmety* and *Leptospira wolffii,* comprising more than 260 serovars (Adler and Moctezuma, 2010). Saprophytic species of leptospires made up of 6 members which include *Leptospira biflexa, Leptospira meyeri Leptospira vanthielii, Leptospira yanagawae Leptospira terpstrae* and *Leptospira wolbachii* and contain more than 60 serovars. Incubation period of *Leptospira* organism is between 1 to 4 days in all animal species (Radostits *et al*., 2006).

Horses have been shown to be infected by different serogroups of *Leptospira* hence they are incidental hosts for most serovars, especially *icterohaemorrhagiae, canicola, pomona and*

ballum,further studies have suggested that horses are maintenance hosts for serovar *bratislava* (Cerri *et al*., 2003; Van den ingh *et al*., 1989). Usually horses become infected by direct transmission via contaminated urine or placental fluids of infected horses, or indirectly from a contaminated environment in which leptospires have been shed by other animal species such as standing water, wet soil, grasses or hays (Bolin, 2000).

Serological testing (enzyme linked immunosorbent assay and microscopic agglutination test) has been the traditional way of diagnosing leptospirosis in the laboratory. Microscopic agglutination test is regarded as the "Gold Standard" for detection of *Leptospira* antibody (Jimenez- Coello *et al*., 2008). Also isolation of the spirochete can be used (OIE, 2004). In recent years, polymerase chain reaction specifically amplifying leptospiral DNA has been used (Leon *et al*., 2006).

Previous studies of leptospirosis on other host in Nigeria gave a prevalence of 18% in human volunteers sampled in Plateau State in Northern Nigeria (Ezeh *et al.*, 1991), 13.5% of people as occupational risk in the eastern Nigeria (Onyemelukwe, 1993), 10.98% in cattle in Zaria, Northern Nigeria (Ngbede *et al*., 2013), 23.5% in sheep in Ibadan, Western Nigeria (Agunloye, 2002), 13.1% in goat in Ibadan, Western Nigeria (Agunloye, 2002), 16.7% in dog in Western Nigeria (Agunloye, 2002).

Brucella is made up of ten species which are non-motile, aerobic, intracellular, gram-negative cocci, cocco-bacilli or short rods (Boussetta, 1991). These species include *Brucella abortus, Brucella melitensis, Brucella ovis, Brucella canis, Brucella suis, Brucella neotomae, Brucella microti, Brucella inopinata, Brucella ceti* and *Brucella pinnipedialis* (Scholz, 2008). Most of these *Brucella* species have a strong host preference for specific animal species (Glynn and

Lynn, 2008) and such pathogenic species are *Brucella melitensis* (goat, sheep), *Brucella abortus* (cattle), *Brucella suis* (pigs), *Brucella canis* (dogs), *Brucella ovis* (sheep), *Brucella pinnipedialis* (marine mammals) (Foster *et al*., 2002).

Equine brucellosis usually involves the cattle pathogen, *Brucella abortus*, although infection with *Brucella suis* has been reported (Nicoletti, 2007). Horses usually become infected by ingestion of *Brucella abortus* via contaminated feed. Most reported cases indicate a history of contact with cattle (Ocholi *et al*., 2004; O'Sullivan, 1981). Brucellosis in horses can be asymptomatic (MacMillan and Cockrem, 1986). The commonest clinical manifestation associated with the disease in horses is septic supra-spinatous bursitis (Fistulous withers) and septic supra-atlantal bursitis (poll evil) (Crawford *et al*., 1990; Cohen *et al*., 1992).

Equine Brucellosis is diagnosed by culturing from exudates in cases of fistulous withers and pull evil but over growth by other bacteria commonly makes the organism difficult to isolate (Nicoletti, 2007). Serological testings such as Card test, (Nicoletti, 2007), plate agglutination test (Dohoo *et al.*, 1986), competitive enzyme linked immunosorbent assay (Perrett *et al*., 2010), tube agglutination complement fixation, coombs antiglobulin mercaptoethanol and agar gel diffusion test can be considered diagnostic for brucellosis infection in horse (Nicoletti,2007*).*

1.2 Theoretical Frame Work

Common source of water is one of the key environmental factors that increase the risk of leptospirosis in horses (Barwick *et al*., 1997). Water can be directly contaminated by a large variety of animal or human excreta and urine or indirectly by soil surface wash by floods, rainfall or run-off (Manciuc *et al*., 2007). Infected individuals shed *Letospira interrogans*

proteoglycan and glycosaminoglycan in urine making transmission from mammalian host to water bodies and vice versa possible (Breiner *et al.*, 2009).

Age of horses has been significantly associated with the presence of *Leptospira* titres with the chance of sero-positivity increasing by approximately 10% with each year of life (Lees and Gale, 1994). Horses during their lifespan encounter leptospires from the environment or from other horses and antibody titre can persist for a long time and they become carrier for life (Baverud *et al.*, 2009). Horses with increasing age and exposure show increased sero-prevalence for serovar *bratislava* (Baverud *et al.*, 2009).

Population density of horses and group management increase the risk of leptospirosis (Barwick *et al*., 1997). This overcrowding increases the rate of contact between animals of all ages, hence transmission of the disease becomes easier from infected horses (Salman and Meyer, 1984).

Appaloosa breed of horse is at increased risk of developing recurrent uveitis and severe vision loss associated with leptospirosis than non-appaloosa breed due to the predilection site it posses for the disease (Dwyer, 1995). Ponies and cold blood has lower sero-prevalence than other breeds of horses (Baverud *et al*., 2009) and the reason is due to management, because grazing horse like cold blood and ponies have greater access to pasture at summer and are less exposed to other kinds of feed that may be contaminated by rodent urine (Baverud *et al*., 2009).

Brucellosis is associated with sex; mares are identified with higher sero-prevalence than stallions (Ahmed and Munir, 1995a) because mares experience comparatively greater physiological stress during pregnancy and lactation hence more susceptible to infection than

males (Wadood *et al*., 2009). In addition, a *Brucella* growth supporting substance, erythritol occur in higher concentration in the uterus, placenta and foetal fluids of mares than seminal vesicle and testis of stallions (Keppie *et al.*, 1965).

Mares and geldings have higher sero-positivity for *Leptospira bratislava* than stallions, reason being that stallions and racing horses are kept individually and therefore will be less exposed to shedding from other horses (Lees and Gale, 1994).

The practice of mixed-farming of horses with other animals such as cattle, pig, dog, donkey, sheep and goat increase sero-positivity to *Leptospira* and *Brucella* in horses (Salman and Meyer,1984; Abo-Shehada, 2009).This is because naturally acquired *Brucella* infection in horses is associated with infected cattle (a maintenance host for *Brucella abortus*) and swine (a maintenance host for *Brucella suis*) and horizontal transfer earlier demonstrated through close contact with infected animal or materials with skin abrasions (Forbes,1990).

Pregnant mares habour more antibodies against leptospirosis because leptospires localize in the uterus, placenta and subsequently in fetus resulting in premature born foals or death of the fetus (Leon *et al*., 2006).

1.3 Statement of Research Problem

In Nigeria, no known reported studies has been documented on Leptospirosis in horses despite the fact that the disease has been reported in this same host in other parts of the world by so many researchers (Barwick *et al*.,1997; Ellis *et al*., 1983a; Rocha *et al*., 2004) . Leptospirosis in horses is characterised by fever, jaundice, anorexia, lethargy and respiratory distress (Van den ingh *et al*., 1989). It is associated with recurrent uveitis (Brandes *et al*., 2007), abortion, stillbirth or weak neonatal foals (Bernard et al., 1993b). Cases of renal and

hepatic dysfunction have been reported (Divers *et al*., 1992; Hathaway *et al*., 1981). A good number of researchers have given much attention to leptospirosis in other species of animal such as cattle, sheep, goat, pig, dog and human (Ngbede *et al*., 2013; Agunloye, 2002; Ezeh and Agba, 1982; Onyemelukwe, 1993).

Studies on brucellosis in Nigeria have focused on domesticated animals with few investigations carried out on horses to determine its epidemiological role (Bale and Kwanashie, 1984; Ehizibolo *et al*., 2011a; Tijjani *et al*., 2011). Leptospirosis and brucellosis cause abortion in horses (Shapiro *et al*., 1999). Horses are prestigious animals and are used in Nigeria in traditional activities like durbar in Northern Nigeria, in the military for ceremonial parade and cadet training, in sports like polo and racing, in recreational activities, as transport animal and in agriculture for draft purpose (Kalkat and Kul, 1983; Hendricks, 2007; Umar *et al*., 2013). This close contact with humans pose a great risk of transmission of *Leptospira* and *Brucella* organisms from horses to man. Therefore,a research to determine the sero-prevalence and risk factors to *Leptospira* and *Brucella* infections in stabled horses in the study is a welcome development.

1.4 Justification of the Study

Horses are given special attention by their owners due to the immerse role they play in sports, cultural festivals, transport, recreational activities, armed forces parade, cadet training and security purpose. In Nigeria, there are paucity of information on Leptospirosis and brucellosis in horse, hence awareness is necessary. Studies on these diseases in Nigeria have been focused mainly on domesticated ruminants and cattle remains at the centre of attention. Few studies have been carried out to survey the prevalence of *Brucella* antibodies in horses despite the endemicity of brucellosis in Nigeria (Ocholi *et al*., 1993; Rikin, 1988).

The close contact which exists between horses or other animals and human can have zoonotic implications. The infected animals can remain asymptomatic (Hathaway et al., 1981) while shedding the organism thus contaminating the environment with the *Leptospira* species, thus serving as a source of infection not only to humans but also to other animals (Romeo et al., 1993). However, factors such as mixed- farming of horses and other animals, population density of horses, common source of drinking water, sex, age breed and so on are all important indices to leptospirosis and brucellosis in horses, evaluation is thus important. This group of animals poses significant economic threat as a result of ill-health treatment cost and reproductive losses.

This research is design to determine the status of *Brucella* and *Leptospira* antibodies among horses and their associated risk factors from army and police stables, polo club stables and other individually owned stables in Kaduna Metropolis. This study is also carried out for provision of base line data information on equine leptospirosis and brucellosis for the purpose of instituting a prevention and control measures.

1.5 Aim of the Study

To determine the sero-prevalence and risk factors of leptospirosis and brucellosis in stabled horses in Kaduna Metropolis.

1.6 Objectives of the Study

The study is designed to determine the:

i. Prevalence of *Leptospira* antibodies in horses in stables within Kaduna Metropolis.

ii. Prevalence of *Brucella* antibodies in horses in stables within Kaduna Metropolis.

iii. Presence of risk factors such as age, sex, breed, presence of other animal species , common source drinking water, wound on the body of horses, presence of rodent, pregnancy and lack of vaccination against leptospirosis and brucellosis in the various stables, using structured questionnaires.

1.7 Research Questions

i. Is antibody to leptospirosis present among stabled horses in Kaduna Metropolis?

ii. Is antibody to brucellosis present among stabled horses in Kaduna Metropolis?

iii. Are *Leptospira* and *Brucella* infections in horses influenced by risk factors such as age, sex, breed, presence of other animal species, common source of drinking water, wound on horse body and presence of veterinary clinic?

