# FOETAL WASTAGE AND DETECTION OF *LEPTOSPIRA* SPECIES IN WASTED FOETUSES FROM COWS IN OLD KANO ABATTOIR, KANO STATE, NIGERIA

**BY**

**Nana Aisha BAKARI**
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**DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

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## DECLARATION

I declare that the work in this dissertation titled **"Foetal Wastage and Detection of *Leptospira species* in wasted Foetuses from Cows in old Kano Abattoir, Kano State, Nigeria"** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria under the supervision of Professor J.K.P. Kwaga and Professor J. Kabir. The information derived from the literature has been duly acknowledged in the list of references provided. No part of this Dissertation was previously presented at this or any other institution.

| | | |
|---|---|---|
| Nana Aisha BAKARI | ..... | ..... |
| Name of student | Signature | Date |

## CERTIFICATION

This dissertation entitled **"Foetal Wastage and Detection of *Leptospira species* in wasted Foetuses from Cows in old Kano Abattoir, Kano State, Nigeria"** by Nana Aisha BAKARI, meets the regulations governing the award of the degree of Master of Science in Public Health and Preventive Medicine of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

| | | |
|---|---|---|
| Prof. J.K.P. Kwaga,<br>Chairman, Supervisory Committee. | ---------- Signature | ---------- Date |
| Prof. J. Kabir,<br>Member, Supervisory Committee. | ---------- Signature | ---------- Date |
| Prof. E.C. Okolocha,<br>Head, Department of Veterinary Public Health and Preventive Medicine,<br>Ahmadu Bello University, Zaria. | ---------- Signature | ---------- Date |
| Prof. K. Bala<br>Dean, School of Postgraduate Studies,<br>Ahmadu Bello University, Zaria. | ---------- Signature | ---------- Date |

## DEDICATION

This piece of work is dedicated to Allah (SWA) for His infinite and everlasting mercy, compassion, love and favour throughout my life and to my parents Alhaji Hammanyero Bakari and Hajiya Khadija Abdullahi for having brought me up, for their continuous love and support. May Allah bless and have mercy on them.

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## ABSTRACT

Foetal wastages constitute an obstacle to livestock production and economic development in Kano State in particular and Nigeria in general. Foetal wastage may serve as source of leptospirosis to abattoir workers, the general public and animals. Leptospirosis is a serious economic and zoonotic disease of animals and humans. Retrospective and cross sectional studies were employed to determine the extent of foetal wastage and occurrence of *Leptospira* species in foetuses using MAT and ELISA from slaughtered pregnant cows in old Kano abattoir, Kano State, Nigeria. The findings revealed that 2.6 % of the 520,805 cows slaughtered in the abattoir during a 10-year period (2005-2014) were pregnant. The period 2010-2014 and the wet season (May to October) had significantly ($p < 0.05$) higher number of foetal wastage compared to 2005-2009 and the dry season (November to April) respectively. Five hundred and sixty one (5.2 %) cases of foetal wastage from 10, 769 slaughtered cows were recorded during the 2 months study period. Of these, 445 (79.3%) were in the second and third trimesters. The monetary losses of wasted foetuses per annum if they were allowed to be calved would be in the range of ₦13, 563, 000 to ₦16, 953,750 and ₦ 673, 200 to ₦ 8, 415, 000 for the 10 years and 2 months study period respectively. Of the 372 sera tested for antibodies using enzyme linked immunosorbent assay, 21 (5.6 %) were positive for *Leptospira* Hardjo antibodies. A seroprevalence rate of 9.5 %, 5.2 % and 0.0 % were obtained for foetuses at 3rd, 2nd and 1st trimester respectively. There was no significant difference ($p > 0.05$) in seropositivity between the three gestational stages. Out of 372 sera tested using microscopic agglutination test, 52 (13.98 %) were positive to one or more Leptospira serovars at serum dilution of 1:10. There was significant difference ($p < 0.05$) observed between age groups and *Leptospira* infection. Amongst the 52 positive samples, 14 (26.9 %), 4 (7.6 %), 2 (3.8 %) and 1 (1.9 %) reacted to Hardjo, Grippotyphosa, Canicola and

Pomona respectively at serum dilution of 1:20 or higher. This study indicates that foetal wastage in old Kano abattoir is on the increase and the presence of *Leptospira* species in these foetuses poses an occupational risk to butchers, abattoir workers, and carnivorous animals that handle or consume infected foetuses/placentas. Therefore, indiscriminate slaughter of pregnant cows in abattoirs should be prevented. This undesirable practice of wasting foetuses can be converted into a business investment sector thereby saving the enormous loss due to foetal wastage, consequently maintaining and even improving the national cattle population as well as creating employment for the citizens, food security for the nation, income earnings to the farmers and the economy as a whole.

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**ABBREVIATIONS AND SYMBOLS**

| | |
|---|---|
| AAGFS | Animal Agriculture And Global Food Supply |
| AFLP | Amplified Fragment Length Polymorphism |
| AHF | Andaman Haemorrhagic Fever |
| AP-PCR | Arbitrary Primed PCR |
| bp | Base Pair |
| BSA | Bovine Serum Albumin |
| CBN | Central Bank Of Nigeria |
| CD4 | Cluster Of Differentiation 4 |
| CD8 | Cluster Of Differentiation 8 |
| CDC | Center For Disease Control And Prevention |
| CMI | Cell Mediated Immunity |
| CSF | Cerebrospinal Fluid |
| DHS | Dihydrostreptomycin |
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EMJH | Ellinghausen Mccullough-Johnson Harris |
| ESAP | Ethiopian Society of Animal Producton |
| FAO | Food And Agricultural Organization |
| FAT | Fluorescent Antibody Test |
| FDL | Federal Department Of Livestock |
| FlaB | Flagellin B |
| GDP | Gross Domestic Product |
| GLP | Glycoprotein |
| HRP | Horse Radish Peroxidase |
| IBR | Infectious Bovine Rhinotracheitis |
| ICSB | International Committee On Systematic Bacteriology |
| IFNγ | Interferon Gamma |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |

| | |
|---|---|
| IL | Interleukin |
| ILS | International Leptospirosis Society |
| IS | Insertion Sequence |
| LBERG | Leptospirosis Burden Epidemiology Reference Group |
| LigB | Ligase B |
| LipL | Lipoprotein From *Leptospira* |
| LLS | Lipopolysaccharide- Like Substances |
| LPS | Lipopolysaccharides |
| MAT | Microscopic Agglutination Test |
| mL | Milliliter |
| MLST | Multilocus Sequence Typing |
| MLVA | Multiple-Locus Variable Number Tandem Repeat Analysis |
| Nm | Nano Meter |
| OIE | Office International Des Epizooties |
| OLRG | Organic Livestock Research Group |
| OmpL1 | *Leptospira* Outer Membrane Protein |
| OMPs | Outer Membrane Protein |
| PAGE | Polyacrylamide Gel Electrophoresis |
| PBS | Phosphate Buffered Saline |
| PCR | Polymerase Chain Reaction |
| PFGE | Pulsed-Field Gel Electrophoresis |
| pH | Power Of Hydrogen/Potential Of Hydrogen |
| RAPD | Random Amplified Polymorphic DNA Fingerprint Analysis |
| rRNA | Ribosomal Ribonucleic Acid |
| SPSS | Statistical Package For Social Sciences |
| TLR | Toll Like Receptors |
| TMB-E 3,3,5,5 | Tetramethylbenzidine |
| TSL | Taxonomic Subcommittee on Leptospira |
| µl | Microliter |
| VNTR | Variable Number of Tandem Repeats |

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background of the Study

Animal production is very paramount to food security and an essential component to the economic development of any nation. Livestock especially cattle are important as producers of meat and milk, which are part of the food chain and are the main sources of animal protein in Nigeria (Cheeke, 1998; AAGFS, 1999; Bamaiyi, 2013). Livestock serve as sources of flexible house hold income for millions of small holder farmers in Asia, Africa and Latin America (Haan *et al.,* 1998), foreign exchange for the economy and employment for the citizens. They are "cash at hand" and empower their owners with purchasing power (ESAP, 2002). Malnutrition is endemic in almost all developing countries as well as in Nigeria (FAO/WHO, 1983). In Nigeria, high population growth rate (Fasoyiro and Taiwo, 2012) , low economic standard of the citizens (Bamaiyi,2013), infectious diseases (Brisibe *et al.,* 1996; Bamaiyi,2012; Bhat *et al.,* 2012), low level of education of the farmers (Adebayo and Adeyola, 2005) and indiscriminate slaughtering of pregnant animals (Garba *et al.,* 1998; Abdulkadir *et al*., 2008; Muhammed *et al.*, 2009; Cadmus and Adesokan, 2010; Ardo *et al.,* 2013) are factors contributing to the shortage of animal proteins.

Foetal wastage is a major constraint to livestock production. It contributes to livestock shortage as well as protein malnutrition in developing countries. Factors contributing to foetal wastage include inadequate meat inspection practices leading to slaughtering of pregnant animals (Garba *et al.,* 1998; Abdulkadir *et a.,* 2008; Muhammed *et al.,* 2009; Cadmus and Adesokan, 2010; Ardo *et al.,* 2013), long dry season of the year in sub-Saharan Africa associated with forage scarcity

leads to increased sales of aged cows and less productive females to meet household cash needs (Toulmin, 1984; Alhaji, 2011). As dry season progresses and stress on cattle increases, herders are compelled to sell pregnant females before they die naturally (Peter, 2013); however, sales at this period hardly consider the pregnancy status and condition of the animals (Toulmin, 1984; Sanusi *et al.,* 2006; Muhammed *et al.,* 2009; Alhaji, 2011; Peter, 2013). Ignorance of the pregnancy status of animals at the time of sale, increased demand for financial resources for farming activities and abortifacient diseases also contribute to foetal wastage (Toulmin, 1984; Glatze, 2002; Alhaji, 2011; Sanusi *et al*., 2006; Muhammed *et al*., 2009; Peter, 2013). The recurrence of spontaneous abortion by pregnant heifers at the feedlots also contributes significantly to foetal wastage (Peter, 2013).

Foetal wastage may create health problems to humans handling it and subsequent consumption of the foetus by humans or animals could increase the public health concern of leptospirosis in the environment. Leptospirosis is caused by a spirochaete bacterium of the genus *Leptospira* and it is an economically important zoonotic bacterial infection of livestock that causes infertility, early embryonic death, abortion, stillbirth and delivery of weak newborn.

Humans become infected with leptospirosis directly through contact with foetuses, tissue and body fluids of infected animals (such as rodents, dogs, cattle and swine) that harbour the pathogen (*Leptospira* spp.), or may be indirectly infected through contaminated water (Heinemann *et al.,* 2000; Tansuphasiri *et al.,* 2006; Adler and de la pena Moctezuma, 2010).

Leptospirosis is caused by a spirochaete bacterium of the genus *Leptospira*. The disease affects fertility in cattle, sheep and goats, causing early embryonic death, abortion, stillbirth and delivery of weak newborn in cattle and pigs. Infection of susceptible cattle by *Leptospira interogans*

serovar Hardjo results in the localization of the organism in the renal tubules and, if the animal is pregnant at the time of infection, leptospires may also localize in the placenta; in some cases this is followed by foetal infection with consequent abortion or premature birth (Ellis and Michna, 1977; Ellis *et al.*, 1982a;1982b; 1986a; 1986c; Subharat *et al.*, 2010; Adler *et al.*, 2011).

### 1.2 Statement of Research Problem

Globally, Nigeria falls among countries with very low per person consumption of animal protein (FAO, 1971; 2006; Aliu *et al.*, 2001; Nwakpu and Osakwe, 2007; Alhaji, 2011). Animal protein in the diet of average Nigerian has been diminishing due to marginal improvement in animal population and productivity (Oyenuga, 1987). Ideally, sound economic livestock management, demand that animals sold for slaughter should be mainly males or reproductively inactive females (Opara *et al.*, 2006; Abdulkadir *et al.*, 2008; Cudworth, 2008; Riehn *et al.*, 2010). Admittedly, in various slaughterhouses in Nigeria, slaughtering of clinically healthy cows at different stages of gestation has been reported (Garba *et al.*, 1998; Abdulkadir *et al.*, 2008; Muhammed *et al.*, 2009; Cadmus and Adesokan, 2010; Addass, 2010; Ardo *et al.*, 2013). Slaughter of pregnant cows will no doubt worsen the already precarious supply of animal protein to the populace and also constitute a grave economic concern to livestock production and food security of the country (Abdullahi, 1985; WHO, 1999; Ahmed *et al.*, 2006; Sanusi *et al.*, 2006).

In recent years, leptospirosis has been identified as a global public health problem because of its role in increased morbidity and mortality in different countries (Radostits *et al*., 2007; Ahmed *et al*., 2006); and there is growing concern in regards to the under-recognized burden of leptospirosis in developing countries (WHO, 1999). Leptospirosis is an important cause of abortion, stillbirth, infertility, poor milk production and death in animals, all of which cause great economic losses (Ellis, 1984). Besides, the disease also constitutes a potential zoonotic threat to

humans exposed to infected cattle (Samina *et al.*, 1997). Swart *et al.* (1983) and Weinstein and Cameron (1991) in their reports have demonstrated a clear association between *Leptospira* infection and occupation.

In Nigeria, where meat workers do not wear adequate protective materials, they are easily exposed to urine, body fluids, foetuses and tissues of potentially infected animals, and are therefore at risk of being infected with any of the *Leptospira* species. This has serious consequences as the clinical manifestation of leptospirosis in humans produce signs similar to malaria and typhoid fever which are endemic to Nigeria (Ngbede *et al.*, 2012b). In addition, the foetuses recovered from slaughtered pregnant animals are often sold for human and pet consumption which may serve as source of the disease to humans and pets as these foetuses may be potential carriers of *Leptospira*.

### 1.3 Justification

Foetal wastage may constitute an obstacle to livestock production and economic development in Kano State in particular and Nigeria in general. Foetal wastage may also pose health risk to health workers, the public and pets in the highly populated Kano metropolis, as they may serve as source of leptospirosis. The information obtained on foetal wastage and its control in our abattoirs may contribute in no small measure to increasing the population of livestock in Kano State in particular and Nigeria in general. Information obtained in this study will help relevant authorities and stakeholders understand the menace of foetal wastage and how consumption of foetuses is promoting this undesirable practice in Nigeria and its impact on the country's economy. It will also contribute to the knowledge on the health risks it poses to abattoir workers as well as the public in Kano State. To the best of our knowledge, there is no data on the prevalence of leptospirosis in foetuses recovered from slaughtered pregnant animals in Nigeria.

Adequate investigation of leptospirosis and foetal wastage in old Kano abattoir, one of the largest abattoirs in Nigeria is necessary and imperative for effective and implementable control measures to be developed. Therefore, given the economic implication of foetal wastage to the economy and food security of our nation and the zoonotic importance of the leptospirosis, as well as the paucity of information on foetal wastage and leptospiral infection in wasted foetuses, there is a need for this study to fill the existing gap on the role of *Leptospira* infection and foetal wastage in old Kano abattoir, Kano state, Nigeria.