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Information on *Leptospira* Infection in Horses

2.1.1 A Brief history of *Leptospira* infection.

Syndromes similar to leptospirosis were recognized as an occupational hazard of rice harvesting workers in ancient China, later in Japan, Australia, and Europe and elsewhere, long before their aetiologies were appreciated (Faine *et al*., 1999). It has been suggested that *Leptospira interrogans* serovar *icterohaemorrhagiae* was introduced to Western Europe in the 18th century by westward extension of the range of *Rattus norvegicus (brown rat)* from Eurasia (Levett, 2001).

The best known of the initial published reports of clinical leptospirosis in humans, which described a characteristic infectious disease, accompanied by splenomegaly, jaundice and nephritis, was made over 100 years ago by Adolf Weil (1886). Weil was by no means the first to describe or distinguish this disease, since clear descriptions of *Leptospira* jaundice appeared earlier in the 19th century. Mathieu (1886) had earlier in the same year reported a similar syndrome, but it was Weil's detailed reports, written in German and circulated among doctors, dealing with the frequent epidemics among troops, which achieved most recognition, resulting in leptospirosis often being referred to as Weil's disease.

The first demonstration of a *spirochete* in Weil's disease occurred when Stimson (Stimson, 1907), using the then recently discovered Levaditi's technique, demonstrated `spiral organisms in kidney tissue of a patient who died of Weil's disease but was mistaken for yellow fever (Faine *et al*., 1999).

2.1.2 Aetiology of *Leptospira* infection in horses.

Leptospirosis is caused by bacteria, also known as leptospire. Leptospires are within the genus *Leptospira*, family *Leptospiraceae*, and order *Spirocheatales* (Faine *et al*., 1999: Magalhaes *et al*., 2010). The genus is classified into two species based on pathological characteristics; *L. interrogans*, comprising all pathogenic strain and *L. biflexa* containing the saprophytic strains isolated from the environment (Levett, 2001).

At present 14 pathogenic *Leptospira* species: *Leptospira alexanderi*, *L. alstonic*, L. *borgpetersenii*, *L. inadai*, *L. interrogans*, *L. fainei*, *L. kirschneri*, *L. weilic* and *L. wolfii*, comprising in turn more than 260 serovars are known (Adler and Moctezuma, 2010). Saprophytic species of *Leptospira* include *Leptospira biflexa*, *L. meyeri*, *L. mayeri*, *L. kmeteyi*, *L. vantheilii*, *L. yanagowae* and *L. wolbachii* and contain more than 60 serovars.

Morphologically, leptospires are thin flexible helical microorganisms, with a 0.1um in diameter and 6 to 20um in length, often with the hook at one or both ends. When unstained, cannot be observed by bright field microscopy but only dark-field and phase contract microscopy. Electron microscopy reveals the three major structural components of *Leptsopira* cell; outer envelope (OE), two periplasmic or axial filaments flagella (PF) and aprotoplasmic cylinder (PC) (Hovind-Hougen, 1986).

2.1.3 Risk factors associated with *Leptospira* infetion in horses.

Several studies have assessed risk factors for exposure to leptospirosis in horses and age of a horse has been identified as one of the risk factors to leptospirosis (Barwick *et al*., 1997). Adult horses have been found in some studies, particularly those between seven (7) and eleven (11) years of age to have the greatest serological evidence of exposure to *Leptospira*

(Park *et al*., 1992) suggesting that *Leptospira* infection would be more likely to occur in older horses. Age of mare might have some epidemiological factor in increasing the time of exposure or susceptibility to *Leptospira* (Park *et al.*, 1992). Horse with increased age and exposed to outdoor life had an increase sero-prevalence for sv *bratislava* (Baverud *et al*., 2009).

Another associated risk factor to leptospirosis is horse breed (Baverud *et al*., 2009). Appaloosa had a significantly higher risk (3.8 times susceptible) of developing uveitis and associated blindness relative to thorough breeds while standard breed had a significantly lower risk of developing uveitis than thorough breeds (Angelo *et al*., 1988; Dwyer, 1995). Leptospirosis is found in all breeds; thoroughbred, standardbred, quarterhorse, saddlebred, arabian and miniature bred but majority of abortion caused by leptospirosis in horses occurred in thoroughbred, though there was no indication of breed predilection reported by Sellnow, (1998).

The risk of infection is shown to increase with horse population density and group management (Barwick *et al*., 1997). High population density leads to increased degree of contact between maintenance and accidental host, hence raising the risk of exposure to the bacteria such as *Leptospira* and *Brucella* (Levett, 2001). Wild life exposure is another factor associated with the risk of *Leptospira* infection in horses. Horse is a maintenance host for L. *bratislava* serovars of *Leptospira* (Ellis *et al*., 1983a). Other maintenance hosts for *bratislava* serovars are within wild mammals populations such as foxes, skunks, hedgehogs, raccoons (Feigin and Anderson, 1975; Hathaway et al., 1983). These act as continuous reservoirs with horizontal transmission taking place within the particular host species (Sellnow, 1998). Contaminated soil and water were factors associated with the risk of exposure to some

serovars such as *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. canicola* in horses (Barwick *et al*., 1998). Horses should be restricted from drinking from municipal drains, ponds or eating from flooded posture as these are easily contaminated by wild life (Wright, 2011). Soil moisture and low acidity favor the survival of *Leptospira* organism (Twigg *et al*., 1969) and in acidic soil (pH 6.2) leptospires survive up to 7 weeks while in rain water flooded soil it survives for about 3 weeks (Levett, 2001).

An animal species may be infected either by parasitic leptospires which are adapted to and carried by members of that animal species or may be infected by leptospires carried by another animal species (Ellis *et al*., 1983a). Extensive serological surveys in horses have revealed that *Leptospira* infection is wide spread worldwide, the predominant serogroups varying depending on the country in which the survey was conducted and the antigens used. The main serogroups of *Leptospira* interrogans isolates so far obtained described in horses in several countries are *sejroe* (sv *hardjo* and *mini*), *icterohaemorrhagiae*, *canicola*, *Austrialis, Bratislava, Lora, Muenchen, Ballum, Promona, autumnalis and grippotyphosa* (Rocha *et al*., 2004; Brem *et al*., 1992; Ingh *et al.*, 1989; VanRiel *et al*., 1977; Poonacha *et al*., 1993; Donahue *et al*., 1995; Kinde *et al.,* 1996; Giorgi *et al*., 1981; Myers, 1976).

The divergence of leptospiral serogroup to which antibodies have been detected and the apparent absence of a predominant serogroup to which antibodies were present in most surveys led to a conclusion that horse did not appear to act as a maintenance host for host-adapted leptospire (Hathaway *et al*., 1981). On the contrary, Ellis (1983a) suggested that serovar *Bratislav*a may be adapted to and maintained by the horse population, based on the very high prevalence of antibodies in horse sera, when tested with a horse isolated Bratislava

strain in Northern Ireland. Horses are susceptible to a wider spectrum of *Leptospira* than other animal species.

In addition, the sero-prevalence of equine leptospirosis recorded in some countries were for example 34.6% in United Kingdom (Hathaway et al., 1981), 21.3% in Ireland (Egan and Yearsley, 1986), 20% in France (Gaumont and Trap, 1986), 36% in Belgium (Desmecht, 1986), 5.6% in Germany (Schonberg *et al*., 1986), 20.6% in USA (Park *et al*., 1992), 57.2% in Argentina (Bordoy, 1985), 41.5% in Australia (Swart *et al*., 1982), 44% in Portugal (Rocha *et al*., 2004). In Nigeria, like most parts of the world, studies concerning leptospirosis have been conducted in cattle, sheep, goat, pig and dog but no report on equine leptospirosis has been documented.

2.1.4 Clincal signs of *Leptospira* infection in horses.

The clinical signs of acute infections can be mild pyrexia with accompanying anorexia. In more severe forms, depression, weakness, lethergy, jaundice, conjuntival suffusion, mucosal petechiae, hemoglobunuria and anaemia may appear to varying degrees lasting 5 to 18 days (Faine *et al*., 1999). Pregnant mares may abort (Kinde *et al*., 1996; Faine *et al*., 1999) and neonatal foals infected in utero may show severe chronic illness and die (Bernard *et al.*, 1993b; Faine *et al*., 1999). Symptoms like pyrexia. Depression and signs of abdominal discomfort with polyuria/polydipsia have been described in a weanling with acute renal disease due to leptospirosis (Hogan *et al*., 1996) severe respiratory distress, diarrhea, unsteady gait with pyrexia and depression and sudden death have also been described in foals with acute leptospiral infections (Ingh *et al*., 1989).

Chronic infection can result in abortion and premature foaling (Ellis et al., 1983b). Some horses may develop periodic ophthalmia, which is recurrent iridocyclitis or uveitis also known as moon blindness (Faine *et al*., 1999).

2.1.5 Diagnosis of *Leptospira* infection in horses.

Definite diagnosis of leptospirosis is usually provided by bacteriologic culture of the infecting organism (Bolin *et al.,* 1989b). The isolation of the infecting strains is the yardstick against which all other antigen detection methods are measured (OIE, 2008). Isolation is followed by typing which is very useful in epidemiological studies to determine which serovars are present within a particular group of animals, animal species and geographical location (OIE, 2000). Culture media are incubated at 28^{o}C to 30^{o}C and examined by dark-field microscope at least fortnightly for 3-6 months (Faine, 1994; OIE, 2000). Leptospires are commonly isolated from urine or kidney of infected animals, even though other specimens such as blood could also be used.The technical difficulties in isolating leptospires from carrier animals or from clinical or pathological material have largely contributed to investigating most equine leptospirosis with serology.

Serological testing is the most widely and frequently used laboratory procedure to confirm the clinical diagnosis, determine herd prevalence or conduct epidemiological studies. A wide variety of serological tests have been described for diagnosis of leptospirosis, but the microscopic agglutination test (MAT) is the reference method for serological diagnosis of the disease against which all other serological test are evaluated (OIE, 2000; Levett, 2003).

Microscopic agglutination test using live antigens is the most widely used laboratory test for the diagnosis of leptospirosis and is the reference method for serological diagnosis of the

disease (OIE, 2000) against which all other serological tests are evaluated (OIE, 2000). The MAT detects agglutinating antibody in serum that reacts with live leptospires in liquid (suspension) medium as antigens, and in general it has high sensitivity and specificity (Cumberland *et al.,* 1999). MAT detects both IgG and IgM classes of antibody (Alder and Faine, 1978). Antibodies in the serum bind to leptospires and cause agglutination, which can be detected using dark-field microscopy.

Another important serological test is enzyme linked immunosorbent assay (ELISA); ELISAs were developed for more specie or serovar specific determination of the presence of its antibody or antigen and due to the deficiencies of the MAT. It is faster, safer and more precise assay for the detection of antileptospiral antibody and in some cases antigen (Adler *et al.,* 1982). ELISAs have been described for detection of serovars Pomona and Tarassovi (Cousins *et al*., 1985) and Hardjo (Adler *et al.,* 1982). One of the major identified roles of ELISA tests in livestock species is the identification of recent infections by the detection of IgM (Cousins *et al.,* 1985.). ELISA tests have been developed using a wide variety of antigen preparations and assay protocols from recombinant lipoproteins such as LipsL32 (Tomich *et al.,* 2007), the outer membrane porin ompL1, LPS (Yan *et al.,* 1999), carbohydrate antigens (Matsunaga *et al.,* 2003), sugar chains, outer membrane protein (Flannery *et al.,* 2001), whole lysed bacteria (Bercovich *et al*., 1990), fixed whole culture extract (Weyant *et al*., 1999), outer sheath proteins (Cho *et al*., 1989) and proteinase-K-treated antigen (Ribeiro *et al.,* 1995). The major benefit of ELISA is that it can be specific for the detection of IgM or IgG antibodies (Cousins et al., 1985).