### 1.4 Aim

To determine the proportion of foetal wastage and occurrence of *Leptospira* species in foetuses from slaughtered pregnant cows in old Kano Abattoir, Kano State, Nigeria.

### 1.5 Objectives

The objectives of this study are to:

i. determine the occurrence of foetal wastage among slaughtered cows in old Kano abattoir, Kano State, Nigeria using passive and active data.
ii. estimate the economic losses due to foetal wastage in old Kano abattoir, Kano State, Nigeria.
iii. determine the proportion of wasted foetuses from slaughtered pregnant cows that may be infected with *Leptospira* species in old Kano State Abattoir using Enzyme Linked Immunosorbent Assay and Microscopic Agglutination Test.

## 1.6 Research Questions

i. Does foetal wastage occur among cows slaughtered at old Kano abattoir, Kano State?

ii. Does foetal wastage constitute an economic loss to livestock production in Kano State?

iii. Are wasted foetuses in old Kano abattoir infected with *Leptospira*?

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Agricultural Production in Nigeria

The role of agriculture in economic development of most countries can hardly be over emphasized (Timmer, 2003). The contribution of agricultural growth to overall poverty reduction has been documented (Sarris, 2004). Nigeria's rich human and natural resources endowment gives it the potential to become Africa's largest economy and a major player in the global economy (Nigerian National Planning Commission, 2004). However, the economic development in Nigeria has been disappointing. Agriculture, though largely under-developed, is the most important sector of the Nigeria economy after oil, of which animal production plays a very crucial part (Okereke, 1982; Lai and Felton, 1986; Anyanwu and Jukes, 1991).

A retrospective look into the Nigerian economy and its development reveals that agriculture was both the main stay of the Nigerian economy, providing employment and source of livelihood for the increasing population and the chief foreign exchange earner (Chigbu, 2005). In the 1960s, agriculture accounted for over 80 percent of the export earnings and employment; about 65 per cent of the Gross Domestic Product (GDP) and about 50 per cent of the government revenue (Balogun, 2000; Federal Republic of Nigeria, 2000; Olagunju, 2007). This contribution to the Nigerian economic growth has however declined over the years (Federal Republic of Nigeria, 2000; Aigbokhan, 2001; Balogun, 2000; CBN, 2003) due to the dominant role of crude oil sector in the economy and in the GDP aggregate share (Ingawa, 1979). Inspite of this, the sector still accounts for about 40% of GDP and provides employment, both formal and informal for about 60 per cent of Nigeria's 170 million people (Aigbokhan, 2001; Onyeahialam, 2002;

Olagunju,2007; Nigeria Agricultural Economy and Policy Report, 2009; Odoemelam, 2011; Population, 2012).

### 2.1.1 The bane of revitalizing the agricultural sector in Nigeria

To fully revitalize agricultural production in Nigeria, the Federal Government had established series of agricultural policies (Aigbokhan, 2001; Akande, 2006) but have not been implemented with resultant hunger and problem of food security has not been guaranteed (Obeta, 1990; Babatunde and Qaim, 2010; Agboola and Balciliar, 2012). The agricultural sector consists of sub-sectors namely, crops, livestock, fishery and forestry. It was discovered among the sub-sectors that crop had major contribution to the agricultural sector with livestock following closely. The contribution of agriculture to economic development lies in providing more food to the rapidly expanding population; increasing the demand for industrial products and thus necessitating the expansion of the secondary and tertiary sectors. To be effective, and attain higher level of productivity and growth in the agricultural sector there is a need to identify the major factors that determine its growth (Aigbokhan, 2001; Akande, 2006).

## 2.2 Economic Importance of Livestock Production

Livestock industry is an economic enterprise and can also be considered as a "survival enterprise" for millions of herd people throughout tropical Africa, especially in the arid, semi-arid, and subhumid areas. Livestock production is very essential to food security and the development of any nation. It provides foreign exchange for the economy, employment for the citizens, and rich source of protein which is essential for human nutrition for the populace. It is a source of prestige, it provides income for the citizens, provides draught power and transportation, it provides raw materials for the clothing and shoe industry and by-products that can be used in

industries such as the cosmetic industry amongst others (Bamaiyi, 2013). Livestock play very important role in Nigerian agriculture contributing about 12.7 percent of the Agricultural GDP (CBN, 1999). In monetary terms, the value of Nigerian livestock resources was conservatively estimated to be approximately six billion US Dollars (Akande *et al.*, 2011).

### 2.2.1 Constraints of livestock production

Livestock production is faced with a number of constraints, which in the long run results in low productivity and reduced profitability. Animal production trends in Nigeria are said to be influenced by factors such as population growth, (Fasoyiro and Taiwo, 2012), urbanization, lower economic standard of the citizens (Bamaiyi, 2013), infectious diseases (Brisibe *et al.*, 1996; Bamaiyi, 2012; Bath *et al*., 2012), level of education of the farmer (Adebayo and Ademola, 2005), season (Toulmin, 1984; Alhaji, 2011; Glatze, 2002) and indiscriminate slaughtering of pregnant animals (Garba *et al.,* 1998; Abdulkadir *et al.*, 2008; Muhammed *et al.*, 2009; Cadmus and Adesokan, 2010; Ardo *et al.,* 2013).

### 2.2.2 Livestock production and food security in Nigeria

Nigeria is the most populous country in Africa with an estimated population of over 170 million as at 2012 (Population, 2012) with over 70% of the population living on less than one dollar a day (Watts, 2006). By the year 2050 the projected population will be 402 million, making Nigeria the fourth most populous country on earth after india, China and the United States (Population, 2012). With a fast growing population, Nigeria is threatened with the problem of food insecurity and poverty which can be addressed with a more developed animal production system in addition to other sectors (Fasoyiro and Taiwo, 2012). The average Nigerian still consumes far less animal protein than his counterpart in the developed world (Aliu *et al*., 2001).

This has been attributed to the infancy of livestock industry, its hydra-headed problems and its low per capita income which leads to consumption of less than 9 grams of animal protein per capital per day as compare to over 50grams per capital per day in North America and Europe (Grigg, 1995; Niang and Jubrin, 2001; Boland *et al.,* 2013).

### 2.2.3 Importance of livestock diseases to economic development

Animal diseases constitute a major constraint to livestock production as well as the economic development. In addition, animal diseases pose health risk to the human population of tropical Africa. Implications of reduced livestock productivity and profitability include; inadequate consumption of protein of animal origin, poverty, unemployment, low contribution to the Nation's GDP among others.

## 2.3 Cattle Production and its Limitation in Nigeria

Nigeria is one of the leading countries in cattle production in sub-Saharan Africa (Ikpi, 1990). Cattle and beef trade provides the largest market in Nigeria with millions of Nigerians making their livelihood from various beef-related enterprises (Umar *et al*., 2008). Cattle are ruminant animals marketed in Nigeria for their meat, milk as well as for hide and skin among other purposes. Of the Nigerian livestock population, cattle contributed about 10 percent, while in monetary terms they are accounted for about 40 percent of livestock total revenue of the country (Mclntyre *et al.,* 1992). However, the supply of beef and milk has failed to keep pace with increasing population. As a result of this, widespread hunger and malnutrition are evident in the country. This calls for the adoption of all possible measures to accelerate the rate of cattle production in the country.

## 2.4 Foetal Wastage

More recently, foetal wastages through the slaughter of pregnant animals has been associated with production problems in Nigeria (Alemede, 2004; Du-Sai *et al.*, 2005; Umar *et al.*, 2006; Nwakpu and Osakwe, 2007; Muhammad *et al.*, 2009; Ngbede *et al*., 2012a; Ardo *et al*., 2013; Oduguwa *et al*., 2013; Bakari *et al*., 2015). Literature has shown that the wastage of the conceptus through haphazard slaughter of pregnant livestock is one of the practices man has ever used against his production endeavour (Garba *et al.,* 1998; Umar *et al.,* 2006; Bello *et al.,* 2008). This has been observed as a key factor responsible for protein malnutrition in some African countries and a possible constraint to future livestock populations on the continent (Nwakpu and Osakwe 2007; Ademola. 2010; Cadmus and Adesokan, 2010).

The Food and Agricultural Organization (FAO) recommended a minimum of 35g/head/day of animal protein for good health in human population world over. However, in Nigeria, this is far beyond reach because the actual average protein consumption is 4.5g/head/day (Niang and Jubrin, 2001; Atsu, 2002). Today, there exists a wide disparity between the FAOs' recommended quantity of animal protein intake and what is consumed in Nigeria. This deficiency may be attributed to several factors that account for inadequate supply of meat in Nigeria (Alade *et al.,* 2011).

### 2.4.1 Economic implications of foetal wastage

The increasing human population is an indication that more cattle for the purpose of meat will be needed, but the destruction of young cattle in the form of foetal wastage due to slaughter of pregnant cows is a great impediment to achieving the much needed increase. Foetal wastage has been reported to account for about 20-25 % of the fall in livestock production in sub-Saharan

Africa (Chaudhari and Paul-Bokko, 2000). Despite reports from different parts of the country on the magnitude of foetal wastage, the problem is yet to receive a meaningful attention from the agency responsible (Idahor *et al.*, 2009; Ibironke, 2010; Addass *et al.*, 2010; Adama *et al.*, 2011; Alhaji, 2011; Cadmus and Adesokan, 2010). Therefore, Nigeria with a cattle population of about 15 million and a growth rate of 0.8 % needs to urgently control this practice which is producing a negative effect on its livestock production sector (Ahemen and Zahradden, 2010).

## 2.5 Leptospirosis

Leptospirosis is a zoonotic bacterial disease with a worldwide distribution, and is an emerging and re-emerging infectious disease in animals and humans (Christopher *et al*., 2011). It is endemic in many countries globally. Both saprophytic and pathogenic species presently exist in nature, but saprophytic species (*Leptospira biflexa),* live in water and soil and do not infect animals (Christopher *et al*., 2011; Skyes, 2011).

### 2.5.1 Historical perspective of leptospirosis

The earliest recognised accounts of leptospirosis were descriptions of severe illness with jaundice and renal involvement in man published by Landouzy (1883), Weil (1886), and Vasiliev (1888). These were clinically distinct from other icteric and nephritic illnesses, but the cause was not determined (Turner, 1966). In 1907, Stimson demonstrated by silver staining the presence of clumps of spirochetes in the kidney tubules of a patient who reportedly died of yellow fever. The spirochetes had hooked ends, and Stimson named them *Spirochaeta interrogans* because of their resemblance to a question mark. Unfortunately, this sentinel observation was overlooked for many years (Faine, 1994).

The etiology of leptospirosis was demonstrated independently in 1915 by Inada and Ido in Japan and by 2 groups of German physicians (Everard, 1996). Soon after the breakthrough that Weil's disease was caused by leptospires several other disease entities were being recognized to have leptospiral aetiology. These include 'nanukayami'or the Japanese seven-day fever, akiyami' the harvest fever and Andaman fever or Andaman haemorrhagic fever (AHF) (Vijayachari *et al.*, 2008).

The same Japanese group that identified leptospires illustrated the zoonotic nature of the disease and rats as vectors. Subsequently several wild and domestic animals were identified as carriers (Faine, 1994). The strain Ictero No. 1 of serovar Icterohaemorrhagiae (Spirochaeta *Icterohaemorrhagiae japonica*) was the first isolate of *Leptospira*, which was recovered by Inada and Ido in 1915 from a patient suffering from Weil's disease. Since then about 268 pathogenic serovars have been described and each one has reference strains (Vijayachari *et al.*, 2008).

The role of the rat as a source of human infection was discovered in 1917 (Ido *et al*., 1917), while, leptospirosis in livestock was recognized some years later (Alston and Broom, 1958). The first report of bovine leptospirosis was published in the former USSR in 1935, where *Leptospira interrogans* Serovar Grippotyphosa was isolated from calves with acute infection (Amatredjo and Campbell, 1975). The first isolation of *L.* Hardjo from cattle was made by Roth and Galton (1960) in America. In October 2010 British rower Andy Holmes died after contracting Weil's disease (Leggat, 2010). His death has raised awareness of the disease among the public and medical professionals (Forbes *et al*., 2012).

## 2.6 Biology, taxonomy, classification and genome of *Leptospira*

### 2.6.1 Biology of *Leptospira* organisms

Leptospires are corkscrew-shaped bacteria, which differ from other spirochaetes by the presence of end hooks. Leptospires are motile, about 0.1 μm in diameter by 6–20 μm in length (Faine *et al*., 1999) requiring the use of dark field microscopy or phase contrast for observation. Morphologically all leptospires are indistinguishable, but the morphology of individual isolates varies with *in vitro* subculture and can be restored by passage in hamsters (Ellis *et al*., 1983). These bacteria are obligate aerobic, with an optimum growth temperature of 28 to 30°C in media containing vitamins, bovine serum albumin and rabbit serum. They produce both catalase and oxidase (Smibert, 1977), they do not resist drought or hypertonicity, however they support alkalinization to pH 7.8. Motility in leptospires is a function of the FlaA and FlaB periplasmic flagella or endoflagella proteins which arise from each end of the bacterium. Leptospires have a typical double membrane structure in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlaid by an outer membrane (Ko *et al*., 2009). Within the outer membrane, the LPS constitute the main antigen for *Leptospira*. It is structurally and immunologically similar to LPS from Gram negative organisms, but has lower endotoxic activity (Shimizu *et al.*, 1987). Their visualization is achieved after metal impregnation (silver staining) or after artificial thickening by immunoperoxidase or immunofluorescence (Andre-Fontaine1992). Double membrane constitution of *Leptospira* supports Gram-negative bacteria whereas the close association of the cytoplasmic membrane with murein cell wall is reminiscent of Gram-positive envelope architecture (Haake, 2000; Levett, 2001; Vijayachari *et al.*, 2008; Ko *et al*., 2009). Thus this bacterium is susceptible to the antibiotics which are used for both Gram-

negative as well as Gram positive bacteria. The bacteria are sensitive to wide range of antibiotics except chloramphenical.

### 2.6.2 Taxonomy of *Leptospira*

The genus *Leptospira* belongs to division Gracillicutes, class Scotobacteria, order Spirochaetales and family, *Leptospiraceae*.

### 2.6.3 Classification of *Leptospira*

The classification and nomenclature of *Leptospira* is complex. Presently two different classification systems – one based on phenotypic characters and the other on the genetic homology are being used.