Other serological tests for *Leptospirha* organism are fluorescent antibody test (FAT). Fluorescent staining of antibody in urine or culture is fast and accurate diagnostic method for

detecting the presence of leptospires and for identifying serotypes (Hodges and Ekdahl, 1973).

Dark field microscopy is another technique available for diagnosis of leptospires.The dark field microscopy is based on the principle of refraction of light. Dark-field microscopy examination of body fluids such as blood, urine, cerebrospinal fluid (CSF), dialysate fluid and organ homogenates has long been used for the demonstration of leptospires, but it is both insensitive and lacking specificity (Ellis, 1992; Levett, 2001). The principle of dark ground microscopy is that the object is illuminated only by light rays that are scattered by the object.

Silver impregnation staining of leptospires is an established method for demonstrating the presence of leptospires in fixed tissues. This staining method is not specific for leptospires and can be technically unreliable (Skilbeck and Chappel, 1987) and has been reported to give many false negative (Ellis, 1992). Another technique of staining is immunoperoxidase staining of leptospires has also been described (Terpstra *et al*., 1983).

Molecular methods for identification have become more widely used and this because of the difficulties associated with serological identification of leptospiral isolates. Molecular typing are of various types such as polymerase chain reaction (PCR) (Levett, 2001). There is evidence that PCR assays are more sensitive than conventional diagnostic methods such as culture and dark field microscopy (Heinemann *et al.,* 2000). Other molecular techniques include: amplified fragment length polymorphism (AFLP) (Slack *et al*., 2006), random amplified polymorphism DNA fingerprint (RAPD) analysis (William *et al*., 1990) and arbitrary primed PCR (AP-PCR) aches (Weyant *et al.,* 1999), restricted fragment length polymorphism (RELP) analysis (Heinemann *et al.,* 1992), 16S rRNA sequencing (Morey *et*

al., 2006) and more recently by multilocus sequence typing (MLST) (Ahmed *et al*., 2006), ribotyping, chromosomal DNA digestion by restriction endonuclease (REA) (Levett, 2001) whole genome DNA-DNA hybridization analysis (Brenner *et al.,* 1999), pulsed field gel electrophoresis (PFGE) (Heinemann *et al*., 1992), insertion sequences bacterial typing methods (IS) and variable number of tandem repeats (VNTR) (Majed *et al.,2005).*

2.1.6 Treatment of *Leptospira* infection in horses.

Correct treatment for *Leptospira* infection in animals depends on the severity, duration of clinical signs and site of infection. Sometimes, the case may be resolved spontaneously or with supportive therapy alone. In general leptospires appear susceptible to some antibiotics such as penicillin, ampicillin, amoxicillin, cefotaxime, ceftiofur, erythromycin and ciprofloxacin (Alt and Bolin, 1996).

In horses, no specific studies have addressed antibiotic therapy for leptospirosis. Antibiotics such as penicillin, oxytetracycline, streptomycin, dihydrostretomycin and erythromycin have been recommended (Bernard, 1993). Horse with clinical leptospirosis maybe azotemic, hence, it is important to consider the potential for nephrotoxicity when selecting a specific antibiotic.

In human patient, deoxycycline and penicillin are recommended for treatment (Levett, 2001).

2.1.7 Prevention and control of *Leptospira* infection in horses

Equine leptospirsis can be completely control by elimination (antibiotic treatment) of the infection in carriers or urinary shedders of leptospires. The infection passes to other animals and to human by direct or indirect contact with infected urine. Limiting exposure to potential carriers such as cattle, swine, rodent and wildlife may help control leptospirosis (Bernard, 1993; Radostits *et al*., 2000).

In other species of animals, vaccination against leptospirosis has been common, but not in horses, though evaluation of the response of horse to vaccination with leptospiral bacteria is being studied (Rohrbach *et al*., 2005).

Preventive measure has been targeted to the leptospire outside the body of its host (Broom, 1952). Hence management and sanitary measures such as provision of sanitary quarters, isolation of sick and aborted animal, quarantine and medication of incoming stock, discouraging open drain and communal drinking trough, adequate control of rodent, discouraging co-grazing and spreading of feed on the ground (Donahue *et al*., 1995).

2.1.8 Public health importance of *Leptospira* infection in horses.

Leptospirosis is one of the most wide spread zoonosis in the world result to its description as a re-emerging disease (McBride *et al*., 2005). The disease is more in geographic area with moist and warm climate. Human leptospirosis has wide spectrum of clinical signs, sometime sub-clinical or mild.

Humans catch infection by direct/indirect contact with tissue/urine of infected animals. Occupation group such as horse handlers, veterinarians, farmers, butchers, laboratory workers, abattoir workers have been established as important risk factors for humans (Levett, 2001). Risk of exposure has been increased by some type of recreational activities such as swimming and hunting (Levett, 2001). Stable staff and related groups should take precautions when handling infected animals to reduce exposure. Protective clothing and latex gloves should be put on when handling urine contaminated materials. Detergent and disinfectants such as iodophors should be used while washing. If already exposed to infection, prophylactic antibiotic therapy may be used.

2.1.9 Situation of *Leptospira* infection in Nigeria

Leptospirosis is a bacterial zoonosis that occurs worldwide, caused by bacteria of the genus Leptospira and affects human and some animals including horses (Greene, 1998). In Nigeria the disease is believed to be underestimated with incidence of six human cases reported annually (CureResearch, 2011). Leptopirosis has been reported as a cause of abortion in Nigeria (Ezeh *et al*., 1991; Onyemelukwe, 1993), although at present, it is not routinely considered in the differential diagnosis of abortion-related cases in both human and animals in Nigeria (Agunloye *et al*., 2001) due to lack of clinical evidence and important diagnostic materials.

Previous serological studies have shown a high percentage of leptosprirosis in man and livestock in different parts of Nigeria (Diallo, 1978; Ezeh and Agba, 1982). Despite these studies and endemicity of the disease in today's Nigeria (Agunloye *et al*., 2000), no investigation has been done or recorded about the true burden of leptospirosis in horses.

Diallo and Dennis (1982) isolated *Leptospira* strains from 6.4% of bovine kidneys of cattle in Zaria, Nigeria. Agunloye (2002) evaluated the presence of *Leptospira* antibodies in sheep and goats by the macroscopic agglutination test (MAT) using a total of seven *Leptospira* serovars. Of 575 animals tested, 17.7% were positive to *Leptospira* by MAT, the prevalence in sheep and goats was 23.5% and 13.1% respectively. The highest reacting *Leptospira* in both species was *L. pomona* with a rate of 25.5%. This was followed by *L. icterohaemorrhagiae* (17.9%) and *L. automnalis* (17.0%), respectively.

In Plateau State, Nigeria, antibodies to *Leptospira* organisms were detected in 18% human volunteers sampled (Ezeh *et al*., 1991). Abattoir workers were particularly at risk with a

prevalence of 30%. Serological examination of sera from human volunteers in Enugu and environs in eastern Nigeria showed that *Leptospira* antibody titres of 1:100 and above were present in 89 (13.5%) of total 661 sera samples (Onyemelukwe, 1993). Coal miners were most at risk with a prevalence rate of 41(46%), followed by the butchers/abattoir workers 26 (29.2%), farmers 18 (20.2%) and hospital laboratory personnel 4(4.5%) (Onyemelukwe, 1993).

At present, there is no specific control strategy against leptospirosis in Nigeria as little is known about the epidemiology of the infection in Nigeria. Vaccination against leptospirosis is not routinely carried out in Nigeria (Agunloye *et al*., 2000).

2.2 Background Information on *Brucella* Infection in Horses

2.2.1 A brief history of *Brucella* infection in horses.

Brucellosis is an infectious disease occurring in many species of animals and man attributed to bacterial organism of the genus *Brucella* (Kubuafor *et al*., 2000). The disease now called brucellosis, under the name 'Mediterranean fever', first came to the attention of British medical officers in Malta during the Crimean war in the 1850s. The casual relationship between organism and disease was first established by Dr. David Bruce in 1887 (Wilkinson, 1993). In 1897, Danish Veterinarian Bernhard Bang isolated the agent, and the additional name 'Bangs's disease' was assigned. In modern usage, 'Bang's disease'' is often shortened as ''Bangs'' when ranchers discuss the disease or the vaccine. Maltese doctor archaeologist, Sir Themistocles Zammit identified unpasteurized milk as the major source of the pathogen in 1905, and it has since become known as Malta fever.

The popular name ‘‘undulant fever’’ originates from the characteristic undulance (or ‘‘wave-like’’ nature) of the fever which rises and falls over weeks in untreated patients. In the 20th century, this name, along with ‘‘brucellosis’’ (after *Brucella*, named for Dr. Bruce), gradually replaced the 19th century names ‘‘Mediterranean fever’’ and ‘‘Malta fever’’. *Brucella suis* was isolated in USA from an aborted porcine fetus (Traum, 1914). Three more species of *Brucella* has been described; Buddle and Boyes (1953) who isolated *Brucella ovis*, the cause of epididymitis and abortion in sheep in New Zealand and Australia; Stoener and Lackman (1957) isolated *Brucella neotomae* from the desert wood rat (Neotoma lepida) in Utah, USA and *Brucella canis* which causes canine epizootic abortion and epididymitis, isolated from Beagle dog in the USA by Carmicheal and Kenny (1968).

2.2.2 Aetiology of *Brucella* infection in horses.

Equine brucellosis is caused by two species of genus *Brucella* (*Brucella abortus* and *Brucella suis*) and three biovars have been isolated from horse, namely, *B.abortus* biovars I (Robertson *et al*., 1973), *B. suis* biovars I (Cook and Kingston, 1988) and *B. suis* biovar 3 (Cvetnic *et al*., 2005). All reported species are pathogenic to human beings (Radostitis *et al*., 1994).

Six major *Brucella* species that infect a variety of animals and humans have been classically characterized: *B. abortus*, *B. melitensis*, *B. canis*, *B. ovis*, and *B. neotamae* (Young, 1995; Corbel, 1997). Recently two new species, *B. ceti* and *B. pinnipedialis* have been isolated from marine species (Foster *et al*., 2007). Most *Brucella* species have a strong host preference, which is evident in their ability to establish chronic infection in individuals and maintain transmission and infection in population of specific animal species (Glynn and Lynn, 2008). Different *Brucella* species with their preferential hosts are as follows: *Brucella melitensis* for sheep/goat; *Brucella abortus* for cattle /bison, *Brucella canis* for dog, and *Brucella suis* for

pigs. However, cross-species infections can occur; for example cattle can be affected by both *B. abortus* and *B. melitensis* at the same time (Abdussalam *and Fein*, 1976). None of the *Brucella* species are adapted to the horse. Equine infections usually involve the cattle pathogen, *B. abortus*, though infection with *B. suis* has been reported (Nicoletti, 2007).