#### *2.6.3.1 Serological classification of Leptospira*

The genus *Leptospira* is divided into two species based on the phenotypic characteristics, namely; *L. interrogans* (pathogenic) and the *L. biflexa* (non-pathogenic). Each species is divided into numerous serovars defined by agglutination after cross-absorption with homologous antigen (Dikken and Kmety, 1978; Johnson and Faine 1984; Kmety and Dikken, 1993). There are more than 300 distinct leptospiral serovars recognised and these are arranged in 25 serogroups (Picardeau, 2013). Serovars that show 10% cross-agglutination (antigenically related) are collected into a serogroup (Faine, 1994). Certain serovars have preferences for particular animal host species and usually replicate relatively asymptomatically in the renal tubules of these preferred animal hosts (WHO, 2003). These animals then become reservoirs for the leptospires; however, leptospires can readily adapt to new host species (WHO, 2003; Green-McKenzie, 2005).

*2.6.3.2 Genotypic classification of Leptospira*

The phenotypic classification of leptospires using serology is a difficult and time consuming procedure. Therefore, the molecular biology techniques were introduced into the field of leptospirosis (Yasuda *et al*., 1987; Ramadass *et al*., 1992). The genomospecies of *Leptospira* do not correspond to the previous two species (*L. interrogans* and *L. biflexa*), and indeed, pathogenic and nonpathogenic serovars occur within the same species. Speciation is based on DNA homology as determined by DNA–DNA hybridization and the guanine-plus-cytosine mol percentages (G+C mol %) content of DNA, but because of technical difficulties, PCR-based methods are commonly used instead (Ahmed *et al.*, 2012a; 2012b). Woodward and Redstone (1994) suggested the PCR combined with restriction fragment length polymorphism as a useful tool for rapid detection and preliminary differentiation of leptospires.

Restriction fragment length polymorphism PCR can amplify the FlaB gene of 23 serovars of the genus *Leptospira*. Each enzyme is said to yield different restriction fragments which can be used for classifying the leptospiral isolates. The genus *Leptospira* now consists of 20 species and includes nine pathogenic, five intermediate and six saprophytic species (Picardaeu, 2013) .The majority of pathogenic serovars are found in the three species with a global distribution of *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri*. The other pathogenic species are *L. alexanderi*, *L. alstonii*, *L. kmetyi*, *L. noguchi*, *L. santarosai* and *L. weilii*. However, according to the Taxonomic Subcommittee on Leptospira (TSL) of the International Committee on Systematic Bacteriology (ICSB), the "official" Taxonomic scheme which is accepted for leptospires is based on serology (Faine, 1994).

### 2.6.4 *Leptospira* genome

A complete sequencing data is now available for the two leptopires (serovar Lai and serovar Copenhegeni). Leptospiral genome has two chromosomes; large chromosome (CI) and small chromosome (CII). The size of the large chromosome ranges from 4,332,241 bp to 4,277,185 bp whereas the size of the small chromosome is in the range of 358,943 bp to 350, 181 bp (Zuerner, 1991). Leptospires contain two sets of 16S and 23S rRNA genes but only one 5S rRNA gene (Fukunaga *et al*., 1989a), and the rRNA genes are widely spaced (Fukunaga *et al*., 1989b; Baril *et al*., 1992). Several repetitive elements have been identified (Zuerner and Bolin, 1988; Woodward and Sullivan, 1991; Boursaux-Eude *et al.,* 1995; Kalambaheti *et al*., 1999; Takahashi *et al*., 1999) of which several are insertion sequences (IS) coding for transposases. IS*1533* has a single open reading frame (Zuerner, 1994), while IS*1500* has four (Boursaux-Eude *et al.*, 1995). Both IS*1500* and IS*1533* are found in many serovars (Zuerner and Bolin, 1990; Boursaux-Eude *et al.*, 1995), but the copy number varies widely between different serovars and among isolates of the same serovar (Boursaux-Eude *et al.,* 1998). Within serovar Icterohaemorrhagiae, the genome appears to be conserved (Herrmann *et al*., 1991; Takahashi *et al*., 1998). This conservation allowed the identification of at least one new serovar by recognition of distinct pulsed-field gel electrophoresis (PFGE) profiles (Herrmann *et al*., 1994).

## 2.7 Epidemiology of Leptospirosis

### 2.7.1 Geographical distribution

Leptospirosis occurs throughout the world and is considered the most widespread zoonosis (WHO, 1999). However, only a few serovars are enzootic in any particular region. The incidence is significantly higher in warm climate countries than in temperate regions (Everard and Everard,

1993; Ratnam, 1994). The magnitude of the disease in tropical and sub-tropical region is largely attributed to climatic and environmental conditions, in addition to the likelihood of contact with a *Leptospira*-contaminated environment caused by local agricultural practices, poor housing and waste disposal. The risk of leptospirosis can become further widespread during rainy seasons in spring and summer. During periods of flooding, leptospirosis may cause severe outbreaks among individuals exposed to the leptospiral contaminated waters (WHO, 2001).

### 2.7.2 Global burden of leptospirosis

The exact global disease burden of lepstospirosis remains unknown due to poor surveillance and difficult diagnosis (Ko *et al.*, 1999), but estimates by the Leptospirosis Burden Epidemiology Reference Group (LBERG) at the World Health Organization have set the number of human cases of severe leptospirosis to over 500,000 per year (Abela-Ridder *et al*., 2010). Human leptospirosis has a wide geographic distribution, with Southeast Asia, Oceania, the Indian subcontinent, Caribbean and Latin America considered as highly endemic to the disease (Pappas *et al*., 2008). In these regions, climatic changes and flooding, poor level of sanitation and high populations of maintenance hosts (e.g., rats) are important determinants of infection. The annual incidence in these areas is 10–100 per 100,000, and this rate rises during outbreaks and in high risk populations (Victoriano *et al.*, 2009).

### 2.7.3 Epidemiologic analysis of leptospirosis

Powerful molecular techniques such as multilocus sequence typing (MLST) and multiple-locus variable number tandem repeat analysis (MLVA) have been applied to the epidemiologic analysis of leptospirosis, but have yet to be widely used (Palmer and Zochowski, 2000; Galloway and Levett, 2008; Ahmed *et al.,* 2006; Salaun *et al.,* 2006; Slack *et al.,* 2006; Thaipadungpanit *et al.,* 2007).

### 2.7.4 Prevalence of leptospirosis in Nigeria

Bovine leptospirosis is endemic in Nigeria (Agunloye *et al.,* 2000). Previous studies have shown high prevalence of leptospirosis in man and animals in different parts of the country (Diallo, 1978; Diallo and Dennis, 1982; Ezeh and Agba, 1982; Ezeh *et al*., 1991; Onyemlukwe,1993; Agunloye *et al*., 1996; 2000; 2002; Ngbede *et al.*, 2012b). The most common serovars reported in Nigeria are: *Leptospira interrogans* serovars Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, and Ballum (Agunloye *et al*., 2002). The following *Leptospira* serovars have been reported in humans in Nigeria: *L.* Icterohaemorrhagiae, Pomoma, and Hardjo (Onyemelukwe, 1993). Infection has been demonstrated in coal miners, butchers and abattoir workers, local farmers and hospital laboratory personnel from various communities in Enugu (Onyemelukwe, 1993). It is widely recognized that the incidence of leptospirosis is remarkably underestimated and the disease underdiagnosed in endemic regions. Despite these studies, the disease is believed to be underestimated in Nigeria with an incidence of six human cases reported annually (CureResearch, 2011). No Surveys on leptospirosis as a cause of febrile illness have been conducted in Nigeria; however, a recent case report from Plateau State proved it to be a diagnosis to consider in febrile, icteric illness (Isa *et al*., 2014) but at present, it is not considered in the differential diagnosis of leptospirosis-like illnesses in both humans and animals in Nigeria (Agunloye *et al.,* 2001). Field reports in Nigeria indicate that abortion is a common feature among livestock but cases are not always subjected to detailed laboratory investigation (Oie *et al*., 1986). Incidence rates of leptospirosis maybe underestimated and underreported due to lack of awareness of the disease, relatively inaccessible rapid diagnostic methods (Bharti *et al.,* 2003) and due to lack of clinical suspicion (Cachay and Vinetz, 2005) by veterinarians and

physicians. A recent risk analysis among abattoir workers revealed low levels of awareness and high Levels of risk-of-exposure-prone practices (Ngbede, *et al*., 2012c).

### 2.8 Host Reservoirs of *Leptospira*

Distinct variations in maintenance hosts and the serovars they carry occur throughout the world (Hartskeerl and Terpstra, 1996). The knowledge of the prevalent serovars and their maintenance hosts is essential to understanding the epidemiology of the disease in any region. Animals may be maintenance hosts of some serovars but incidental hosts of others, infection with which may cause severe or fatal disease (Bharti *et al*., 2003). Most often, serovars present in a particular region are associated with one or more animals that inhabit the area and act as maintainance host (Levett, 2001). An animal infected with a host-adapted serovar of the organism, is a 'maintenance' or 'reservoir' host. Exposure of susceptible animals to non host-adapted serovars results in accidental or incidental disease. Each serovar is adapted to a particular maintenance host, although they may cause disease in any mammalian species (Bharti *et al.,* 2003). A serovar behaves differently within its maintenance host species than it does in others, incidental or accidental hosts.

A maintenance host is defined as a species in which infection is endemic and is usually transferred from animal to animal by direct contact. A maintenance host is characterized by high susceptibility to infection, endemic transmission within the host species, relatively low pathogenicity of the organism to the host, a low antibody response to infection, with difficulties in diagnosis, a tendency of the organism to cause chronic rather than acute disease, producing insidious economic loss through, reproductive losses, persistence of the serovar in the kidney and sometimes the genital tract (Little, 1986; Radostits, 2007).

Some common serovars and their maintainance hosts include: Serovar Hardjo-bovis in cattle (North America), Hardjo-prajitno in cattle and pigs (Europe), Bratislava in horses, Pomona (genotype kennewicki) in pig, skunk, racoon, opossum, Grippotyphosa in racoon, opossum, squirrel and Icterohemorrhagiae in brown rats, Canicola in dogs (Hathaway, 1981; Ellis, 1992; Faine et al., 1999). Some common leptospiral serovars and their accidental hosts are as follows: Hardjo in sheep and man, Pomona in sheep and cattle, Grippotyphosa in sheep and cattle Icterohemorrhagiae in cattle and pigs.

## 2.9 Method of Transmission of *Leptospira*

Infection of susceptible host by *Leptospira interogans* results in localization of the organism in the renal tubules and if the animal is pregnant at the time of infection, leptospires may also localize in the placenta; in some cases, this is followed by foetal infection with subsequent abortion or premature birth (Babudieri and D'Amore, 1958; Ellis and Michna, 1977; Ellis *et al*., 1982a;1982b; Ellis *et al*., 1986a). Infection of animals and humans can occur directly from exposure to infective tissues, body fluids or urine of acutely infected or asymptomatic carrier animals, or indirectly from environmental leptospires, originating in the urine of carrier animals (Faine 1994). The extent to which infection is transmitted depends on many factors, including climate, population density, and the degree of contact between maintenance and accidental hosts (Levett, 2001). Damp alkaline soil, vegetation, and mud with a temperature >22°C promote leptospires survival and increase the likelihood of leptospiral transmission (Green-McKenzie, 2005).

### 2.9.1 Direct transmission of *Leptospira*

Direct transmission refers to the immediate transfer of *Leptospira* species from a reservoir host to a susceptible host. Direct transmission among animals can be transplacental (Ellis *et al* 1978; 1985; 1986c), haematogenous (Murphy and Jensen, 1969; Ellis and Michna, 1977), by sexual contact (Brown *et al.*, 2003; Vijayachari *et al.*, 2008) and vaginal fluid of infected animal (Lilenbaum *et al.*,2008a) or by suckling milk from infected mother (Bolin and Koellner, 1988; Lilenbaum *et al.*,2008b). Direct transmission from animals to humans is common amongst the occupational groups who handle animals and animal tissue such as butchers, veterinarians, cattle and pig farmers, rodent control workers**.** Human-to-human transmission through breast-feeding has been recorded (Bolin and Koellner, 1988).

### 2.9.2 Indirect transmission

Indirect transmission refers to transfer of the organism through the environment, animate-vector or inanimate-vehicle intermediaries, from a reservoir to a susceptible host. It can occur through the ingestion of feed or water contaminated with urine, faeces, fluid from aborted or stillborn foetuses, and vaginal discharges after parturition (CDC, 2003; Corney, 2003; OIE, 2008). Risk factors such as poor sewage disposal, stagnant water, dumping of waste in bodies of water and recreational activities in water, predisposes to contracting infection with zoonotic species of *Leptospira* (Onyemelukwe, 1993). High risk groups include abattoir workers, farmers, veterinarians, rodent control workers, international travelers and other people swimming in contaminated water, and any other occupations that bring people in contact with animals (Onyemelukwe, 1993; Levett, 2001; Cachay and Vinetz, 2005).

## 2.10 Virulence Factors Associated with *Leptospira*

Pathogens possess a number of virulence factors, and delineating and understanding the function of these determinants is paramount to understanding the pathogenesis of the diseases they cause (Jason *et al.*, 2014). The mechanisms by which pathogenic *Leptospira* cause disease remain largely unknown (Evangelista and Coburn, 2010). Targeted gene knockouts still remain out of grasp for most *Leptospira* researchers and no replicative plasmid vector is available for pathogenic *Leptospira* (Jason *et al.*, 2014). The known leptospiral virulence factors have been extensively reviewed (Ko, *et al.*, 2009; Adler, *et al.*, 2011; Narayanavari, *et al*., 2012) and include lipopolysaccharides (LPS), flagella, hemolysin, outer membrane protein (OMPs) and other surface proteins as well as adhesion molecules (Ko, *et al.*, 2009; Adler *et al.*, 2011; Narayanavari *et al*., 2012). In addition, hemolysins and sphingomyelinases may play a role during infection, although there are conflicting reports regarding their true contributions to overall virulence (Narayanavari, *et al*., 2012).

### 2.10.1 Pathogenesis of Leptospirosis

Leptospires enter into the body of a susceptible host through mucous membrane or abraded skin. After 4 to 10 days, the host becomes bacteraemic, this period lasting from hours to 7 days, and may be characterised by pyrexia, leptospires in the milk and anorexia. However, clinical symptoms will depend on the susceptibility of the host, the serotype of *Leptospira* and the virulence of the organisms (Thiermann, 1984). After the number of the leptospires in the blood and tissues reach a critical level, lesions due to the action of leptospiral toxin and consequent symptoms appear (Faine, 1994). Capillary damage is common to all serotypes and during the septicaemic phase, petechial haemorrhages in mucosae are a common expression of this (Radostits *et al*., 2007). Immunity appears to be solely humoural with circulating antibodies opsonising leptospires, causing cessation

of bacteraemia. With the appearance of circulating antibodies, leptospires localise and persist in a number of organs, especially in the proximal renal tubes and in the female genital tract (Ellis and Michna, 1977; Thiermann, 1982; Ellis *et al*., 1982a; 1982b; 1986a). The duration of such localisation and urinary shedding will depend on host-serovar adaptation. When leptospires are infecting a definite host, infection of these organs will be long-term, perhaps even for life. In the case of accidental host infections, colonisation is short-term and urinary shedding may not occur (Thiermann, 1984; Ellis, 1984). In the case of *L.* Pomona infection, intravascular haemolysis and interstitial nephritis are important parts of the disease, whereas *L.* Hardjo produces no haemolysin and causes no interstitial nephritis (Hathaway and Marshall, 1980; Radostits *et al*., 1994). *Leptospira* Hardjo is capable of growing in the pregnant and non-pregnant uterus and lactating mammary gland so that, it produces septicaemia and then mastitis and/or abortion (Thiermann, 1982; Ellis *et al*., 1986a; 1986b).