2.2.3 Risk factors of *Brucella* infection in horses.

Environment of horses has been linked as a risk factor to brucellosis. *Brucella* organism survive in the environment for weeks, so horse grazing pasture recently occupied by infected cattle are at risk (Jean-Pierre and Hinchcliff, 2011). The bacteria often survive desiccation and can survives freezing temperature for over two years (Public Health Agency of Canada, 2009). Facilities and pasture can remain contaminated for long periods, but direct sunlight reduces the bacteria's survival significantly (Health Protection Agency United Kingdom, 2009). New *Brucella* strain or species may emerge and existing *Brucella* species adapt to changing social, cultural trade and agricultural environment (Godfroid *et al*., 2005). Risk of infection with *Brucella abortus* in horses should be considered in cases of supraspinatus bursa also known as fistulous withers (Wesse, 2002). Associated risks of spread are present in cases with draining tracts or during surgical debridement. Some clinicians recommend against surgical debridement in seropositive horses (Hawkins and Fessler, 2000).

Age is one of the intrinsic factors which influence the susceptibility to *Brucella* infection, as it is more associated with sexual maturity with a predilection for ungulate, placenta, foetal fluid and testes (Radostits *et al*., 2000; Solorio-Rivera *et al*., 2007). Agab (1997), Ahmed and Munir (1995b) also noted that the antibody titer against *Brucella abortus* appears to be associated with age. It may be possible that after entry, the organism localizes itself in the regional lymphnodes and remain there without provoking antibody production until the

animal is conceived and start secreting erythritol which stimulates and supports the growth of *Brucella* organisms (Keppie *et al*., 1965). Usually young animals are protected by maternal immunity until when the immunity wanes, thus susceptibility seems to be low among them (Farouk *et al*., 2011). In horses, sex has been identified as associated risk factor to brucellosis due to numerous reports on higher prevalence in females than in males (Ahmed and Munir, 1995a; Solmaz *et al*., 2004). Females experience comparatively greater physiological stress during pregnancy and lactation due to which they are susceptible to infection (Wadood *et al*., 2009). Besides, a *Brucella* growth supporting substance erythritol which occurs in higher concentration in the placenta and foetal fluids of females than in seminal vesicle and testis of males could probably be responsible for the observed disparity of infection rates (Keppie *et al*., 1965).

Additionally, horse drinking water from common pond that was also used by cattle, dog and other animals is associated risk factor for equine brucellosis (Acosta-Gonzalez *et al*., 2006). In endemic region especially, sheltering the horses and donkey with cows and other ruminants increase brucellosis seropositivity rate (Abo-Shehada, 2009). Thus the close association between donkey and ruminant exposes donkey and horse to many ruminant pathogens including Brucella species (Abo-Shehada, 2009). Recent history of abortion in stable with more than fifty-one females has been incriminated as a factor in the spread of brucellosis (Negreiro *et al*., 2009; Villar *et al.*, 2009). Aborted materials and infected vaginal discharges of cattle, swine could be a factor in the spread of *Brucella* infection to horse and vice versa (Baek *et al*., 2003). Diverse animal species are a risk factor for equine brucellosis from epidemiologic studies investigating the role of diverse species. There is evidence that wild mammals such as white tailed deer, opossums, raccoons (Boeer *et al*., 1980) hares (Girando *et*

al., 1985), pampas deer (Mathias *et al*., 1999) do serve as reservoirs of *Brucella abortus*. Horse is also a potential reservoir for *Brucella abortus* (Acosta-Gonzalez *et al*., 2006). Finally, feeding horses with raw cow's milk is associated risk factor to equine brucellosis (Musa, 2004).

Brucellosis distribution is worldwide, though it is more common in countries with poorly standardized animal and public health programme (Capasso, 2002). It has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and new Zealand (Geering *et al.*, 1995) but still remains an uncontrollable problem in regions of high endemicity such as Africa, the Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002). Brucellosis is endemic in Nigeria and has been reported sporadically since 1921 (Ocholi *et al*., 1993).

However, very few studies on the seroprevalence of *Brucella* species in horses have been reported worldwide. The reported seroprevalence vary. 12.89% in India (Sharma *et al*., 1979); 8-16% in the UK (MacMillan, 1985); 5.78% in Pakistan (Ahmed and Munir, 1995a); 0.00% in Eritrea (Omer *et al*., 2000); 0.2% in tropical region of Mexico (Acosta-Gonzalez *et al*., 2006); 5.88% in Egypt (Montasser *et al.*, 1999); 2.5% in North-East Iran (Tahamtan *et al.*, 2010) .

The routes of infection are multiple i.e., food-borne, occupational /recreational, linked to travel and even to bioterrorism (Bossi *et al*., 2004).

2.2.4 Clinical signs of *Brucella* infection in horses.

In equine brucellosis, horses may become infected with *Brucella abortus* (MacMillan *et al*., 1982). In some cases infection may remain asymptomatic (Denny, 1973) while in other infections it is associated with a variety of clinical manifestations including generalized

infection (Cvetnic *et al*., 2005). However, in horses that develop clinical signs of infection, the organism usually localised in bursae causing septic bursitis (Denny, 1973), tendon sheaths causing septic teno-synovitis (Carrigan *et al*., 1987), and joints causing septic arthritis (Ocholi *et al*., 2004). Less commonly, cases of vertebral osteomyelitis (Collins *et al*., 1971), abortion in mare and infertility in stallion (Carmichael, 1990). The commonest clinical disease associated with *Brucella* species infection in horses are septic supra-spinatous bursitis (fistulous withers), septic supra- atlantal bursitis (poll evil), non-specific lameness due to joint infection or rarely,mid to late- term abortions in mares (Hinton et al.,1977).

Chronic draining sinuses occur in both conditions (Crawford *et al*., 1990). Fistulous withers is a chronic inflammatory disease of the supra-spinatus bursa and associated tissues (Cohen *et al*., 1992). Clinical signs of supra-spinous bursitis (fistulous withers) include singular or multiple draining tracts or diffuse swelling of the withers without drainage. Early signs include localized heat, pain and swelling of the bursa. There may be lethargy and general stiffness. In most cases the bursa ruptures and purulent exudate is discharged from one or more fistulae. These fistulae may heal over time, but may subsequently reform. Extensive fibrosis may occur (Cohen *et al*., 1992). Poll evil (septic supra-atlantal bursitis) causes similar clinical signs to fistulous withers in the poll region. Mid to late term abortion in mares has been reported, but this appear to be rare (Hinton *et al*., 1977).

2.2.5 Diagnosis of *Brucella* infection in horses.

Brucellosis most frequently involves Brucella abortus and sometimes *Brucella suis* and *Brucella canis* in horse population (Ribeiro *et al*., 2008; Nicoletti, 2007). The resumptive diagnosis of equine brucellosis is based on the presenting clinical signs. However, other causes of chronic draining abscesses have to be ruled out. Other microorganism such as

Actinomyces bovis have been isolated together with *Brucella abortus* from poll evil and fistulous withers (Quinn *et al*., 1999). Nevertheless the isolation of B. *abortus* or B. *suis* or B.*canis* serves to confirm the diagnosis. Isolation of *Brucella* bacteria is the only unequivocal method for the diagnosis of brucellosis (Alton *et al*., 1988). The spleen and lymph nodes are the most reliable samples for isolation purpose in necropsied animal (Marin *et al*., 1996). Microscopic examination of smear from virginal swabs, placentas, aborted foetuses and fistulous, swabs stained with the stamp modification of the Zichl Nelsen method are recommended (Alton *et al*., 1988). Various culture media or selective media with antibiotics are incubated in an atmosphere of 10% CO_2 at 37^{o}c for 3-9 days (Kudzas and Horse, 1953). Selective media are needed for isolation purpose to avoid the growth of contaminant (Corbel *et al*., 1985). Farrell's selective medium is recommended for such (Alton *et al*., 1988).

Animal Inoculation is another method of diagnosis. Guinea pigs are more susceptible to *Brucella* than any other laboratory animal; however, mice are susceptible and may be substituted for guinea pigs (Avong, 2000). A suspension of suspected material (foetal, living, intestinal content, placenta, blood, uterine discharge and milk) is infected intra peritoneally and the animal necropsied three to six week later. If positive, *Brucella abortus* is recovered from spleen by culturing on the appropriate media (Alton *et al*., 1988). Because of the difficulty that may be encountered in attempts to culture *B. abortus* from horses with fistulous withers, concomitant serologic testing for detection of specific antibodies is recommended.

Serology is a definative diagnosis of brucella infection in horses (Ribeiro *et al*., 2008). The procedures are divided into two categories; the conventional tests and primary binding assays. One of the major enzyme immunoassays (EIAs) used in brucellosis diagnosis is the indirect

enzyme link immunoassays (i-ELISAs). ELISAs are methods that involve the immobilization of one of the active components on the solid phase, and i-ELISAs are those in which the antigen is bound to a solid phase, usually a polystyrene microtitre plate so that antibody, if present, in a sample, bind to the immobilized antigen and may be detected by an appropriate anti-global linked-enzyme conjugate which in combination with a chromogenic substrate gives a coloured reaction indicative of the presence of antibody in the sample (Marin, 1999).

Another method of ELISAs is the competitive ELISA (Perrett *et al*., 2010). In this test, *Brucella* antigen is immobilized as the i-ELISA. Following that, the serum under test and a monoclonal antibody directed against an epitope on the antigen are co-incubated. This anti *Brucella* monoclonal antibody is conjugated to an enzyme, the presence of which is detected if it binds to the antigen. This will occur if there is no antibody in the serum sample which is bound preferentially. Moreover, the estimation of the results can either be visual or be made with rather simple spectrophotometer (ELISA Reader) (Voller *et al*., 1976).

Other Serodiagnosis of horse brucellosis includes rose bengal plate test (RBPT), serum agglutination test (SAT), fluorescent antibody test (FAT), complement fixation test (CFT) , milk ring test (MRT), 2-marcapto-ethanol test (2-MET), the rivanol (acridine) test, agar gel diffusion and coombs test (Denny,1972; MacMillan, 1985). In recent years, molecular methods such as PCR have been suggested as alternative gold standard tests to confirm brucellosis (Bricker, 2002). However, such method may be expensive to rely upon in routine diagnosis of equine brucellosis.

2.2.6 Treatment of *Brucella* infection in horses.

Treatment of brucellosis in horses generally involves a combination of systemic antimicrobials and local surgical rainage/debridement of infected tissue. *Brucella* species are generally sensitive to tetracyclines, chloramphenicol, Streptomycin and sulphonamides, there may be insufficient penetration into infected tissue to achieve resolution of infection (Nicoletti, 2007). Moreover, polymicrobial infections are common. There has been a report of successful treatment of three horses using clofazimine (Knottenbelt *et al*., 1989). In animals, most time relapse occurs after treatment of these facultative intracellular bacteria (FAO, 2004). Treatment in human brucellosis; tetracycline and streptomycin is most potent antibiotics. WHO gave a recommendation for the treatment of acute *Brucella* infection in adult with a combination of rifampicin and doxycycline daily for a period of six weeks (WHO, 1986). Meningoencephalitis complication in children may be treated by trimethoprim and rifampicin therapy (Lubani *et al*., 1989).

2.2.7 Prevention and control of *Brucella* infection in horses.

Brucella infection has been eradicated or brought under control in some countries by programmes of blood testing, slaughter, surveillance and vaccination (Godfroid et al., 2004). Horses acquire *Brucella abortus* and *Brucella suis* infection from cattle and pig, therefore, prevention of infection involve prompt recorgnition of potential cases and reduction or elimination of animal contact (Denny, 1973; Cromlet and Bernhanu, 1979). Infected horses represent a source of infection to man and vice versa (Acha and Szyfres, 1987), thus barrier precautions (gloves, protective cloth, eye wear and surgical mask) and careful handling of laboratory materials should be practice. Management system like the use of common pastures and water sources should be discontinued (Bertu *et al*., 2012). Vaccine are not available for

use in horses, though *Brucella* strain19 vaccine has been described but with serious adverse effects including death (Denny, 1973; Cohen *et al*., 1992).

Epidermiological evidence shows that brucellosis is present in different species of domestic livestock including horses in Nigeria (Ehizibolo, 2011b), but unfortunately, there is no control policy in Nigeria for this disease with a potential for huge economic and medical impact (Ehizibolo *et al*., 2011b)

2.2.8 Public health significance of *Brucella* infection in horses.

Brucellosis remains one of the world important zoonotic diseases. Horses usually acquire *Brucella abortus* infection from cattle (Denny, 1973) or *Brucella suis* infection from pig (Nicoletti, 2007), therefore, horses should not be housed or pastured with seropositive cattle or pig.

Horses infected by *Brucella* species may represent a source of infection to human (Acha and Szyfres, 1987), although documented cases of this route of infection are rare (Jalil, 2008) .Most cases of *Brucella* infection is caused by occupational exposure to infected animals and the disease is known as occupational disease of those working with infected animals or their tissues such as horse handlers, veterinarians, abattoir workers, farm workers (Bossi *et al*., 2004). Brucellosis is responsible for numerous economic losses and considerable human morbidity in endemic region especially developing countries (Bossi *et al.,* 2004).

Brucella suis and *Brucella melitensis* pathogens are potential agent of biological terrorism (Yagupsky and Baron, 2005).

2.2.9 Situation of *Brucella* infection in Nigeria.

Epidemological evidence shows that in Nigeria brucellosis is found in animals including cattles, sheeps, and goats, dogs and horses (Adamu and Ajogi, 1999; Osinubi *et al*., 2004; Ehizibolo *et al*., 2011a). Serological surveys of *Brucella* antibodies in Nigeria indicate a prevelance ranging from 0.4-48.8% in cattle (Erganis *et al*., 2005), 1.4-16.1% in sheep and goat (Bertus *et al*., 2008; Okewole *et al*., 1988), 4.4-7.5% in dogs (Agunloye *et al*., 1999; Osinubi *et al.,* 2004), 2.8% in swine (Bale and Nuru, 1995) and 2.0% in poultry (Adesiyun and Abdu,1984).

Reports on equine brucellosis are rare (Ocholi *et al*., 2004) despite the endemicity of this disease in Nigeria (Rikin, 1988; Ocholi *et al*., 1993). The first evidence of brucellosis in horse in Nigeria was reported by Bale in 1984. The few reports on equine brucellosis in Nigeria have focused on clinical cases (Ocholi *et al*., 2004; Oladosu *et al*., 1986) and to our notice, only few serological studies; Bale and Kwanashie, 1984; Ehizibolo *et al*., 2011a; Tijjani *et al*., 2011 have been conducted in Nigeria. Serological survey by Bale and Kwanashie (1984) on sero-prevalence of brucellosis among horses in northern Nigeria revealed 8.4%, that by Ehizibolo *et al*., (2011a) on seroprevalence of brucellosis in horse stables in two northern states of Nigeria revealed 14.7%, while that of Tijjani *et al*., (2011) on serological survey of *Brucella* antibodies in horses in Maiduguri Metropolis, Borno state, Nigeria revealed 28.7% sero-positivity. Some other clinical cases are Oladosu *et al*., (1984) on isolation of *Brucella* from aborted foetus in Ibadan, western Nigeria, Ocholi *et al*., (2004) reported a case of Carpal bursitis associated with *Brucella abortus* in horse in Nigeria. In Nigeria, only *Brucella abortus* has been isolated from horse (Ocholi *et al*., 2004). Unfortunately, there is no control policy in Nigeria for brucellosis with a potential for huge economic and medical impact (Mai *et al*., 2012; Cadmus *et al*., 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study area is Kaduna Metropolis. The study area is comprised of Kaduna North, Kaduna South, part of Chikun and part of Igabi Local Government Areas. Igabi Local Government Area is located on longitude $10^0$47''N and Latitude $7^0$46''E, covers a land mass of 3,727 Km^2 and has a population of 430, 753 (NPC, 2006). Kaduna North is located on longitude $11^0$32''N and latitude $8^0$2''E, has a population of 364,575 (NPC, 2006) and covers an area of 72 Km^2. Kaduna South Local Government Area cover a land mass of 59Km^2 and has a population of 402,390 (NPC, 2006) while Chikun Local Government Area cover a land mass of 4,645Km^2 and has a population of 368,250 (NPC, 2006). This study area is located in the Northern Guinea Sanvannah zone and the predominant occupation by the people in this area is farming.

The area is known for a lot of recreational activities including the Kaduna Polo club and the Fifth Chukker Polo club. The Deebee farm provides horse riding trainings for people. The farm also has stables which are leased out for people to keep horses. Other stables include the Nigeria Police Force and the Nigeria Defence Academy where horses are kept for cadets training, parades and special ceremonies.

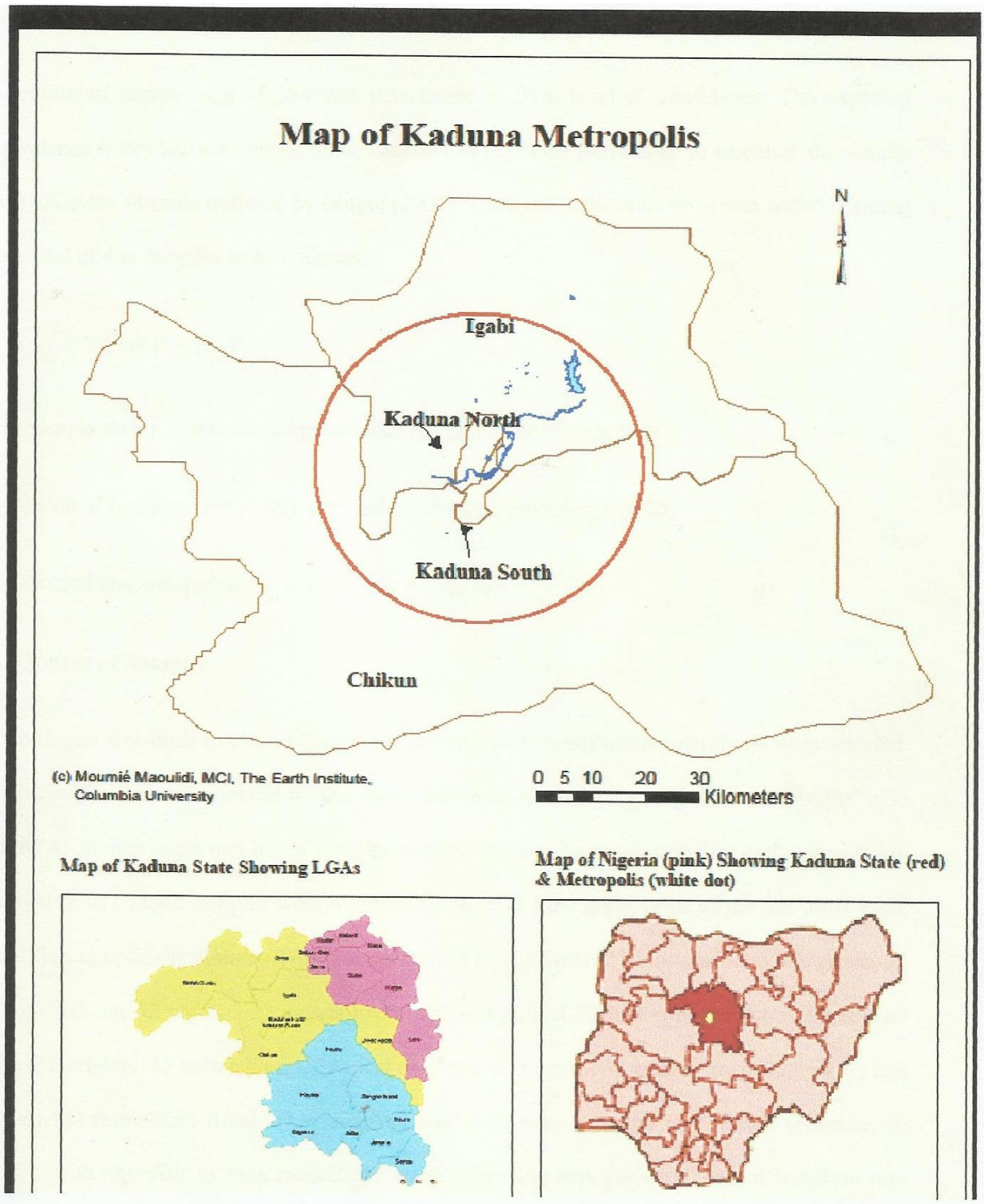


Figure 3.1: Map of Kaduna Metropolis consisting of Kaduna North, Kaduna South, parts of Igabi and Chikun and Chikun LGAs. The red circle represents 30km radius defining the study area (MCI, 2011).

3.2 Study Population

This study is targeted towards horse population, stable environment and horse handlers in Kaduna Metropolis. Consequently, horses in seven stables in Kaduna town were used and the different stables form the various study units. These stables were, Nigeria Defense Academy stable, Nigeria Police force stable, Kaduna Polo Club stable, Angwan Doki stable, Al-amin stable, 5th Chukker game stable and Deebee farm stable. The study inclusions were horses of all ages, sexes and breeds. Horses outside Kaduna Metropolis were excluded. Non-horse handlers were also excluded in the questionnaire. Horse handlers (stable grooms, stable cleaners, horse riders, farriers and horse trainers), veterinarians, stable managers, and horse owners were included in the study.

3.3 Study Design

A cross-sectional study was carried out to sample a total of two hundred and eighty four (284) horses of all ages, sexes and breeds, in the seven different stables within Kaduna Metropolis. Age of each sampled animal was determined using the conventional dental technique (Wosu, 2002) and recorded, while sexes and breeds determination were done under close observation by noting the individual characteristics of the animals. History of previous abortion of mares and vaccination of stabled horses against leptospirosis and brucellosis were ascertained before sampling. Sample collection lasted for a period of 3 weeks.

Questionnaires were administered to stable personnels in two ways, to horse handlers, horse owners and veterinary medical personnels immediately after sampling in the various stable units at the time of visit.

3.4 Sample Collection

3.4.1 Sample size

The total numbers of horses in each of the seven stables were 44, 34, 34, 184, 184, 56 and 30. Stable owner's consent was obtained. Convenient sampling method was used for the study and samples were obtained from 50% of the horses in each stable, giving a total sample size of 284 horses.

3.4.2 Sampling procedure

The history of the horses was taken before restrained and sample collected. Blood (10ml) was collected from each horse via jugular venipuncture and dispensed into sterile, well labeled 10ml sample bottle containing no anti-coagulant and kept in a slanted position to clot. The serum was decanted into a well labeled, 5ml serum bottle after separation from the clot and then transported in cooler containing ice packs to the Veterinary Public Health laboratory for further processing.

Each serum was centrifuged at 4,000 revolusions per minute (revs/min) for 10 minutes and the serum dispensed into serum vials and stored at -20^0C until use. Age, sex and breed of each sampled horse were recorded.