## 2.11 Clinical Manifestations of Leptospirosis in Animals

Leptospirosis in cattle may appear as acute, subacute or chronic forms and is usually associated with Pomona or Hardjo (Radostits, 2007). The number of animals clinically affected in a cattle herd depends on the host-serovar adaptation, and the susceptibility of the herd to the infecting serovar. In all animals the incubation period is from 3 to 7 days (Thiermann, 1984).

### 2.11.1 Clinical manifestations associated with *Leptospira* Pomona

Calves up to 1 month old are most susceptible to acute leptospirosis. Acute leptospirosis in cattle is characterised by one or more symptoms of fever, haemolytic anaemia, haemoglobinuria, hepatitis, jaundice, interstitial nephritis, meningitis, and agalactia. The case-fatality rate is high and if recovery occurs, convalescence is prolonged. In adult cattle, abortion due to the systemic

reaction may occur at the acute stage of the disease (Radostits, 2007). Subacute leptospirosis differs from the acute form only in degrees. Nearly the same signs are observed in a number of affected animals but not all of the signs necessarily being present in one animal. Abortion usually occurs 3-4 weeks later after infection. One of the characteristic findings is the marked drop in milk production and the appearance of blood-stained or yellow-orange, thick milk in all four quarters without apparent physical change in the udder. Chronic leptospirosis is mild and may be restricted to abortion. Severe 'storms' of abortions occur most commonly in groups of cattle which are at the same stage of pregnancy when they are exposed to infection. The abortion usually occurs during the last trimester of pregnancy (Ellis *et al*., 1986b). Beside abortion, there is no depression of reproductive efficiency in cattle affected by leptospirosis. Many animals in the group develop positive agglutination titers without clinical illness. Animals which have recovered from acute leptospirosis may develop a carrier condition in which leptospires grow and remain in the renal tubes for periods of days to years. These excretory animals are the central points of distribution of leptospires to other animals or people (Faine, 1994). However, *L.* Hardjo can also persist in other organs, notably the genital tract of cattle (Ellis *et al*., 1986a; 1986b).

### 2.11.2 Clinical manifestations associated with *Leptospira* Hardjo

In *L.* Hardjo infection, there is a sudden onset of fever, anorexia, immobility and agalactia. The milk is yellow to orange and may contain clots. The udder is flabby, has no heat or pain, and all four quarters are equally affected (Durfee and Allen, 1980; Tripathy *et al*., 1985a; 1985b). This may affect up to 50 % of cows at one time, causing a precipitate fall in the herd's milk yield (Higgins *et al*., 1980). Abortion may occur several weeks later, but may also occur as the only evidence of the disease (Ellis *et al*., 1985b). In some areas or circumstances it is the principal clinical manifestation of leptospirosis due to *L.* Hardjo, and the principal cause of abortion in cattle

(Sullivan, 1970a; Sullivan, 1970b). However, as natural immunity develops in adult cows, only heifers become newly infected, and the only sign is abortion. It is also possible that many cows in a herd show subclinical infections with *L.* Hardjo in which only drop in milk yield may be detectable (Radostits *et al*., 2007).

### 2.11.3 Abortion in leptospirosis infection

Following systemic invasion by leptospires, abortion may occur due to foetal death, with or without placental degeneration. Abortion usually occurs several weeks after septicaemia because of the time required to produce the changes in the foetus, which is usually autolyzed at birth. Abortion occurs most commonly in the second half of pregnancy, due probably to the greater ease of invasion of the placenta at this stage, but may occur at any time from four months on (Radostits, 2007). Although abortion occurs commonly in both cattle and horses after either the acute or the subacute form of the disease, abortion without prior clinical illness is also common. This is particularly the case in sows and occurs to a less extent in cows and mares; this may be due to degenerative changes in the placental epithelium. Leptospires are rarely present in the aborted foetuses, however if the aborted foetus has survived the infection long enough to produce antibodies, these may be detectable. Experimental infection of serologically negative pregnant cattle with a north Queensland strain of *L. borgpetersenii* serovar Hardjo resulted in seroconversion and shedding of the organism in the urine (Smith *et a1*., 1997). Elective caesarean sections were done 6 weeks after challenge. No evidence of *L.* Hardjo infection of the foetuses occurred. Some of the foetuses had histopathological lesions consistent with *Neospora* spp. infection.

## 2.12 Differential Clinical Diagnosis

The acute and subacute forms of leptospirosis in cattle need to be differentiated from diseases causing haemolytic anemia with or without haemoglobinuria. They include babesiosis, anaplasmosis, rape and kale poisoning, postparturient haemoglobinuria, bacillary haemoglobinuria, and the acute haemolytic anaemia which occurs in calves after drinking large quantities of water (Radostits *et al.,* 2007). Chronic leptospirosis causing abortion must be differentiated from all other causes of abortion in cattle such as infectious bovine rhinotracheitis; protozoal abortion (*Sarcocytis* spp., *Toxoplasma gondii* and *Neospora caninum*). Less common causes are brucellosis, bovine virus diarrhea, pine needle abortion, mycotic placentitis, *Campylobacter*, *Actinomyces pyogenes*, *Ureaplasma* (Radostits, 2007). Leptospiral agalactia needs to be differentiated from *Mycoplasma bovis* mastitis, tick-born fever, sudden water deprivation, acute infectious bovine rhinotracheitis (IBR) and cold cow syndrome (Ellis, 1986b).

## 2.13 Host Immune Responses to *Leptospira* Species

It was previously thought that protective immunity against leptospirosis was sufficiently provided by antibodies (Faine *et al.,* 1999) since antibodies against leptospiral lipopolysaccharides (LPS) give passive protection in some animal models protecting against a number of strains and species of *Leptospira* (Masuzawa *et el*., 1990; Naiman *et al.,* 2001). However, studies have shown that cell mediated immunity has a role in protection from leptospirosis (Bolin *et al*., 1989; 1991; Ellis *et al*., 2000; Naiman et *al*., 2001; Brown *et al*., 2003; Chassin *et al*., 2009)

### 2.13.1 Humoral response

The mechanism of resistance to leptospirosis is mediated largely by the humoral response (Adler and Faine, 1977; Levett, 2001; Ko, *et al.*, 2009). Similar to other Gram-negative bacteria, the antibodies produced during leptospiral infection were found to be agglutinating (Jost *et al.*, 1986). The antibodies produced are mainly directed against the leptospiral LPS and some outer membrane proteins (OMP) (Faine *et al*., 1999; Adler and de la Pena- Moctezuma, 2004). Leptospiral OMPs OmpL1 and LipL41 were protective immunogens when used in combination (Haake *et al*., 1999). Other surface-exposed proteins such as LigB have potential immunoprotective properties, but testing of these has been limited to only a few (Yan *et al*., 2009).

The first serological response with *L.* Hardjo infection is the production of immunoglobulin M (IgM) antibodies (Adler *et al*., 1982; Cousins *et al*., 1985; Raostits *et al*., 1994). These rise rapidly but commonly decline to undetectable concentrations by 4 weeks after infection. Within 1-2 weeks of infection, $IgG_1$ antibodies appear, and at 3 months they represent 80% of antibodies detected in the microscopic agglutination test (MAT) (Raostits *et al*., 2007). The MAT titer peaks 11-21 d after infection but may vary from a titre of 3200 to an undetectable concentration. The MAT titer declines gradually over 11 months but the persistence is variable. IgM will retard bacterial growth, but will not kill or clear all the organisms. IgG causes lysis of circulating leptospires by complement activation, or by mediated phagocytosis in the macrophages and granulocytes (Naiman *et al*., 2002; Cinco, 2010).

### 2.13.2 Cell mediated immunity

Cattle vaccinated against serovar Hardjo with pentavalent vaccines were found vulnerable to infection with serovar Hardjo despite the presence of high titers of anti-LPS antibody (Bolin *et al*., 1989; 1991). Moreover, recently developed vaccines that protect against *L. borgpetersenii* serovar Hardjo including renal colonization and urinary shedding (Bolin and Alt, 2001; Naiman *et al*., 2001) and protect against transplacental infection of the foetus induce a type 1, or cell-mediated immune response (Ellis *et al*., 2000; Naiman *et al*., 2001). Pheripheral blood mononuclear cells (PBMCs) from cattle immunized with killed *L. borgpetersenii* serovar Hardjo -vaccine proliferated and produced IFNγ after *in vitro* stimulation with *Leptospira* antigens (Naiman *et al*., 2001). CD4+ cells were the main source of IFNγ but CD8+ also produced it (Brown *et al*., 2003).

The presence of both TLR2 and TLR4 are necessary for an effective innate immune defense against *Leptospira* (Werts *et al*., 2001; Nahori *et al*., 2005; Viriyakosol *et al.*, 2006; Chassin *et al*., 2009). The presence of TLR4 was shown to be necessary for early production of IgM by B cells, in response to leptospire LPS (Chassin *et al*., 2009). The absence of TLR4 in mice infected with *L. interrogans* serovar Icterohaemorrhagiae has been shown to lead to severe leptospirosis with clinical syndromes of jaundice and pulmonary hemorrhage, as well as accumulation of leptospires in kidneys and lungs (Viriyakosol *et al.,* 2006). Pheripheral blood mononuclear cells from non vaccinated cattle also responded to *Leptospira* antigens but the response was lower than that of the vaccinated animals (Naiman *et al*., 2002). While natural infection may induce a type 1 response, it is too weak to prevent establishment of chronic infection (Naiman *et al*., 2002).

## 2.14 Calf Immunity

Antibodies are natural proteins in the body, and their structure is thought to be safe for transfer to the foetus (Englund *et al*., 2003). Antibody transfer from mother to foetus is directly influenced by the type of placenta in a given species. Transfer across the maternal-foetal barrier depends on the thickness and extent of the barrier as well as the concentration gradient or presence of active transport mechanisms (Rosso, 1981; Jones *et al.*, 2007; Donnelly and Campling, 2008). The placenta can be classified by the number of membranes (layers of tissue of both maternal and foetal origin) that separate the maternal and foetal circulatory systems in the placental barrier region. This yields four types of placentas: epitheliochorial, synepitheliochorial, endotheliochorial and haemochorial (Leiser and Kaufmann, 1994).

In humans, lagomorphs and rodents, which have a hemochorial placenta, immunoglobulins are transferred mainly to the foetus via the placenta by the neonatal Fc receptor (FcRn) (Story *et al*., 1994; Marques *et al.*, 2007; Hironaka *et al.*, 2003; Palmeira *et al*., 2012). In ungulates, including horses and pigs (epitheliochorial placenta), ruminants (synepitheliochorial placenta), offspring are born agammaglobulinemic and immunoglobulin transmission occurs via mammary colostrum secretions. In rats mice and dogs (endotheliochorial placenta), antibodies are transferred both via placenta and colostrum (Giguère and Polkes, 2005; Crisman and Scarratt, 2008; Tizard, 2009).

The prenatal transfer of immunoglobulins to the bovine foetus is limited by the syndesmochorial placental structure. Consequently, calves are essentially agammaglobulinemic at birth (Brambell, 1970; Weaver, 2000; Pires, 2009). However, the bovine foetus is able to respond immunologically to many antigens from an early age (Schultz, 1973). Therefore, serological

examination of foetal sera could be useful in identifying agents which may have infected a foetus, provided that the foetus is of an immunologically competent age and survives infection long enough to produce detectable levels of circulating antibody (Ellis *et al*., 1978b). The immunological capability of the bovine foetus has been examined by Butler, (1969), Butler *et al.* (1971), Osburn and Hoskins (1971), Dunne *et al.* (1973), Hubbert *et al.* (1973), Schultz(1973), Schultz *et al* (1971) and Horner *et al* (1973). Osburn and Hoskins, (1971) injected viable cultures of *Vibrio fetus* into the placental cavities of cows between 122 and 249 days of pregnancy. Elevated levels of IgM with questionable antibody specificity were observed in the late-term foetuses. Horner, (1973) detected IgG in serum of bovine foetuses from a tropical and temperate environment. Specific antibody was detected for BVD mucosal disease virus in 7 of 26 foetal calf sera. Gould, (1978) detected antibodies to bovine syncytial virus (BSV) and Respiratory Syncytial virus (RSV) in bovine foetal serum. Ellis *et al*. (1978b) detected serum Ig in 81.5% of aborted foetuses and 32.8% of non-aborted foetuses.

## 2.15 Diagnosis of Leptospirosis

### 2.15.1 Dark-field microscopy

Microscopic examination of the centrifuged urine using dark-field illumination is considered to be a convenient and rapid diagnostic test. However, dark-field microscopic (DFM) examination is insensitive and requires a skilled observer to differentiate leptospires from artefacts (Bolin *et al*., 1989b). Dark-field microscopic examination of body fluids such as blood, urine, CSF, and dialysate fluid has been used but is both insensitive and lacking specificity. Approximately104 leptospires/ml is necessary for one cell per field to be visible by dark-field microscopy (Turner, 1970). Direct dark-field microscopy of blood is also subject to misinterpretation of fibrin or

protein threads, which may show Brownian motion (Faine et al., 1999; Turner, 1970; Wolf, 1954).

### 2.15.2 Isolation of leptospires

The isolation of the infecting strains of *Leptospira* is the yardstick against which all other antigen detection methods are measured (OIE, 2008). The isolation of leptospires is one of the most specific methods of demonstrating their presence, provided that antibiotic residues are absent, that tissue autolysis is not advanced, that tissues are processed for culture rapidly after collection, and in the case of urine at a suitable pH (OIE, 2014). For selecting proper tissues or samples for isolation purposes, it is important to first determine the stage of the disease. In cases of acute disease, isolation should focus on blood samples. In the chronic form after the development of serological responses, isolation should be attempted from the urine. In the case of clinical disease or with aborted foetus, isolation should be attempted from kidney, liver and the aqueous humour (Thiermann, 1984; Faine, 1994).