3.5 Assessment of Risk Factors

A pretested (using seven stables), structured questionnaire consisting of open and close-ended questions were designed and administered to seventy seven (77) horses handlers in the seven stables units to determine the presence of risk factors to leptospirosis and brucellosis (Appendix 1). Questions such as source of horses, number of horses in the stable, breed, sex,

age, management practices, hygienic practices, zoographic, presence of clinical signs, vaccination history of the horses against leptospirosis and brucellosis were asked.

3.6 Laboratory Analysis for the Field Samples

3.6.1 Source of ELISA kits.

Leptospira indirect -ELISA kit for the *Leptospira* IgG test was obtained from Diagnostic Automations Inc. UK and *Brucella* competitive- ELISA kit for *Brucella* antibodies test was obtained from Animal Health Veterinary Laboratories Agent (AHVLA), UK.

3.6.2 Indirect ELISA procedure for *Leptospira* IgG.

The i-ELISA test was performed based on the manufacturers recommendation which are as follows: Four microtitre plate, each with 96 wells coated with *Leptospira* antigen; twelve wells arranged horizontally (1-12 wells) and eight wells arranged vertically (A-H) were broken off and were placed in strip holder 100µl of pre-diluted negative control was added to well number I and 100µl of pre-diluted positive control was added to well number II. The test sera (10µl) of tested serum were diluted at 1:40m 390µl of dilution buffer (containing 30 ml buffered protein solution)and 100µl of the samples were dispensed into each of the remaining wells and were incubated at room temperature for 10 minutes then were washed thrice. After the last washed step, the wells were slapped on a clean absorbent towel to remove excess wash buffer. Then 2 drops of enzyme conjugate were added to each well and was incubated at room temperature for 10 minutes, then were washed thrice. After the last washed step, the wells were slapped on a clean absorbent towel to remove wash buffer and 2 drops of the Chromogen® (tetramethylbenzidine) were added to each well and were incubated at room temperature for 5 minutes. Two drops of the stop solution (1M phosphoric acid) were added to each well, the wells were mixed by gently tapping the sides of the strip holder index finger

for approximately 15 seconds. The experiment was read one hour of adding stop solution with an ELISA plate read at 450nm.

Based on the manufacturer's recommendation, the test results were interpreted as; negative (non-reactive) when the samples value is 0.0 to 0.4 OD units or - -, - - -, indicating antibody is not present in the sample and positive (strong reactive) when the samples value is 0.50D units and above or ++ ,+++, indicating the presence of specific antibody.

3.6.3 Competitive ELISA procedure for *Brucella* antibody.

The c-ELISA test was performed according to the manufacturer's recommendation. Four microtitire plates, each with 96 wells coated with *Brucella abortus* antigen; twelve wells average horizontally (1-12 wells) while vertically, eight wells were arranged (A-H).

The test serum (20µl) was added per well. Columns 11 and 12 were left for controls. 20µl of the negative control were added to wells A11, A12, B11, B12, C11 and C12. The positive control (20µl) was added to well F11 F12, G11, G12, H11 and H12. Serum was not added to the remaining wells which acted as the conjugate control. Immediately dispensed into all wells 100µl of the prepared conjugate solution which gives a final serum dilution of 1/6. The plate is then vigorously shaken for 2 minutes in order to mix the serum and conjugate solution. The plate were covered with the lid and incubated at room temperature at 160 revs/min. Contents of the plate were shaken out and rinse 5 times with wash solution (a mixture of ampoule of sodium phosphate, 1ml of tween20 and 10 litre of distill water) and then was thoroughly dried by tapping on absorbent paper towel. Microtitre plate reader was switched on and the unit was allowed to stabilize for 10 minutes. Before use, immediately substrate and chromogen solution were prepared by dissolving one table of urea hydrogen peroxide in 12ml of distilled water and when dissolved OPD tablet was added and mixed

thoroughly. 100µl were added into all the wells and the plate was left at room temperature for 10 minutes. The stop solution (100µl) was added to all wells to slow the reaction. Condensation was removed from the bottom of the plate with absorbent paper towel and plate was read at 450nm within 1 hour. Optical density (OD) readings was printed out. Also, the plate was inspected visually to determine whether a sample was positive or negative.

Based on the manufacturer's recommendation, lack of color development indicated that the sample tested was positive. A positive or negative cut off were calculated as 60% of the mean of optical density (OD) of the 4 conjugate control wells. Any tested sample that gave an OD equal to or below this value was regarded as positive.

3.7 Data Analysis

All the data obtained from the various investigations were presented using descriptive statistics with the aid of Statistical Package for Social Sciences (SPSS) version 17 through multivariate logistic regression. Chi-square, odds ratio and confidence interval to check for associations and relative risk between sex (male and female), horse age (0-5yrs, 6-10yrs, 11-15 yrs and 16 yrs and above etc) to specific reactors. Values of $p < 0.05$ were considered statistically significant. The percentage of horses positive (% positive) for antibodies to *Leptospira* and *Brucella* species were calculated by dividing the total number of seropositive horses by the total number of horses sampled and multiply by 100.

$$\text{Seroprevalence} = \frac{\text{Number of seropositive horses X 100}}{\text{Number of horses tested}}$$

CHAPTER FOUR

RESULTS

Serologically, antibodies to *Leptospira* and *Brucella species* were detected in sera of stabled horses in Kaduna Metropolis which were examined using indirect enzyme linked immunosorbent assay (i–ELISA) and competitive enzyme linked immunosorbent assay (c – ELISA) respectively. These are other major findings revealed in this study;

Of the 284 horses sampled, 188 (66.2%) were aged 6-10 years old, while 67 (23.6%), 24 (8.5%) and 5 (1.8%) were aged 0-5 years, 11-15 years, and 16 years and above, respectively. A total of 184 (64.8%) were females (mares) while 100 (35.2%) were stallions. Distribution of horses by breed showed that 133 (46.8%) were of Argentine breed, while 103 (36.3%)\, 43 (15.1%) and 5 (1.8%) were Arewa, Sudan and South African breeds respectively. The distribution of horses by stables in Kaduna Metropolis used in this study were 17(6.0%), 28(9.9%), 22(7.7%), 93(32.7), 15(5.3%), 17(6.0%) and 92(32.4) for KadP, Al-amin, NDA, 5^{TH} Chukker, Deebee Farm, NPF and Angwan Doki stable respectively (Table 4.1). Physical examination revealed that 25 (8.8%) of the horses had wounds on their body while 259 (91.2%) of the horses does not have wound on their body (Table 4.1).

Horses aged 16 and above years had the highest seroprevalence (60%), while those aged 11-15 years had the least seroprevalence of leptospirosis (16.7%). There was no significance association ($p>0.05$) between the presence of antibodies to *Leptospira* species and the age of horses sampled (Table 4.2)

There was also no significant association ($p > 0.05$) between the presence of *Leptospira* species antibodies and the sex of the horses sampled. Although, males (stallions) had a higher seroprevalence (34.0%) than females (mares) (23.4%) (Table4. 2).

There was also no signicant association ($p>0.05$) between the the breed of horses and the presence of antibodies to *Leptospira* species. The S/African breed had the highest seroprevalence (60%), followed closely by the Argentine breed. The Sudan and Arewa breeds had the same seroprevalence of 23.3% (Table 4.2).

About 29.8% of the horses that were watered from a common source of water were seropositive to *Leptospira* species antibodies, while 21.5% of the horses which do not have a common water source were seropositive for *Leptospira* species antibodies. There was no significant association ($p >0.05$) between having a common source of drinking water and the presence of leptospira antibodies in the horses (Table 4.2).

Furthermore, there was also no signicant association ($p >0.05$) between the presence of *Leptospira* species antibodies and the presence of other animals on the stables. Horses with and without other animals on the stable had seroprevalence of 27.1% and 27.2% respectively (Table 4.2).

There was, however, a significant association ($p< 0.05$) between the presence of *Leptospira* species antibodies and the presence of wound on the horses. Horses with wound had a higher prevalence of antibodies to *Leptospira* species (52.0%) than those horses without wound (24.7%) (Table 4.2).

Horses that were fed with both home and commercial feed, had a higher seroprevalence (37.0%) than those horses fed home-made feed (15.4%). However, there was a significant association ($p < 0.05$) between the presence of antibodies of *Leptospira* species and the type of feed fed to the horses (Table 4.2).

There was a significant association ($p<0.05$) between the presence of antibodies to *Leptospira* species antibodies and the presence of veterinary clinic in the facilities. Horses with no veterinary clinic in its stable had a lower seroprevalence (20.0%), than those with veterinary clinic (33.6%) (Table 4.2).

In Table 4.3, no association between sex and prevalence of antibody to leptospirosis was recorded. Not having open water source for the horses (OR = 0.040, 95% CI 0.013 , 0.124), having no wounds on the body (OR = 0.286, 95% CI 0.104, 0.787), being feed only home made feed (OR = 0.069, 95% CI 0.029, 0.167) and not having veterinary clinic in the stable (OR = 0.134, 95% CI 0.052, 0.347) were significantly protective for a horse being seropositive to *Leptospira* spp (Table 4.3).

There was no significant association ($p>0.05$) between the presence of *Brucella* species antibodies and age of the horses sampled. Horses aged 16 years and above had the highest seroprevalence (80%) while horses aged 0-5, 6-10 and 11-15 had seroprevalence of 44.8%, 56.9% and 45.8% respectively (Table 4.4).

Male horses had a higher seroprevalence (57.0%) than female horses (51.6%) with seroprevalence of antibodies to *Brucella* species (Table 4.4). However, there was no significant association ($p>0.05$) between the presence of antibodies to *Brucella* species and the sex of the horses.

Also, there was a significant association ($p < 0.05$) between the presence of antibodies to *Brucella* species and the breed of horses. The South African breeds also had the highest seroprevalence (80%) followed closely by Argentine breed (72.9%) (Table 4.4).

There was a significant association ($p<0.05$) between having a common source of drinking water and presence of antibodies to Brucella species. Horses with common source of drinking water had a seroprevalence of 41.4%, while those without a common source of drinking water had a seroprevalenc of 78.5% (Table 4.4).Horses in stables with other species of animals had a *Brucella* species antibodies seroprevalence of 38.1%, while those without any other animals had a seroprevalence of 80.6%. There was a significant association ($p<0.05$) between the presence of other animals on the stable and having antibodies to *Brucella* species (Table 4.4).

There was no significant association ($p > 0.05$) between the presence of *Brucella* species antibodies in horses sampled and the presence of wound on the horses. Horses with wound had a higher seroprevalence of 64.0% than horses without wound (52.5%) (Table 4.4).Horses fed or kept on both home-made and commercial feed had a higher seroprevalence of *Brucella* antibodies (76.0%) than those kept on home-made feed (26.9%). There was a significant association ($p < 0.05$) between the presence of *Brucella* species antibodies and the type of feed fed to the horses (Table 4.4).

There was also a significant association ($p < 0.05$) between the presence of *Brucella* species antibodies in horses and the presence of veterinary clinic in the facilities. Horses with veterinary clinic in their stables had a higher seroprevalence (75.2%) than horses with no clinic in their facility (29.6%) (Table 4.4).

Not having open source of water (OR=5.18, 95% CI 2.92, 9.17), being fed with home made feed (OR=0.81, 95% CI 0.015, 0.425) and not having veterinary clinic in the stable (OR = 0.126, 95% CI 0.040, 0.402) were protective to being positive for *Brucella* spp. (Table 4.5).

Table 4.6 shows the seroprevalence of *Leptospira* species antibodies in horses based on the stables sampled. The overall seroprevalence for *Leptospira* species antibodies in the horses sampled was 27.1% (77/284). Al-amin, NDA and Kadpolo club stables had a seroprevalence of 78.6%, 77.3% and 76.5% respectively. While NPF had none of their horses seropositive to *Leptospira* species antibodies.

Table 4.6 also shows the seroprevalence of *Brucella* species antibodies in horses based on the stables sampled. The overall seroprevalence for *Brucella* species antibodies was 53.5% (152/284). All the horses sampled from NDA stables were positive for *Brucella* species antibodies, while, Al-amin and Kadpolo club stables had seroprevalence of 96.4% and 94.1% respectively. Deebee stable had none of its horses seropositive to *Brucella* species antibodies (Table 4.6).

Among the mares sampled (184), 7 (16.3%) of those with history of abortion were seropositive to *Leptospira* antibodies while 12 (12.6%) of the mares that had aborted previously were positive to *Brucella* antibodies (Table 4.7). The association was, however, not statistically significant ($p>0.05$) (Table 4.7).

Table 4.1: Distribution of Horses Sampled by their Zoograhpic Characteristics (n=284) .

Zoographic characteristics	Frequency	Percentage
Age of horses		
0-5	67	23.6
6-10	188	66.2
11-15	24	8.6
16 and above	5	1.8
Sex of horses		
Female	184	64.8
Male	100	35.2
Breed of horses		
Sudan	43	15.1
South Africa	5	1.8
Argentina	133	46.8
Arewa	103	36.3
Stable of horse		
KadP	17	6.0
Al-amin	28	9.9
NDA	22	7.7
5^{TH} Chukker	93	32.7
Deebee Farm	15	5.3
NPF	17	6.0
Angwan Doki	92	32.4
Horses with wound		
Yes	25	8.8
No	259	91.2

Table 4.2: Risk Factors of *Leptospira* Infection in Horses from Seven Stables in Kaduna Metropolis, Nigeria on January, 2013 (n=284, np = 77).

Zoographic Characteristics	Total no Sampled	Total No Positive (%h)	Chi Square	P- Value
Age				
0-5	67	19(28.4)	4.114	0.249
6-10	188	51(27.1)		
11-15	24	4(16.7)		
16 and above	5	3(60.0)		
Sex				
Female	184	43(23.4)	3.705	0.054
Male	100	34(34.0)		
Breed				
Sudan	43	10(23.3)	4.408	0.221
S/African	5	3(60.0)		
Argentine	133	40(30.1)		
Arewa	103	24(23.3)		
Common source drinking water				
No	93	20(21.5)	2.200	0.138
Yes	191	57(29.8)		
Presence of other animal in the stable				
No	103	28(27.2)	0.000	0.984
Yes	181	49(27.1)		
Horse with wound				
No	259	64(24.7)	8.592	0.003
Yes	25	13(52.0)		
Feed type				
Home-made	130	20(15.4)	16.687	0.0001
Both Home and Commercial	156	57(37.0)		
Presence of vet. Clinic				
No	135	27(20.0)	6.587	0.010
Yes	149	50(33.6)		

n = total number of sampled horses, np = total number of sampled positives horses

Table 4.3 Odds Ratio (95% CI) from Multivariate Logistic Regressions comparing Seropositive and Seronegative Horses for *Leptospira interrogans* in Kaduna Metropolis (n = 284; np = 77).

Antibody of Leptospira spps								
		Positive				Negative		
Zoographic Characrerisitc	Total no sampled	Total no positive (%)	OR	95% confidence interval Lower bound	Upper bound	OR	95% confidence interval Lower bound	Upper bound
Female	184	43(23.4)	0.875	0.428	1.789	1.143	0.559	2.336
Male	100	34(34.0)	-	-	-	-	-	-
No open water source	93	20(21.5)	0.040	0.013	0.124	25.222	8.047	79.052*
Open water source	191	57(29.8)	-	-	-	-	-	-
No wound	259	64(24.7)	0.286	0.104	0.787	3.495	1.271	9.609*
Wound	25	13(52.0)	-	-	-	-	-	-
Home made feed	130	20(15.4)	0.069	0.029	0.167	4.391	5.994	34.551*
Commercial feed	156	57(37.0)	-	-	-	-	-	-
No vet clinic	135	27(20.0)	0134	0.052	0.347	7.467	2.879	19.367*
Vet clinic	149	50(33.6)	-	-	-	-	-	-

*$p < 0.05$, n = total no of sampled horses, np = total no of sampled positive horses.

Table 4.4 Risk Factors of *Brucella Infection* in Horses from Seven Stables in Kaduna Metropolis, Nigeria on January, 2013 (n = 284, np = 152).

Zoographic Characteristic	Total no Sample	Total No Positive (%)	Chi-Square Value	P. Value
Age				
0-5	67	30(44,8)	4.90	0.179
6-10	188	107(56.9)		
11-15	24	11(45.8)		
16 and above	5	4(80.0)		
Sex				
Female	184	95(51.6)	0.751	0.386
Male	100	57(57.0)		
Breed				
Sudan	43	17(39.5)	42.356	0.0001
S/African	5	4(80.0)		
Argentine	133	97(72.9)		
Arewa	103	34(33.0)		
Common source drinking water				
No	93	73(78.5)	34.669	0.0001
Yes	191	79(41.4)		
Presence of other animal in the stable				
No	103	83(80.6)	47.577	0.0001
Yes	181	69(38.1)		
Horse with wound				
No	259	136(52.5)	1.210	0.271
Yes	25	16(64.0)		
Feed type				
Home-made	130	35(26.9)	68.180	0.0001
Both Home and Commercial	156	117(76.0)		
Presence of vet. Clinic				
No	135	40(29.6)	59.043	0.001
Yes	149	112(75.2)		

n = total number of sampled horses, np = total number of sampled positives ho

Table 4.5 Odds Ratios (95% CI) from Multinominal Logistic Regressions comparing Seropositive and Seronegative *Brucella Species* in Horse Stable in Kaduna Metropolis (n = 284, np = 152).

			Antibody of Brucella spps					
			Positive			**Negative**		
Zoographic Characrerisitc	Total no sampled	Total no positive (%)	OR	95% confidence interval Lower bound	Upper bound	OR	95% confidence interval Lower bound	Upper bound
Female	184	95(51.6)	0.882	0.429	1.811	1.134	0.552	2.330
Male	100	57(57.0)	-	-	-	-	-	-
No open water source	93	73(78.5)	3.437E-9	8.22E-10	1.472E-8	2.910E-8	6.791E-7	1.247E-9
Open water source	191	79(41.4)	-	-	-	-	-	-
Home made feed	130	35(26.9)	0.081	0.015	0.425	12.350	2.353	64.809*
Commercial feed	156	117(76.0)	-	-	-	-	-	-
No vet clinic	135	40(29.6)	0.126	0.040	0.402	7.926	2.488	25.251*
Vet clinic	149	112(75.2)	-	-	-	-	-	-
0-5 (yrs)	67	30(44.8)	0.969	0.036	26.365	1.032	0.08	28.078
6-10	188	107(44.8)	1.102	0.042	28.698	0.907	0.035	23.629
11-15	24	11(45.8)	0.803	0.030	21.199	1.245	0.047	.32.865
16 & above	5	4(80.0)	-	-	-	-	-	-
Sudan	43	17(39.5)	2.748	0.860	8.786	0.364	0.114	1.163
S/Africa	5	4(80.0)	15.210	0.719	321.868	0.066	0.003	1.391
Argentine	133	97(72.9)	1.272	0.184	8.807	0.786	0.114	5.440
Arewa	103	34(33.0)	-	-	-	-	-	-
No other animals	103	83(80.6)	8.579E-7	8.579E-7	8.59E-7	1.166E-8	1.166E-8	1.166E-8
other animals	181	69(38.1)	-	-	-	-	-	-

*$p < 0.05$, n = total no of sampled horses, np = total no of sampled positive horses.

Table 4.6: Seroprevalence of *Leptospira* and *Brucella* Antibodies of Horses Based on Stables Sampled in Kaduna Metropolis, Nigeria, January, 2013.

Stable	Total No Sampled	Leptospira Positive (%)	*Brucella* Positive (%)
Kadpolo club	17	13(76.5)	16(94.1)
Al-amin	28	22(78.6)	27(96.4)
NDA	22	17(77.3)	22(100.0)
5th Chukker Game	93	20(21.5)	73(78.5)
Deebee farm	15	1(6.7)	0(0.0)
NPF	17	0(0.0)	1(5.9)
AngwanDoki	92	4(.3)	13(14.1)
Total	284	77(27.1)	152(53.5)

Table 4.7: Association between Previous History of Abortion and Seroprevalence of *Leptospira interrogans* and *Brucella abortus* Antibodies among Mares in Kaduna Metropolis (n=184).

Seroprevalence	Abortion history		Total	Chi square	P-value
	No	Yes			
***Leptospira* spp**					
Positive	36 (83.7)	7 (16.3)	43 (100.0)	0.996	0.318
Negative	126 (89.4)	15 (10.6)	141 (100.0)		
***Brucella* spp**					
Positive	83 (87.5)	12 (12.6)	95 (100.0)	0.085	0.771
Negative	79 (88.8)	10 (11.2)	89 (100.0)		

CHAPTER FIVE

DISCUSSION

The results of this study have shown that antibodies to *Leptospira* species are present in horses on stables in Kaduna Metropolis and environs with an overall seroprevalence rate of 27.1%. Vaccination against leptospirosis is not routinely carried out in Nigeria (Agunloye *et al*., 2000) and none of the sampled stable in Kaduna Metropolis had reportedly used *Leptospira* vaccine. Therefore the demonstration of antibodies to *Leptospira* in horse in the study area is suggestive of natural exposure to the organism. It may also be due to possible differences in the way horses are managed which may result in seroconversion in horses (Mohammed *et al*., 1996).

The higher seroprevalence in age groups 16years and above (60.0%) seen in this study may suggest a possible development of immunity through earlier contact and re-exposure to infection which might finally result in some tolerance and residual titre in older horses. Other studies have reported high prevalence in adult horses (Park *et al*., 1992; Mohammed *et al*., 1996).

Male horses had a higher percentage of seroprevalence (34.0%) than female horses (23.4%). This result does not agree with several serological studies on equine leptospirosis that reported high proportion of titres in female horses (Kitson-Pggot and Prescott, 1987; Park *et al*., 1992; Mohammed *et al*., 1996). This may be due to differences in the management and the way of keeping the female horse, particularly, the ones sent for brucellosis testing destined to be served and breed.

Horses with common source of drinking water had higher seroprevalence (29.8%) of *Leptospira* antibody than those without (21.5%). This may be because there might be contamination of the water by co-drinking horses and other animals like cattle and swine (Theirmann, 1984).

Horses with wounds had higher seroprevalence of antibodies to *Leptospira* species (52.0%) than those without wound on their body (24.7%).This also agrees with the multivariate regression result that not having wounds on the body of the horses is protective to being seropositive to *Leptospira* species.