The quality of the medium is an important factor in isolating leptospires successfully. Media based on polysorbate-80 and bovine serum albumin (BSA), like EMJH (Johnson *et al*., 1973), T80-40LH medium (Ellis *et al*., 1985a) are the most useful for the isolation of leptospires from infected animals (Thiermann, 1984; Ellis, 1986). Supplements have been recommended by many workers to increase the rate of isolation and to control contamination (Adler *et al*., 1986). The addition of fresh rabbit serum and 5-fluorouracil (Ris and Hamel, 1978; Ellis *et al*., 1982c; Oie *et al*., 1986), as well as the extensive dilution of the sample in 1 % BSA solution are highly recommended (Thiermann *et al*., 1985). For isolation purpose these media are usually used in a semisolid form obtained by adding purified agar to a final concentration of 0.1 to 0.2% agar (Ellis, 1986). Culture media are

incubated at 28°C to 30°C and examined by dark-field microscope at least fortnightly for 3-6 months (Ellis, 1986; Faine, 1994).

Culture of the organism from blood, urine and milk may be attempted by injection of fresh samples directly into hamsters or guinea-pigs on the farm or using special media (Radostits *et al*., 1994). Isolation of leptospires from the urine of vaccinated cows is usually unsuccessful. This difficulty may be because of the presence of antibodies or other substances in the urine of vaccinated cows which interfere with the growth of leptospires (Bolin *et al*., 1989b).

Isolated leptospires are identified to serovar level by traditional serologic methods or by molecular methods, such as pulse field gel electrophoresis (Galloway and Levett, 2008). These techniques are limited in availability to a few reference laboratories.

The major advantage of bacteriologic culture is that leptospires of any serovar can be detected and subsequently identified. However, bacteriologic culture procedures are too expensive and too slow for routine use, because fresh samples are necessary and 4 to 6 months may be required for conclusive results (Bolin *et al*., 1989b). Ellis *et al*. (1986d) reported that even in a fresh foetus the positive identification of leptospires in lesions is very difficult, especially with *L.* Hardjo which is very fastidious in its cultural requirements. Genotype *hardjoprajitno* strains are much more difficult to isolate than are genotype *hardjobovis* strains (Ellis *et al*., 1986d).

**Limitations of isolation of leptospires:** Bacteriologic culture procedures are too expensive and too slow for routine use, because fresh samples are necessary and 4 to 6 months may be required for conclusive results (Bolin *et al*., 1989c). Ellis *et al*. (1986d) reported that even in a fresh foetus the positive identification of leptospire in lesions is very difficult, especially with *L. hardjo* which is

very fastidious in its cultural requirements. The genotype *hardjoprajitno* strains are much more difficult to isolate than are genotype *hardjobovis* strains (Bolin *et al*., 1989c).

### 2.15.3 Serological test

#### *2.15.3.1 Microscopic agglutination test*

Microscopic agglutination test (MAT) is the gold standard for serodiagnosis of leptospirosis in both animals and humans. It is the reference test against which all other serological tests are evaluated (OIE, 2000; Lavett, 2003). The MAT detects both IgM and IgG antibodies, but it is less useful in diagnosis of chronic disease in maintenance hosts since antibody response to infection may be negligible in chronic infections or may persist from subclinical infections (Radostits, 1994). The MAT detects agglutinating antibody in serum that react with live antigen suspensions of leptospiral serovars. After incubation, the serum-antigen mixtures are examined by dark field- microscopy for agglutination, and the titers are determined (Wolff, 1954; Galton *et al*., 1965; Cole *et al*., 1973; Sulzer and Jones. 1973; Sulzer and Jones. 1978; Faine, 1982; Alexander, 1986; Kaufmann and Weyant, 1995)

The MAT consists of two consecutive steps. The first step screens for the responsible serogroup; and, the second step quantitatively determines the serum titer for every test antigen. The end point is the highest dilution of serum at which 50 % agglutination occurs. Because of the difficulty in detecting when 50 % of the leptospires are agglutinated, the end point is determined by the presence of approximately 50 % free, unagglutinated leptospires compared to the control suspension (Faine, 1982). However cut-off titer for single MAT vary from one geographical area to the other. There is no standard titer that is considered indicative of infection during single sampling of animals. Some workers consider 1:100 as positive while others accept 1:80, 1:200,

1:400 as diagnostic for recent infection (Smith *et al*., 1994; WHO, 2003). The high degree of cross reaction between different serovars is because they possess the same agglutinating epitopes (Faine *et al*., 1999) and this complicates the interpretation of the result. MAT sensitivity is relatively low (Broughton *et al*., 1984; Limmathorotsaku *et al*., 2012).

Study conducted by Ellis *et al.* (1981) on 200 randomly selected cattle at abattoir indicated that 46.4% of renal carriers had antibody titres of less than 1/100 and 9.6% had no detectable MAT titre against *L.* Hardjo.

**Limitations of microscopic agglutination test:** The test has limitations in diagnosis of chronic infection in individual animals, both in the diagnosis of abortion (Ellis *et al*., 1982a; 1982c) and in the identification of renal or genital carriers (Ellis, 1986). This is particularly true with the host-adapted leptospiral infections, e.g. serovar Hardjo infection in cattle: when a titre of 1/100 or greater is taken as significant, the sensitivity of the test is only 41 %, and even when the minimum significant titre is reduced to 1/10, the sensitivity of the test is only 67 % (Ellis, 1986). The demonstration of antibodies in foetal blood is diagnostic, but the titres are often very low, i.e. 1/10, requiring a modified testing procedure for most laboratories (OIE, 2014). The sensitivity of the test can be improved by using local isolates rather than reference strains (Turner, 1968; Torten, 1979; Faine, 1982) but reference strains assist in the interpretation of results between laboratories (OIE, 2014). The MAT is a complex test to control, perform, and interpret (Turner, 1968). Live cultures of all serovars required for use as antigens must be maintained (OIE, 2014). The repeated weekly subculture of large numbers of strains presents hazards for laboratory workers, and laboratory-acquired infections have been reported (Alexander *et al.,* 1952; Pike, 1976).

**Advantages of microscopic agglutination test:** High specificity is the major advantage of the MAT (WHO, 2003). Antibodies against other bacteria usually do not cross-react with *Leptospira* to a significant extent. However, there is significant serological cross-reactivity between serovars and serogroups. Therefore, serology cannot be used to identify definitively the infecting serovar in an individual infection or outbreak, which requires isolation of the agent. Formalinized antigens have been used in the MAT to overcome some of the difficulties associated with the use of live antigens. Titers obtained with these antigens are somewhat lower, and more cross-reactions are detected (Gochenour *et al*., 1958; Manev 1976; Sulzer and Jones, 1978; Faine, 1982; Palmer *et al*., 1987; Woo *et al*., 1997). Agglutination of formalin-treated antigens is qualitatively different from that seen with live antigens (Alexander, 1986).

*2.15.3.2 Enzyme linked immunosorbent assay*

The Enzyme Linked Immunosorbent Assay (ELISA) test is potentially the most useful serological test (Ellis, 1986). The test is highly sensitive, easy to perform and can be specific for IgM or IgG antibodies (Hartman *et al*., 1984; 1986; Cousins *et al*., 1985; Smith *et al*., 1994). The ELISA test is much more accurate than the other serological tests and has many advantages from the point of view of laboratory practice (Thiermann and Garrett, 1983; Cho *et al*., 1989). The test was developed due to deficiency of MAT, it uses a killed antigen and results can be read objectively rather than subjectively (Cousins *et al*., 1985). It has convenient technical features including automation, and can be used efficiently as a screening test for large numbers of serum samples (Radostits, 2007).

An ELISA that measures bovine, porcine, ovine and canine IgG and IgM against various leptospiral serovars has been developed and evaluated in Europe (Adler *et al.,* 1981; Hartman *et al.,* 1984; Cousins *et al.,* 1985; Hartman *et al.,* 1986; Cho *et al.,* 1989; Trueba *et al.,* 1990; Mendoza and Prescott, 1992; Surujballi *et al.,* 1997; Yan *et al.,* 1999; Ribotta *et al.,* 2000;

Surujballi and Elmgren, 2000; Surujballi and Mallory, 2001). The high level of sensitivity, and specificity obtained for the ELISA-IgG, indicated that this test would be a good alternative to the MAT for detection of leptospirosis (Cousins *et al*., 1986). Some difficulty encountered is in interpreting the significance of titres of antibody in serum. Although there is no marked difference between vaccinal and infection titres (Trubea *et al*., 1990), it is apparent from a heifer trial that infection titres generally persist much longer (Broughton *et al*., 1984). A commercially available ELISA and the Immunocomb Leptospirosis Kit which detect *L.* Hardjo antibodies have been compared with the MAT. The Immunocomb and ELISA tests both exceeded the positive results obtained with the MAT (Woodward *et al*., 1997)

Enzyme Linked Immunosorbent Assays for detection of anti-leptospiral antibodies have been developed using a number of different antigen preparations (Theirmann and Garett, 1983; Cho *et al*., 1989; Bercovich et al., 1990; Ribeino *et al*., 1995; Yan *et al*., 1999; Ribotta *et al*., 2000; Tomich *et al*., 2007), assay protocols and assay platforms, including plate tests and dipstick tests. The antigen used dictates the specificity of the ELISA.

**Limitations of enzyme linked immunosorbent assay:** Recombinant OMP-based ELISAs are broadly reactive to antibodies to all pathogenic leptospires and so are of no value in epidemiological investigations. In contrast, lipopolysaccharide antigen-based ELISAs are serogroup specific and have value in epidemiological investigations and control schemes. Problems with validation are a major constraint in assessing most ELISAs. Almost all have been validated against the MAT (using MAT titres of 1/100 or greater), which is an imperfect test, having a sensitivity of less than 50 % in some chronic infections. Human investigators have attempted to over-come this problem by the use of Bayesian latent class models and random-

effects meta-analysis studies (Limmathurotsakul *et al*., 2012; Signorini *et al*., 2013), but the best validation possible is using sequencial sera from culture positive cases (Goris *et al*., 2012).

The major disadvantages are that some ELISA systems use the genus specific antigen which is not suitable for serovar or serogroup identification of leptospirosis (Ribotta *et al*., 2000), thus making them less specific than MAT (Cho *et al*., 1989). ELISA in which the plates are coated with Pomona antigen have been shown to produce cross reactivity in some species such as canine, bovine and humans irrespective of the antigen used (Cousin *el al*., 1985; Langston *et al*., 2003). The cross reactivity produced by some of these ELISA system have been reported to most probably be due to antibody produced against their inner envelop antigen of *Leptospira* sharing some common cross reactivity antigen (Cho *et al*., 1989). Some ELISA cannot differentiate between infected and vaccinated animals (Ribotta *et al*., 2000).

*2.15.3.3 Fluorescent antibody test*

Fluorescent antibody test (FAT) staining of antigen in urine or cultures is a fast and accurate diagnostic method for detecting the presence of leptospires and for identifying serotypes (Hodges and Ekdahl, 1973). The FAT is a very useful technique for demonstrating leptospires in tissues from animals (including foetuses) which have died of leptospirosis (Cook *et al*., 1972; Kirkbride and Halley, 1982). This test may be used with fresh or frozen tissues and urine and aids in the discrimination of leptospires from artefacts (Bolin *et al*., 1989c). Studies of Smith *et al*. (1966) showed that the FAT was superior to culture and histopathological methods in demonstrating the presence of leptospires in autolysed materials. Fluorescent antibody test was comparable to cultural methods when used on specimens containing relatively large numbers of leptospires (Boulanger and Robertson, 1961).

Using incident-light illumination with high magnification oil immersion objectives and low power eye pieces gave the best results in diagnosing leptospirosis in aborted foetuses (Ellis *et al*., 1982c). The FA-positive, culture-negative cases are usually the result of the overgrowth of cultures by contaminating microorganisms (Ellis *et al*., 1982c). It has been proposed that autolyzed, dehydrated, or mummified foetuses (Smith *et al.*, 1966) and formalin-fixed tissues (Cook *et al.*, 1971) can be satisfactory specimens for the FAT. The FAT has been investigated as a serotyping method that would eliminate the time required for growth of isolates and absorption test (Dacres, 1961; Hodges and Ekdahl, 1973)

### 2.15.4 Molecular diagnosis of *Leptospira*

The first approach of direct probing on DNA extracted or purified from biological specimens was limited by a relatively high threshold of detection of DNA and uncertainty about the specificities of the probes (WHO, 2003). Leptospiral DNA has been detected in clinical material by dot-blotting (Millar *et al*., 1987; Terpstra *et al*., 1986; Fach *et al*., 1991) and in situ hybridization (Terpstra *et al*., 1987). A recombinant probe specific for pathogenic serovars was prepared from serovar Lai (da Silva *et al*., 1990). Probes specific for serovar Hardjobovis were developed (LeFebvre, 1987; Zuerner and Bolin, 1988; Van *et al*., 1989) and applied to the detection of leptospires in bovine urine (Bolin *et al*., 1989). In a comparative study, Bolin *et al.* (1989b) showed that DNA hybridization was more sensitive than either FAT or culture techniques for the diagnosis of bovine leptospirosis. However, the sensitivity of $^{32}$P-labeled probes was approximately 103 leptospires (Terpstra *et al*., 1986; Millar *et al*., 1987; Zuerner and Bolin, 1988), much lower than the sensitivity of PCR, and probes have not been used extensively for diagnosis since PCR became available.

Polymerase chain reaction (PCR)-based assays are increasingly used for the detection of leptospires in tissues and body fluids of animals because of their perceived sensitivity and capacity to give an early diagnosis (Van Eys *et al*., 1989; Woodward *et al*., 1991). Real-time PCR is faster than regular PCR and less sensitive to contamination (Picardeau, 2013). Leptospiral DNA has been amplified from serum (Gravekamp *et al*., 1991; Brown *et al.,* 1995; Merien *et al*., 1995), urine (Bal *et al*., 1994; Brown *et al*., 1995; Merien *et al*., 1995), aqueous humor (Merien *et al*., 1993), CSF (Merien *et al*., 1995; Romero *et al*., 1998; Vinetz *et al*., 1996), and a number of tissues obtained at autopsy (Brown *et al*., 2003). An assay based on the IS*1533* insertion sequence (Zuerner *et al*., 1995) facilitated both detection and identification of serovars directly from urine. Woodward and Redstone (1994) developed a PCR to amplify the *flaB* gene of 23 serovars of the genus *Leptospira*.