Horses that were fed on home-made feed had a lower seroprevalence (15.4%) to *Leptospira* than those fed on both home and commercial feed (37.0%). This also agrees with the multivariate logistic regression result that feeding horses with home-made feed is protective to being seropositive to *Leptospira* species. This finding is at par with the report that higher prevalence of antibodies to *Leptospira* species occurred in horses fed on home-made feed. Reason for this was that brown rat urine was being identified to have contaminanted home-made feed than the commercially formulated feed given to horses (Maghami *et al*., 1977).

The overall seroprevalence of *Brucella* antibodies in horse in Kaduna Metropolis for this study was 53.5%. This is higher than the seroprevalence of previous studies in Nigeria such as 28.7% in Maiduguri, Borno State (Tijjani *et al*., 2011), 14.7% in two northern states (Ehizibolo *et al*., 2011) and 4.8% in Northern Nigeria (Bale and Kwanashie, 1984). The higher seroprevalence in this study may be attributed to the high sensitivity of the diagnostic technique (i-ELISA) used by this study compared to the methods used in previous studies such as RBPT and M.SAT (Tijjani *et al*., 2011), RBPT (Ehizibolo *et al*., 2011) RBPT and

SAT (Bale and Kwanashie, 1984). This may also be due to lack of brucellosis control programme in livestock in Nigeria, and the considerable low hygiene practices and awareness among horse handlers.

The seroprevalence of *Brucella* antibodies recorded in this study was comparable to those reported in other countries of the world such as 60.56% in horses in Turkey (Solmaz *et al*., 2004), 20.7% in Pakistan (Wadood *et al*., 2009), 8-16% in United Kingdom (MacMillan, 1985).

There was a higher seroprevalence (80.0%) of *Brucella* antibodies recorded in older horses aged 16 years and above when compared to younger age groups of 0-5 years, 6-10 years and 11-15 years. The pattern of seroprevalence increment for each age group in this study is in agreement with the *Brucella* serological result of the study done in the horse in Pakistan (Wadood *et al.,* 2009). This recorded low prevalence in young animals may be explained on the basis that the animals may habour the organism without expressing any detectable antibodies until their first parturition or abortion. It may be possible that after entry, the organism localizes in the regional lymph nodes without provoking antibody production until the animal is conceived and start secreting erythritol which stimulates and supports the growth of *Brucella* organism (Keppie *et al.*, 1965).

The study revealed a higher seroprevalence of brucellosis in males (57.0%) compared to female horses (51.6%) but the difference was not significant. This higher seroprevalence of Brucellosis among male is not in consonant with earlier reports by Ahmed and Munir, (1995a), Wadood *et al*., (2009) and Solmaz *et al*., (2004) who reported that the occurrence of brucellosis is not associated with sex. The higher prevalence rate in mares was attributed to

the fact that females remain in close association and discharge from aborted and parturition by infected mares can result to transmission of infection to healthy animals.

Horses in stables without presence of common source of drinking water had a higher seroprevalence (78.5%) when compared with horses in the stables with presence of common source drinking water (41.4%). This is surprising because common source of drinking water has been identified to increase the likelihood of spreading the infection (Cadmus *et al*., 2006).

This was further supported by the multivariate logistic regression analysis which showed that not having an open water source that is accessible to other animals, being fed on home-made feed and not having a veterinary clinic in the stable were protective to being positive for *Brucella* species.

Horses in the stables with the presence of animals other than horses had a lower seropositive rate (38.1%) than horses in stable without other animals (80.6%). However, multivariate logistic regression result showed that not having other animals within the stable is protective to being positive for *Brucella* species. This finding agrees with reports of Omer *et al*., (2000) and Cadmus *et al*., (2006) that interaction between animals could result to increased transmission of brucellosis and leptospirosis.

Al-amin and NDA stable had the highest seroprevalence to *Leptospira* compare to other stable. This could be as a result of the fact that age group of all the horses in Al-amin stable were, between 0-5 and 6-10years.

Studies have shown that adult horses, particularly between 7-11 years of age, had the greatest serological evidence of exposure to *Leptospira* (Park *et al.,* 1992). Also foals have been found

to seroconvert at low titres *bratislava* between four and eight months of age (Kitson-piggot and Prescott, 1987), illness, high or increasing MAT titres to *bratislava* were found in foals and serovar lora was isolated from blood in one foal (Ingh *et al.,* 1989) and in another study, *bratislava* have been isolated from kidney of a healthy foal, indicating that horses can be infected and become carriers of *Leptospira* at a young age (Rocha *et al.,* 2004). These observations above explain why Al-amin stable in this study had the highest *Leptospira* seroprevalence because all the horses sampled in the stable fell between 0-5, and 6-10 years of age. Again highest seroprevalence in Al-amin stable may be due to the way their horses were kept/managed. Different management systems have been shown to interfere with the seroprevalence rates and likelihood of seroconversion particularly to *bratislava*in horses (Mohammed *et al.,* 1996).

The highest seroprevalence of NDA stable to *Brucella* antibodies may be as a result of improper disposal of *Brucella* infected aborted material that remain around the stable because it was observed that 66.7% of the mares in NDA stable had history of abortion. The above observation is in line with higher prevalence of brucellosis recorded in stables where infected aborted foetus was not disposed properly (Ahmed *and Munir,* 1995a; Baek et al., 2003). Pregnant mare harbor more antibodies to *Brucella* species due to the role of erythritol, a polyhydric acid found in higher concentration in placenta and fetal fluid of mare which has been noted to increase the susceptibility and spread of brucellosis (Keppie *et al.,* 1965).

In this study, *Brucella* seroprevalence is very high compared to the previous serological studies. This could be due to the sampling diagnostic techniques used; ELISA method used in this study is more sensitive and specific in detection of *Brucella* antibodies compared to the previous studies that used RBPT (Ehizibolo *et al.,* 2011), SAT (Bale and Kwanashie, 1984).

The disease is believed to still be endemic across the northern part of the country. Improved management practices, adequate awareness creation on the economic and public health impact of the diseases should be advocated in the prevention and control strategy.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has shown a *Leptospira* species antibody seroprevalence of 27.1% while that of *Brucella* species was 53.5% among stabled horses in Kaduna Metropolis. Older horses aged 16 and above had a higher seroprevalence of *Leptospira* species antibody, while male (stallion) had a higher seroprevalence compared to females. South African breed of horses also had higher seroprevalence *Leptospira* specie antibodies compared to other breeds. However there were no significant association ($p>0.05$) between the presence of *Leptospira* specie antibody and the age, sex and breed of horses in these stables. There were significant association ($p<0.05$) between the presence of *Leptospira* species antibody in horses sampled and some risk factors like presence of wound in the body of horses, type of feed , presence of veterinary clinic in the stable and common source of drinking water. There was also no significant association ($p>0.05$) between the presence of *Brucella* species antibodies in the horses in the stables and age and sex of the horses. Horses aged 16 and above had a higher (80.0%) seroprevalence of *Brucella* antibody than younger age group, males and South African breeds also had higher seroprevalence of 34% and 60% respectively. There were also significant association ($p<0.05$) between risk factors like wound on horses, type of feed fed to the horses, presence of veterinary clinic, common source of drinking water, breed of horses and presence of other animals in the stable.

6.2 Recommendations

Common source of drinking water is a risk for transmission of both *Leptospira* and *Brucella* infection in stabled horses, thus, potable drinking water should be provided for individual horse.

Wounds on the body of horses is significantly associated with leptosprosis and brucellosis hence, proper and prompt management and treatment of all wounds on the body of horses is important.

Siting veterinary clinic in the stable predisposes environment to contamination and spread of leptosprosis and brucellosis, therefore, veterinary clinic should not be established within horse stable in Kaduna Metropolis.

Feeding horses with a combination of home and commercial made feeds is associated with higher seropositivity of both *Leptospria* and *Brucella* infections, thus, horses should be fed with home – made feed alone.

Horse breed is an associated risk factor to seroprevalence of brucellosis, therefore exotic breeds of horses imported into Nigeria should be screened against the disease.

The practice of mixing other animals like cattles, pigs, dogs, sheeps and goats with horses in the stable should be discontinued because it exposes healthy horses to *Brucella* infected animals.

Control measures should be instituted in Kaduna Metropolis to stamp out or eradicate these infections among stabled horses.

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APPENDICES

APPENDIX I

RESEARCH QUESTIONNAIRE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, KADUNA.

INSTRUCTIONS: Please kindly tick yes or no in the following boxes and specify where necessary. The information obtained will be used for academic purpose only and absolute confidentiality will be ensured.

1. Date:----------
2. Location (town):----------
3. Sex of respondent: Male ( ) Female ( )
4. Educational Qualification: None ( ) Primary ( ) Secondary ( ) Tertiary ( ) Others ( )
5. Type of duty performed on the Stable: ----------
6. Breeds of horse: ----------
7. Apart from horses, is there any other animal in the Stable and its surrounding? YES ( ) NO ( )
8. If question 7 above is yes, what type of animal(s)? specify: ----------
9. What is the total number of horses in the Stable? ( ), Specify males ( ), females ( )
10. What are the age brackets of the horses in your stable?

    Below 5yrs ( ), Above 5 yrs ( )
11. Are the majority of horses in your stable females? YES ( ) No ( )
12. What type of feed does the stable use? Home-made feed ( ), Commercial feed ( ) or Both ( )
13. Are there presences of rodents among the stabled horses? YES ( ), NO ( )

14. Is there open source of water supply in the stable? YES ( ), NO ( )

15. Are your horses vaccinated against leptospirosis? YES ( ), NO ( )

16. Are your horses vaccinated against brucellosis? YES ( ), NO ( )

17. How do you breed your horses?Artificial insemination ( ), Natural mating ( ) or both ( )

18. If natural mating, where do you source your semen/ stallion? ----------

19. Have you ever recorded any case of abortion in your stable? YES ( ), NO ( )

20. If question 19 above is yes, at what stage of the pregnancy did the abortion occur? Between the early- term ( ) the mid- term ( ) or the late-term ( )

21. Was the cause of the abortion diagnosed? YES ( ), NO ( )

22. If question 21 above is yes, what was the diagnosis?----------

23. And who diagnosed it ?----------

24. Have your mating ever resulted to pregnancy? YES ( ), NO ( )

25. Have your conception ever resulted to safe delivery of life foal? YES ( ), NO ( )

26. Have your conception resulted to stillbirth YES ( ), NO ( )

27. Do you have a Veterinary Clinic attached to your stable? YES ( ), NO ( )

28. Do you have a Veterinary Doctor attached to your stable? YES ( ), NO ( )

29. If question 28 above is yes, how often does he/ she visit? Daily ( ), Frequently ( ), Regularly ( ).

30. If question 28 above is no, how then do you treat your horses?---------- ----------

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31. During rainy season, are there moist soils present in the stable environment? YES ( ), NO ( )

32. Is your stable and herd large in size? YES ( ), NO ( )

33. Are there open wound on the body of the stabled horses? YES (0), NO ( )

34. Do the stable practice co-grazing among horses? YES ( ), NO ( )

35. Do you wear protective clothing when performing your job in the stable?

YES ( ), NO ( ).

36. If question 35 above is yes, what type of clothing? ----------

37. If question 35 above is no, what are your reasons?----------

38. Are you exposed to animal feaces/ Urine/ blood/tissue during work? YES ( ),

NO ( ). If yes, by which means? ----------

39. How often are you exposed to the animals/ their feces/ urine/ blood/ tissue?

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40. 40.Do you wash your hands with detergent and disinfectant after handling/ exposure

to the animals/ their feces/ urine/ blood/ tissue? YES ( ), NO ( ).