A limitation of PCR-based diagnosis of leptospirosis is the inability of most PCR assays to identify the infecting serovar, although some primer sets may permit further identification to the species or strain level if the PCR amplicons are sequenced. Strategies designed to overcome this obstacle have included restriction endonuclease digestion of PCR products (Brown and Levett, 1997; Savio *et al*., 1994; Levett, 2001), direct sequencing of amplicons (Oliveira *et al*., 1995), ribotyping (Pe´rolat *et al*., 1990; Pe´rolat *et al*., 1993; Pe´rolat *et al.,* 1994; 288, Tamai *et al*., 1988), PFGE (Herrmann *et al*., 1991; Herrmann *et al*., 1992; Herrmann, 1993), single-strand conformation analysis (SSCP) (Matthias, 2000; Wu *et al*., 1996), Amplified fragment length polymorphism (AFLP) (Slack *et al*., 2006), Random amplified polymorphism DNA fingerprint (RAPD) analysis (William *et al.,* 1990), Arbitrary primed PCR(AP-PCR) (Weyant *et al.,* 1999), 16S rRNA sequencing (Morey *et al.,* 2006), Variable number of tandem repeats (VNTR) (Majed et al., 2005) and Multilocus sequence typing (MLST) (Ahmed *et al.,* 2006). PCR has been used

to distinguish pathogenic from nonpathogenic serovars (Murgia *et al*., 1997; Parma *et al*., 1997; Woo *et al*., 1997)

### 2.16 Gross Pathological Findings in *Leptospira* infected Cattle

In spite of the large volume of literature on post-mortem pathology of leptospirosis in cattle, no definite pathognomonic lesions have been described (Alston and Broom, 1958; Van der Hoeden, 1964; Murphy and Jensen, 1969; Amatredjo and Campbell, 1975). The main pathological signs which may be observed in the acute form are; anaemia, jaundice, haemoglobinuria and subserous and submucosal haemorrhages. There may be ulcers and haemorrhages in the abomasal mucosa in cattle, and if haemoglobinuria has been severe there may be associated pulmonary oedema and emphysema (Radostits *et al*., 2007). In the subacute and chronic form of the disease, the characteristic finding is a progressive interstitial nephritis manifested by small, white, raised areas in the renal cortex (Smith and Armsrong, 1975; Davidson and Hirsh, 1980; McCaughan *et al*., 1980; Baker *et al*., 1989; Wilson *et al*., 1998; Faine, 1999). However, the extent and severity of lesions depend on the infecting serovar, age of animal and the stage of the disease and some chronic carriers have grossly normal kidneys (Faine *et al*., 1999). As with all leptospiral infections, the disease and lesions in calves are much more severe than in adult cattle (Baskerville, 1986). Although there is little detailed histopathology in the literature, it appears that some of the lesions are common in several infecting serovars.

Aborted bovine foetuses are usually autolysed to the point where no lesions or bacteria can be demonstrated (Radostits *et al*., 2007). However, there are no lesions which are consistently found in aborted foetuses or their membranes. Placental oedema, placentitis and nephritis occur inconsistently. The placental lesions are more likely to have caused foetal death but they are not

unique to leptospirosis and may be masked by the decomposition which usually affects aborted bovine placentas (Boulton, 1993). Liver lesions differ markedly in their severity between calves and adult animals and are particularly a feature of *L.* Pomona and *L.* Copenhageni infections, which produce small areas of hepatic necrosis in cattle (Baskerville, 1986).

### 2.17 Treatment of *Leptospira* Infection

The primary aim of treatment in all leptospiral infections is to control the infection before irreparable damage to the liver and kidneys occurs. The secondary aim of treatment is to control the leptospiruria of carrier animals and render them safe to remain in the group. Antibiotics used in the treatment of leptospirosis include the tetracyclines, penicillin/ampicillin, dihydrostreptomycin, streptomycin, fluoroquinolones, doxycycline, ampicillin, amoxicillin, and erythromycin (Guidugli *et al*., 2004). Recommended treatments for acute leptospirosis in cattle are the use of intramuscular injections of dihydrostreptomycin (12.5mg/kg, twice daily) or tetracycline (10-15mg/kg, twice daily). In the case of chronic leptospirosis due to *L.* Pomona, a single intramuscular injection of 25mg/kg body weight dihydrostreptomycin (DHS) can be used for the treatment of renal carrier cattle. This does not, however, remove *L.* Hardjo from the kidney and genital tract of 7 of 10 bovine carriers, based on cultural studies (Ellis *et al*., 1985a). Supportive treatment and management of complications such as, renal failure, hepatic complications, haemorrhages and CNS disease is also necessary. The efficacy of treatment may depend on the serovars involved and fluid therapy. In beef herds, further abortions may be prevented by treatment of all animals with antibiotics and vaccination of the entire herd, if leptospirosis is diagnosed early during an outbreak. In dairy cattle, only infected animals are usually treated, due to the potential loss of milk sales (Guidugli *et al*., 2004). Minocycline has been reported to result in excellent efficacy when administered for the treatment of leptospirosis intravenously (Sakamoto *et al*., 2001).

## 2.18 Prevention and Control of Leptospirosis

Leptospirosis vaccines are available for pigs, cattle and dogs. Although the vaccines prevent disease, they do not completely prevent infection or the shedding of the organisms and immunity is largely serovar specific, and hence vaccines are protective only against the included serovars or closely related serovars. Prophylactic treatment of exposed animals with antibiotics can also prevent the disease (Vinetz, 2001). Prevention is also largely dependent on sanitary measures that may be difficult to implement, especially in developing countries (Bharti *et al.,* 2003). Patients and animals with history of exposure to water bodies, presence of symptoms characteristic of severe leptospirosis, and unexplained fever and flu-like symptoms, should be screened (Myrstad *et al*., 2012). Sanitation and the prevention of contact with contaminated environments or infected wildlife, particularly rodents, can decrease the risk of infections. Animals should not be allowed to drink from or enter contaminated water bodies.

In cattle, infections are usually introduced through contamination of the environment or by contact with infected sheep or pigs. Other control measures include isolation and treatment of infected animals, rodent control, prevention of contact with wildlife, and other reservoir animals, and prevention of animals gaining access to contaminated bodies of water (OLRG, 2000). Vaccination and good management practices like separation of young animals from older animals, can eradicate leptospirosis from a herd over a period of years. Eradication can also be achieved in closed herds by vaccination and testing of animals over a five - year period, in cattle, sheep and pigs (CDC, 2003). To reduce urinary shedding, or eventually eliminate renal leptospirosis, animals may be treated with streptomycin, oxytetracycline, tylosin or erythromycin (Alt and Bolin, 1996). Furthermore, antimicrobial agents in the feed, such as oxytetracycline or chlortetracycline, may reduce clinical signs but will not eliminate carriers (Faine *et al.,* 1999).

Human vaccines are available for workers in high risk professions in some countries but not yet available in Nigeria. Immunization is relatively serovar specific and protects only against serovars in the vaccine or closely related serovars, similar to the situation in animals. Yearly vaccination is required and the side effects of immunization include painful swelling. Doxycycline has been used in humans for short term prophylaxis (OIE, 2004).

The control of infections in livestock and pet animals reduces the risk of human disease, but the existence of wildlife reservoirs complicates prevention. Rodent control can be important in preventing human infections, particularly in urban areas. Avoidance of contact with contaminated or potentially contaminated bodies of water can decrease the risk of infection. Domestic animals should not be allowed to urinate in water that humans can come in contact with. Draining wet areas may also decrease the incidence of the disease and food should be protected from sources of infection. Personal hygiene and protective clothing are important control measures in high risk groups. Gloves and face shields can help prevent infections, when working with infected animals or tissues. Rubber boots can decrease risk of infection in sewer workers and agricultural workers, who may wade in urine contaminated water (OLRG, 2000b).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

Kano State is a state in north-Western Nigeria, lying at $11^0$30'N and $8^0$30'E with an area of 20,131 $km^2$ (Fig. 3.1). It has 44 Local government areas and human population of 9,401,288 (2006, NPC) and cattle population of about 580,390 (FDL, 2010). The dry season lasts from November to Mid-April while the rainy season lasts between 5-6 months starting from mid-April. The old Kano abattoir is located at Fagge LGA at $12^0$0'N and $8^0$31'E. The abattoir is the major source of meat for the people of Kano State and its environs.

### 3.2 Sample Size Determination

A sample size of 372 was determined using the prevalence of 4.6% reported by Ellis *et al.* (1982b) at 2.5% precision and 97.5% confidence level using Win Episcope.

### 3.3 Data Collection

Retrospective study was done using previous records (2005-2014) in the abattoir on foetal wastages. Crosssectional study on cases of foetal wastages was carried out through collection, counting and ageing of foetuses obtained during slaughter at the abattoir from November 2014 - January 2015 and blood samples were also collected from the foetuses.

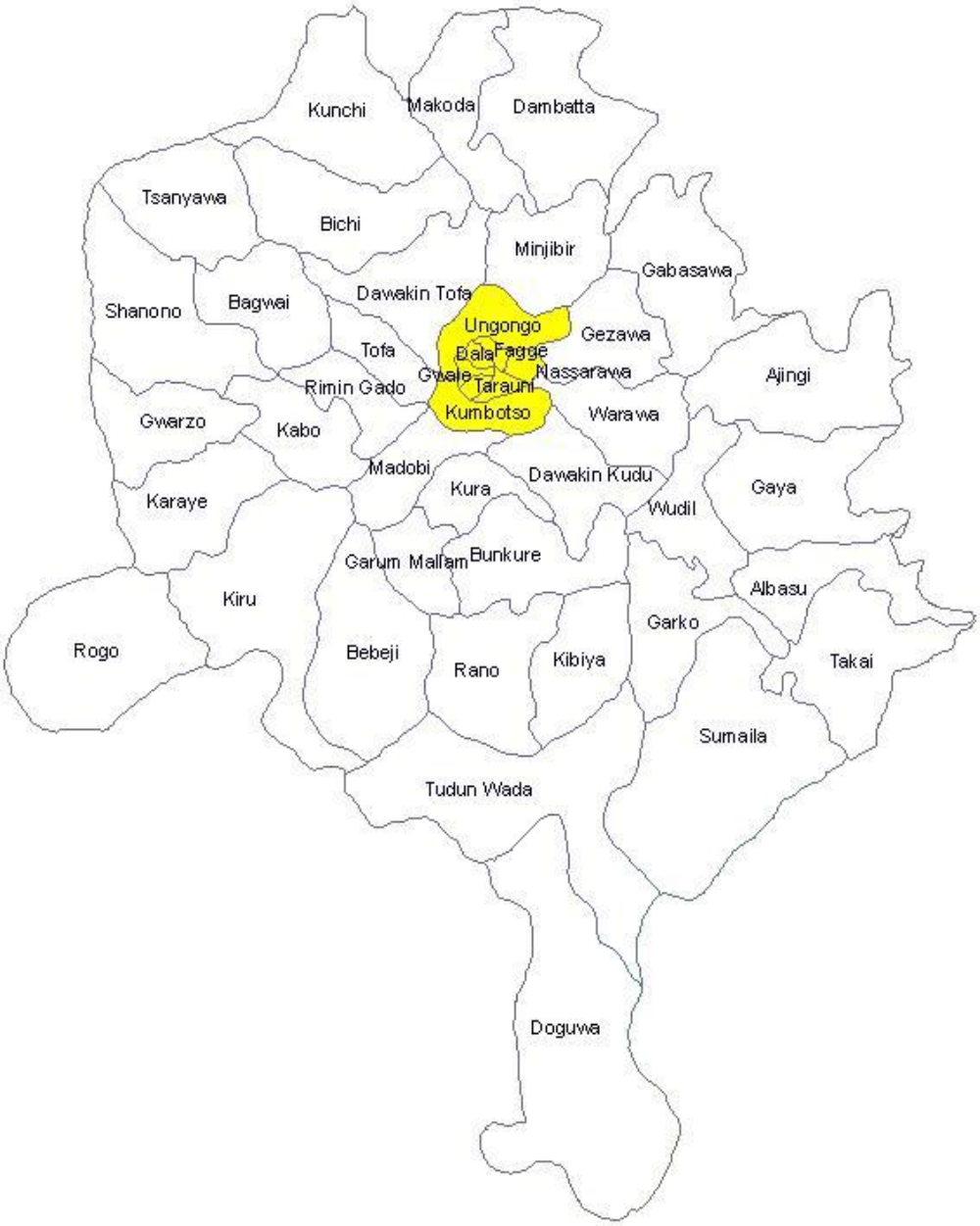


Figure 3.1: Map Showing Kano State, Nigeria, showing forty four Local Government Areas (Source: www.Kanostateemhe.com.ng).

### 3.3.1 Retrospective study

Records of meat inspection available at the Kano State Ministry for Agriculture for the period starting January 2005 to December 2014 were used for the retrospective study. Monthly and annual reports from the abattoir were used to generate information on the total cattle slaughtered and foetal wastages recorded.

### 3.3.2 Cross sectional study

The old Kano abattoir was visited every day of the week (7 days in a week) for a period of two (2) months. Information on the total number and age of foetal wastage observed was collected per day by counting and measuring the foetal crown to rump length respectively. Serum samples of foetuses obtained at slaughter were collected from the abattoir for further laboratory processing to detect the presence of antibodies to *Leptospira* spp.

*3.3.2.1 Estimation of age of foetus*

Ages of the foetuses obtained at slaughter were determined by measuring crown to rump length of the foetus as described by Noakes (1986). The ages of all the foetuses recovered were classified according to the method of Ardo *et al*. (2013) into three gestational stages as follow:

i. First trimester $\leq 93$ days
ii. Second trimester 94–187 days
iii. Third trimester $\geq 188$ days

*3.3.2.2. Estimation of losses due to foetal wastage*

To estimate the cost of each foetus obtained at slaughter from the abattoir, a market survey was carried out in Kano State markets and its environs to determine the monetary value of a living male or female calf in order to calculate the economic losses (financial implication accruing) due

to foetal wastage in Kano State. The average cost of a female calf was N15, 000 and that of the male calf cost N12, 000. The economic loss was estimated at slaughter according to the method of Babatunde *et al*. (2011) using the formula below.

(i) ELB (₦) = $N_0 * P_0$

Where ELB= Economic loss at birth, $N_0$= Number of foetuses at slaughter, $P_0$ = average price of calf at birth.

*3.3.2.3 Collection of foetal serum and Transportation*

Blood sample was obtained from each foetus by direct aspiration of the blood from the heart in the thoracic cavity. Heart blood was collected with a needle and syringe (21 gauge) via the fourth intercostal space and placed in a 5 ml plain vacutainers and centrifuged at 1000 rpm for 10 minutes at a private laboratory in Kano (N-Tawa). The serum was collected into 5 ml plain vacutainers and stored frozen until further laboratory processing.

Three hundred and seventy-two serum samples collected were placed into sterile screw-capped containers and stored on ice before transportation. The serum samples were transported to the Diagnostic Division of the National Veterinary Research Institute, Vom Plateau State, where the laboratory processing was carried out for Microscopic Agglutination Test (MAT) and also to the Bacterial Zoonoses Laboratory, Deparment of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for the Enzyme Linked Immunosorbent Assay (ELISA).

## 3.4 Detection of Antibodies to Leptospirosis

Serologic testing carried out in the two laboratories were the Microscopic Agglutination Test (MAT) and the Enzyme Linked Immunosorbent Assay (ELISA) test.

### 3.4.1 Microscopic agglutination test

Microscopic Agglutination Test (MAT) was performed according to the method described by Herr *et al*. (1987) to check for antibodies in the foetal sera using 8 live serovars of *Leptospira* species as source of antigen. The serovars were obtained from the Serology Department of Onderstepoort Veterinary Institute, South Africa. The 8 leptospiral serovars used were; Icterohaemorrhagiae, Tarassovi, Pomona, Grippotyphosa, Australis, Canicola, Hardjo, and Mini. Foetal sera were checked for antibodies to the 8 serovars. The sera were screened at a dilution of 1:10. Serial 2-fold dilutions of the sera that agglutinated with leptospires at the 1:10 dilution and the endpoint MAT titer was determined.

*3.4.1.1 Test Procedure*

The Microscopic agglutination Test (MAT) test was carried out as follow:

i. the serum was diluted 1:10 in PBS.
ii. the wells of column 1 were filled with 25µl of PBS while 25µl of diluted serum was added to wells of column 2-12.
iii. twenty-five micro litre antigen was added into all 96 wells using a 25µl eppendorf pipette and a new tip for each serotype and mixed gently on a microplate titre plate shaker for 30 seconds and incubated for $37^0$C for 2 hours.
iv. the test results were read by placing one drop of sample on a microscopic slide with a pipette and examined under dark field microscope at x200 magnification without a cover slip for agglutination.
v. 100% agglutination was taken as a positive reaction (Ellis *et al*., 1978a) and reacting sera were titrated with corresponding antigen. Control column had 0% agglutination.

*3.4.1.2 Titration of reacting sera*

The titration of reactive sera was done following the protocol below:

i. the serum was diluted 1:10 in PBS.

ii. using a microdropper, 25µl PBS was dropped in all wells and 25µl of diluted serum was added to wells of row 1.

iii. the microdiluter was set on 25µl and 1:2 dilution, 25µl was taken up from row 1 and diluted up to row 8, 25µl was taken out from row 8. This process resulted in eight, two-fold dilutions covering the range 1:20 -1:2560.

iv. twenty-five micro litre antigens were placed in rows 1-8. New tips were used for each serotype.

v. plates were shaken gently for 30 seconds. After this, the lids of the serology plates were replaced and the plates were placed in plastic bags and incubated in dark at $37^0$C for 2hrs.

vi. the test results were read by putting one drop of sample on a microscopic slide with a pipette and examined under dark field microscope at x200 manification without a cover slip

vii. the end point of an agglutination reaction was deemed to be the dilution at which approximately 50% of the organisms have been agglutinated.

**3.4.2 Enzyme linked immunosorbent assay**

An Enzyme Linked Immunosorbent Assay (ELISA) test kit (Linnodee, Animal care, Ballycare, Ireland) was used to screen the sera for antibodies to *Leptospira* spp. Serovar Harjo. The ELISA has a sensitivity of 94.1 %, specificity of 94.8 % and Kappa index of 0.9 %. The test was carried out in accordance with the recommendations of the manufacturer.

*3.5.2.1 Test procedure*

The Enzyme Linked Immunosorbent Assay (ELISA) test was carried out as follow:

i. sera were diluted in the sample diluents at 1:50 and 100µl were dispensed to each well.

ii. positive and negative controls were diluted at 1:50 in the sample diluents and 100ul dispensed into duplicate wells.

iii. the plates were sealed and incubated at $37^{O}C$ for 1 hour in the incubator and then washed four times with the diluted wash buffer.

iv. the concentrated conjugate was diluted at 1:1000 in diluted wash buffer just before use and 100µl of the diluted conjugate was added per well.

v. the plates were sealed and incubated at $37^{o}C$ for 40min after which the plates were washed four times with the diluted wash buffer.

vi. then, 100µl of the substrate (3'3'5'5'-tetramethylbenzidine- ELISA (TMB-E) was added to each well and the plates were incubated in the dark at room temperature for 10 min.

vii. 50ul of the stop solution (1M $H_2SO_4$) was added to each well and the plates read at 450nm with a correction of 630nm

viii. the positivity of a sample was determined using the following formula:

a. Ratio = $\frac{\text{Mean Sample Optical Density (OD) – Mean Negative control OD}}{\text{Mean Positive control OD – Mean Negative control OD}}$

ix. foetal sera with a ratio that exceeded 0.12 were considered seropositive.

## 3.5 Data Analyses

The data obtained from the study were analyzed using Odds ratio to test for association between foetal wastage, season and year and Fisher's exact test to test for association between

leptospirosis and age of foetuse with the aid of Win episcope and SPSS version 16 respectively. Results were presented in the form of charts, graphs and tables. Values of $p < 0.05$ were considered significant.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Annual Distribution of Foetal Wastage in Old Kano Abattoir from 2005-2014

A total of 13,563(2.6 %) cases of foetal wastage from a total of 520,805 slaughtered cows were obtained from previous records within the period 2005-2014. The year 2013 had the highest proportion of foetuses wasted 2281(4.8 %) in relation to the number of cows slaughtered (Table 4.1). The period 2010-2014 had significantly ($p < 0.05$) higher number of foetal wastage compared to 2005-2009 (Table 4. 2).

### 4.2 Average Monthly Seasonal Variation of Foetal Wastage in the Old Kano Abattoir from 2005-2014

The monthly seasonal variation of foetal wastage obtained from records in the old Kano abattoir during retrospective study is as shown in Figure 4.1. There was a peak of foetal wastage in August. There was a significant ($p < 0.05$) association between seasons and the number of foetal wastages recorded (Table 4.2) with more cases of foetal wastage reported during the rainy season (May to October) than in the dry season (November to April).

### 4.3 Age Distribution of Foetal wastage in Old Kano Abattoir from November 2014 January, 2015.

The age distribution of foetal wastage obtained during the two months study period ($24^{th}$ November, 2014 and $25^{th}$ January, 2015) is as shown in Table 4.3. Five hundred and sixty one (5.2%) cases of foetal wastage from a total of 10769 slaughtered cows were recorded, out of these cases, 116 (20.7 %), 316 (56.3%) and 129 (23 %) occurred during their first, second and third trimester respectively. In all the cases of foetal wastage recorded, 445 (79.3%) occurred during their second and third trimester.

**Table 4.1: Annual distribution of foetal wastage in old Kano abattoir, Kano State, Nigeria between January 2005- November 2014.**

| YEARS | TAS | TBS | TCS | TFW | POFW | TCS:TFW |
|---|---|---|---|---|---|---|
| 2005 | 97,696 | 44770 | 52926 | 789 | 1.49 | 67:1 |
| 2006 | 92,478 | 42408 | 50070 | 852 | 1.70 | 59:1 |
| 2007 | 83,674 | 38367 | 45307 | 1041 | 2.30 | 44:1 |
| 2008 | 78,138 | 35831 | 42307 | 867 | 2.05 | 49:1 |
| 2009 | 91,551 | 41928 | 49623 | 1166 | 2.35 | 43:1 |
| 2010 | 104,159 | 40985 | 63174 | 1900 | 3.01 | 33:1 |
| 2011 | 94,545 | 36329 | 58216 | 1961 | 3.37 | 30:1 |
| 2012 | 76,944 | 29882 | 47062 | 1846 | 3.92 | 26:1 |
| 2013 | 80,504 | 33071 | 47433 | 2281 | 4.81 | 21:1 |
| 2014 | 110,523 | 45836 | 64687 | 860 | 1.33 | 75:1 |
| **TOTAL** | 910,212 | 389,407 | 520,805 | 13,563 | 2.60 | 38:1 |

*Keys: TAS = Total number of animals slaughtered, TBS = Total bulls slaughtered, TCS = Total cows slaughtered; TFW= Total number of foetuses wasted, POFW=Percentage of foetal wastage.*

**Table 4.2: Distribution of foetal wastage by season and years in old Kano Abattoir, Kano State, Nigeria between January, 2005- November, 2014.**

| Variables | Present | Absent | Specific rate | OR | 95%CI | P value |
|---|---|---|---|---|---|---|
| **Season** | | | | | | |
| **Wet season** | 7013 | 239165 | 2.85 | 1.2 | 1.160-1.242 | 0.001 |
| **Dry season** | 6550 | 268077 | 2.39 | ref | | |
| **Years** | | | | | | |
| **2005-2009** | 4715 | 235518 | 1.96 | ref | | 0.001 |
| **2010-2014** | 8848 | 271724 | 3.15 | 1.627 | 1.569-1.686 | |

*Keys: OR=Odd's ratio; CI=Confidence interval*

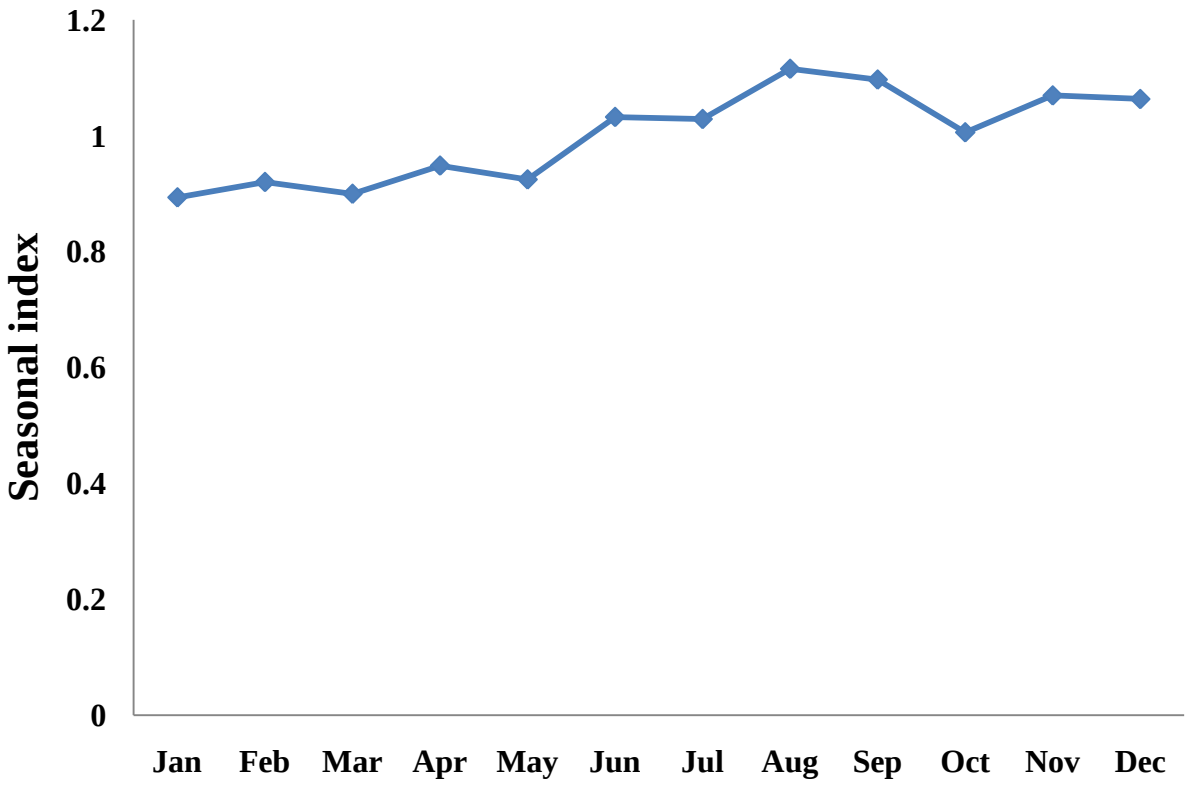


**Figure 4.1: Monthly seasonal variation of foetuses wasted in Old Kano Abattoir, Nigeria (cumulative from 2005-2014).**

**Table 4.3: Age distribution of foetal wastage over two months period (November 2014 to January, 2015) in the old Kano abattoir, Kano State, Nigeria**

| Period | TCS | TFW(%) | 1-3 months(%) | 4-6 months(%) | 7-9 months(%) | TCS:TFW |
|---|---|---|---|---|---|---|
| **Nov-Dec, 2014** | 5720 | 263(4.6) | 36(13.7) | 149(56.7) | 78(29.7) | 22:1 |
| **Dec- Jan, 2015** | 5049 | 298(5.9) | 80(26.8) | 167(56) | 51(17.2) | 17:1 |
| **Total** | 10769 | 561(5.2) | 116(20.7) | 316(56.3) | 129(23) | 19:1 |

*Keys: TCS=Total cows slaughtered, TFW= Total number of foetuses wasted, POFW=Percentage of foetal wastage.*

### 4.4 Estimation of Losses Due to Foetal Wastage in Old Kano Abattoir

During the study, a cost estimation of foetal wastage in the old Kano abattoir was undertaken. It was observed that if calves were sold immediately after birth (i.e. after receiving colostrum) and assuming no pre-natal mortality and no still births, a total of 13563 calves would have been lost in 10 years at an estimated cost of ₦ 162,756,000 ($ 817,869 at exchange rate of US $ 1 to 199 at time of study) or ₦ 203,445,000 ($ 1,022,337) if a calf was sold at ₦12,000- ₦15,000 depending on the weight of the calf. The estimated cost of 561 cases of foetal wastage recorded for the two month period was put at ₦6,732,000 ($ 33,829) or ₦ 8,415,000 ($ 42,286) .

### 4.5 Seroprevalence of *Leptospira* Hardjo in Serum of Wasted Foetuses from Old Kano Abattoir.

**(a) Using Enzyme Linked Immunosorbent Assay**

Table 4.4 shows the seroprevalence of *Leptospira* Hardjo from wasted foetuses obtained from old Kano Abattoir using ELISA. Of the 372 sera tested for antibodies using enzyme linked immunosorbent assay, 21 (5.6 %) were positive for *Leptospira* Hardjo antibodies. In addition, 32 (8.6 %), 256 (68.8 %) and 84 (22.6 %) of the wasted foetuses sampled were at the $1^{st}$, $2^{nd}$ and $3^{rd}$ trimester respectively. Moreover, a seroprevalence rate of 9.5 %, 5.2 % and 0.0 % were obtained for foetuses at $3^{rd}$, $2^{nd}$ and $1^{st}$ trimester respectively. There was no significant difference ($p > 0.05$) in seropositivity between the three gestational stages.

**(b) Using Microscopic Agglutination Test**

Sera of foetuses obtained at slaughter from old Kano abattoir were tested for leptospiral antibodies by the microscopic agglutination test (MAT). Out of 372 sera tested, 52 (13.98 %) were positive to one or more serovars at serum dilution of 1:10.

**Table 4.4: Serological prevalence of *Leptospira* Hardjo using ELISA in bovine foetuses sampled from Kano Abattoir, Kano, Nigeria.**

| Estimated foetal age | Number Tested | Positive (%) | Negative (%) | P value |
|---|---|---|---|---|
| **1-3 months** | 32 | 0(0.00%) | 32 | 0.126 |
| **4-6 months** | 256 | 13(5.1%) | 243 | |
| **7-10 months** | 84 | 8(9.5) | 76 | |
| **Total** | 372 | 21 (5.6%) | 351 | |

*Fisher's exact value= 3.90*

Of the 52 sera that were positive for leptospiral antibodies, 10 (2.69 %), 10 (2.69%), 10 (2.69 %), 7 (1.88 %), 5 (1.34 %), 4 (1.08 %), 3 (0.81 %) and 3 (0.81 %) were positive to Hardjo, Pomona, Grippotyphosa, Canicola, Icterohaemorrhagiae, Australis, Tarassovi and Mini serovars respectively (Table 4.5). There was significant difference ($p < 0.05$) observed between the age groups and *Leptospira* infection (Table 4. 6). Amongst the 52 positive samples, 14 (26.9 %), 4 (7.6 %), 2 (3.8 %) and 1 (1.9 %) reacted to Hardjo, Grippotyhosa, Canicola and Pomona respectively at serum dilution of 1:20 and above (Table 4.7).

**Table 4.5: Age distribution of Microscopic Agglutination Test titre (1:10) to 8 No. *Leptospira* serovars in bovine foetuses in old kano abattoir**

| | | Age distribution | | | |
|---|---|---|---|---|---|
| **Antigen** | **Total sera tested** | **1-3mo.** | **4-6 mo.** | **7-9mo.** | **Total positive (%)** |
| ***L*. Icterohaemorrhagiae** | 372 | 0 | 3 | 2 | 5(1.34) |
| ***L*.Tarassovi** | 372 | 0 | 2 | 1 | 3(0.81) |
| ***L*.Pomona** | 372 | 0 | 10 | 0 | 10(2.69) |
| ***L*. Grippotyphosa** | 372 | 0 | 6 | 4 | 10(2.69) |
| ***L*. Australis** | 372 | 0 | 4 | 0 | 4(1.08) |
| ***L*. Canicola** | 372 | 0 | 7 | 0 | 7(1.88) |
| ***L*. Hardjo** | 372 | 0 | 8 | 2 | 10(2.69) |
| ***L*. Mini** | 372 | 0 | 3 | 0 | 3(0.81) |
| **Total** | 372 | 0 | 43 | 9 | 52(13.98) |

**Table 4.6: Serological prevalence of *Leptospira interogans* species at Microscopic Agglutination Test titre of 1:10 in bovine foetuses sampled from old Kano abattoir.**

| Variable (Age) | Total Number | Positive (%) | Negative (%) | P value |
|---|---|---|---|---|
| | | | | 0.022 |
| **1-3 months** | 32 | 0(0.00) | 32 | |
| **4-6 months** | 256 | 43(16.80) | 213 | |
| **7-10 months** | 84 | 9 (10.71) | 75 | |
| **Total** | 372 | 52(13.98) | 320 | |

*Chi Square value= 7.635*

# CHAPTER FIVE

## 5.0 DISCUSSION

Foetal wastage and occurence of *Leptospira* species antibodies in foetuses from slaughtered pregnant cows in Old Kano abattoir; Kano State, Nigeria was studied. The findings revealed that 2.6% of cows slaughtered in the old Kano abattoir during the 10 year period were pregnant. This was lower than reported in other parts of the country (Wosu, 1988; Nwakpu and Osakwe, 2007; Alhaji, 2011; Ngbede *et al*., 2012; Ardo *et al*., 2013; Bakari *et al*., 2015) and in neighbouring countries of Cameroon and Ghana (Ndi *et al*., 1993; Bah *et al*., 2010; Atawalna *et al*., 2013). The period 2010-2014 had significantly higher number of foetuses detected compared to the 2005-2009 period, which may indicate that foetal wastage in old Kano abattoir is on the increase. The reason behind this could be that reproductive diseases and pressure on family income among other factors which predisposed farmers to sell pregnant animals for slaughter. The rise in foetal wastage during the wet season was in agreement with the work of Ojo, (1978); Alhaji. (2011) and Adama *et al.* (2011). This Period was characterized by increased farming activities and increased demand for financial resources for these activities (Glatze, 2002). Majority of those who keep these animals are subsistence farmers, therefore they tend to dispose some of their animals especially those with history of reproductive problems such as dystocia, recurrent abortion, agalactia and history of rejection of calves. Besides, they prefer selling their cows when pregnant in order for such cows to attract better price (Personal communication, Mallam Musa Abdu, 15$^{th}$ Jan, 2015).

Most of the foetuses wasted in this study were in the second and third trimesters (79.2 %), a finding which is consistent with the reports of Wosu (1988), Ndi *et al.* (1993), Fayemi *et al.* (2008) and Babatunde *et al.* (2011) who found that 74 %, 64.1 %, 75.7 % and 77.2 % of the foetuses recovered

respectively were in the second and third trimesters. This observation suggests that slaughtering of pregnant cows must be for reasons other than ignorance of pregnancy status of the animal as pregnancy in the 2nd and 3rd trimester are relatively easy to detect (Ndi, 1990). However, the number of parity can also affect the enlargement of the uterus, but it is very difficult for the Fulani man to claim ignorance of the pregnancy status of their female animals at these stages. Therefore, selling of cows carrying pregnancy of 4-9 months may be due to the fact that pregnant cattle assume a bulky posture or conformation thus attracting better price (FAO, 1993).

From the results of this study, the monetary losses if foetuses were allowed to be calved were enormous. Per annum, ₦16,275,600 or ₦20,344,500 was lost due to foetal wastage, which poses a significant threat not only to the farmers and the livestock sub-sector but also to the economy of the nation particularly the depleting situation of animal proteins. This undesirable practice of wasting foetuses can be converted into a vibrant business investment sector therby saving the enormous loss due to foetal wastage, consequently maintaining and even improving the of the national cattle population as well as creating employment for the citizens,food security for the nation, income earning to the farmers and the economy as a whole.

A seroprevalence of 13.98 % for 8 serovars of *Leptospira interrogans* and 5.2% for *Leptospira* Serovar Hardjo were demonstrated using MAT and ELISA respectively. The presence of antibodies to *Leptospira* in this group of foetuses suggests antigenic stimulation since normally the sera of bovine foetuses or precolostral calves are devoid of maternally derived immunoglobulin (Brambell, 1970). Bovine foetus is able to respond immunologically to many antigens from an early age (Schultz, 1973). Transplacental transmission of leptospires to foetuses has been demonstrated by Ellis *et al.* (1982a). The infected foetuses in this study may have

survived the acute bacteraemic phase of infection or still, infection of the foetuses could have caused abortion, premature live birth or still birth if the pregnancy had not been terminated by slaughter. Studies have shown that some foetuses were able to survive experimental and natural foetal infection with *Leptospira* and produce specific antibodies (Fennestad and Borg-Peterson 1957; 1958; 1962a; 1962b; Ellis and Michna, 1976; Ellis and Michna, 1977); however, they are mostly delivered weak or prematurely (Ellis and Michna, 1976; 1977). Although, most foetuses are aborted before they are able to produce antibodies (Fennestad and Borg-Peterson 1957; 1958; 1962b; Ellis *et al.,* 1982b), some have been shown to produce antibodies before they are aborted (Kirkbride *et al.,* 1977; Ellis *et al.,* 1978a). Ellis *et al*. (1982b) demonstrated leptospires in 4.6% of viable (abattoir) foetuses but none had detectable leptospiral antibodies. Ellis and Michna (1976) demonstrated the presence of leptospires in aborted foetuses and in a premature calf. From experimental and clinical evidence, *Leptospira interrogans* serovar Hardjo has been suspected as an abortifacient in cattle (Fennestad and Borg-Peterson 1957; 1958; 1962b; Ellis *et al.,* 1982a). Serovar Icterohaemrrhagiae has been demonstrated to cause abortion (Ellis *et al*., 1978a). In Nigeria, although the epidemiology of *Leptospira interogans* has been studied in different parts of the country (Diallo, 1978; Diallo and Dennis, 1982; Ezeh and Agba, 1982; Ezeh, 1991; Onyemlukwe, 1993; Agunloye *et al*., 1996; 2000; 2002; Ngbede *et al.*, 2012), little or no attention has been paid to the potential occupational hazard or abortifacient role of this organism in foetuses.

Higher seroprevalence of leptospirosis was seen in the second and third trimester. This could probably be due to the greater ease of invasion of the placenta at these age or due to the fact that at the second and third trimester the foetuses are immunologically competent and were able to survive infection long enough to produce detectable levels of circulating antibody. This may be

the reason that none of the 1st trimester foetuses was positive. Ellis *et al.* (1978) and Fennestad and Borg-Petersen (1962b) demonstrated leptospiral antibodies in foetuses that were in the second trimester.

Only three sera were positive at titre of >100 by MAT (Appendix VIII). The reason for this low titre could be that the immune system of a foetus is not strong enough to produce very high titre of antibodies. Low titre to *Leptospira* in serum of aborted and premature live calf has been demonstrated by Ellis and Michna (1977) and Ellis *et al.* (1978a).

These infected foetuses may serve as sources of infection to humans who handle and consume them or to animals fed these foetuses. Although, there is limited data on the proportion of foetuses going for human consumption, the practice is going on and has largely been ignored. This prevailing practice is of great public health concern and probably, these infected foetuses may serve as sources of infection to humans and animals.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

This study has established that foetal wastage is still common and is on the increase in old Kano abattoir. Foetal wastage was observed to be more prevalent during the farming and wet season than in other seasons of the year. In addition, it was observed that pregnant cows within their second trimester of pregnancy were more frequently slaughtered than those within their first or third trimester of pregnancy. Furthermore, this study revealed that there was one case of foetal wastage in every nineteen cows slaughtered in old Kano abattoir during the two months study period. An estimated cost of ₦162,756,000 or ₦203,445,000 was lost due to foetal wastage, these was of great economic consequences to both the farmers and the livestock sub-sector. More importantly, it may lead to a huge depletion of the future productive herd or baseline parent stock thereby decreasing the source of animal protein available to Nigerians which will further impede the nation's quest to attain the FAO/WHO recommended protein intake for the populace.

This study further revealed a seroprevalence of 5.2 % and 13.9 % for *Leptospira* serovar Hardjo and *Leptospira interrogans* species in wasted foetuses using ELISA and MAT respectively in Old Kano Abattoir. The Presence of *Leptospira* species in foetuses poses an occupational and health risk for abattoir workers, meat processors, the public and also pet animals that come in contact with or consume infected foetuses/placentas. Abattoir workers are at risk of being infected by the organism as they do not generally wear adequate personal protective equipment, such as gloves and boots. It may also be correct to presume that, the detection of *Leptospira*

*interogans* in wasted foetuses in old Kano abattoir may be involved in causing bovine abortion/stillbirth or birth of weak calves observed in some farms in Nigeria.

## 6.2 Recommendations

Based on the aforementioned conclusion, the following recommendations are hereby made:

i. Government should be enlightened on the effect of foetal wastage on the national herd population with emphasis on the need to save, maintain and even improve cattle population.

ii. Government should enforce the existing laws regulating the slaughter of pregnant animals to eliminate or minimize the indiscriminate slaughter of pregnant cows in abattoirs with powers to prosecute persons found guilty and putting up funds for compensation.

iii. Effective and workable measures to discourage farmers and cattle owners from selling pregnant cows should be developed by relevant agencies/bodies/stakeholders by educating them on the economic implication of slaughtering pregnant cows through enlightenment campaigns.

iv. Abattoirs and slaughter houses should have qualified veterinarians and there should be strict ante-mortem examination of cows to be slaughtered so as to discourage the slaughter of pregnant cows.

v. Educational and public enlightenment on the zoonotic implications of handling or consumption of potential *Leptospira* infected foetuses in abattoirs should be routinely conducted for abattoir workers and the public in general.

vi. Abattoir workers should be encouraged to wear personal protective clothing to avoid direct contact with potentially infected foetuses.

vii. Further studies to determine the rate of foetal wastage and seroprevalence of *Leptospira* species in wasted foetuses should be undertaken in abattoirs nationwide to establish national figures for foetal wastage and prevalence of leptospirosis in Nigeria.

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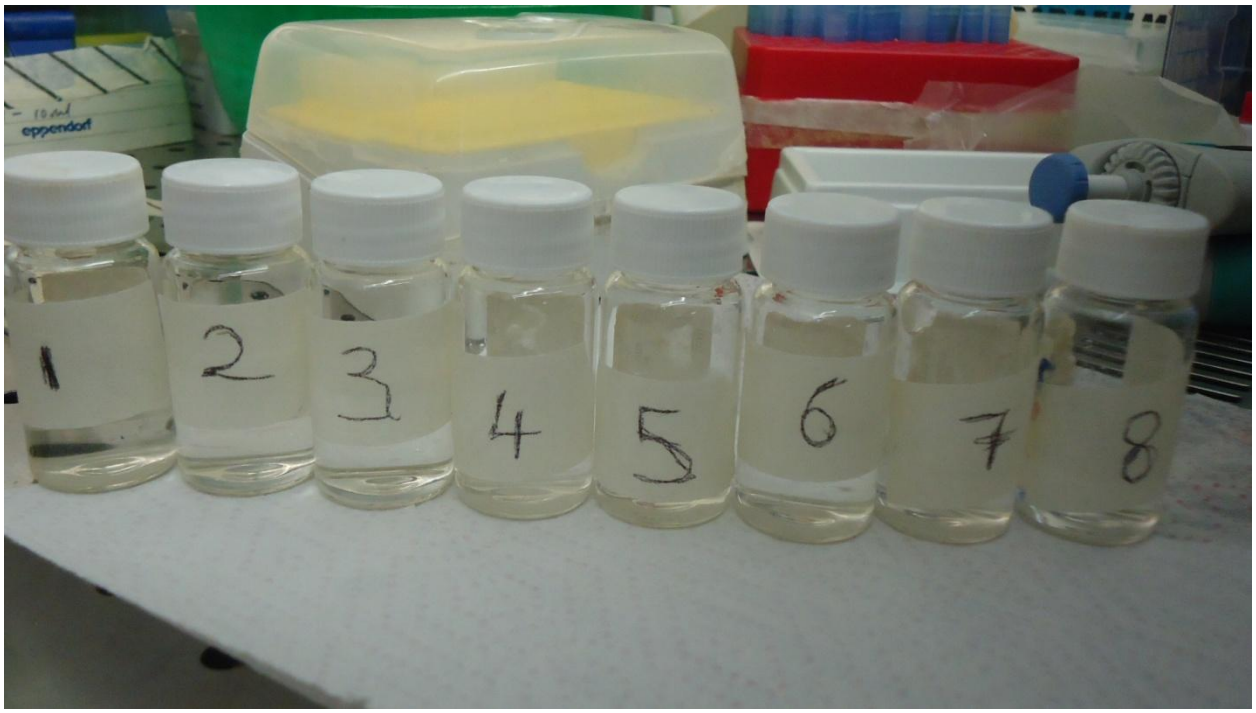
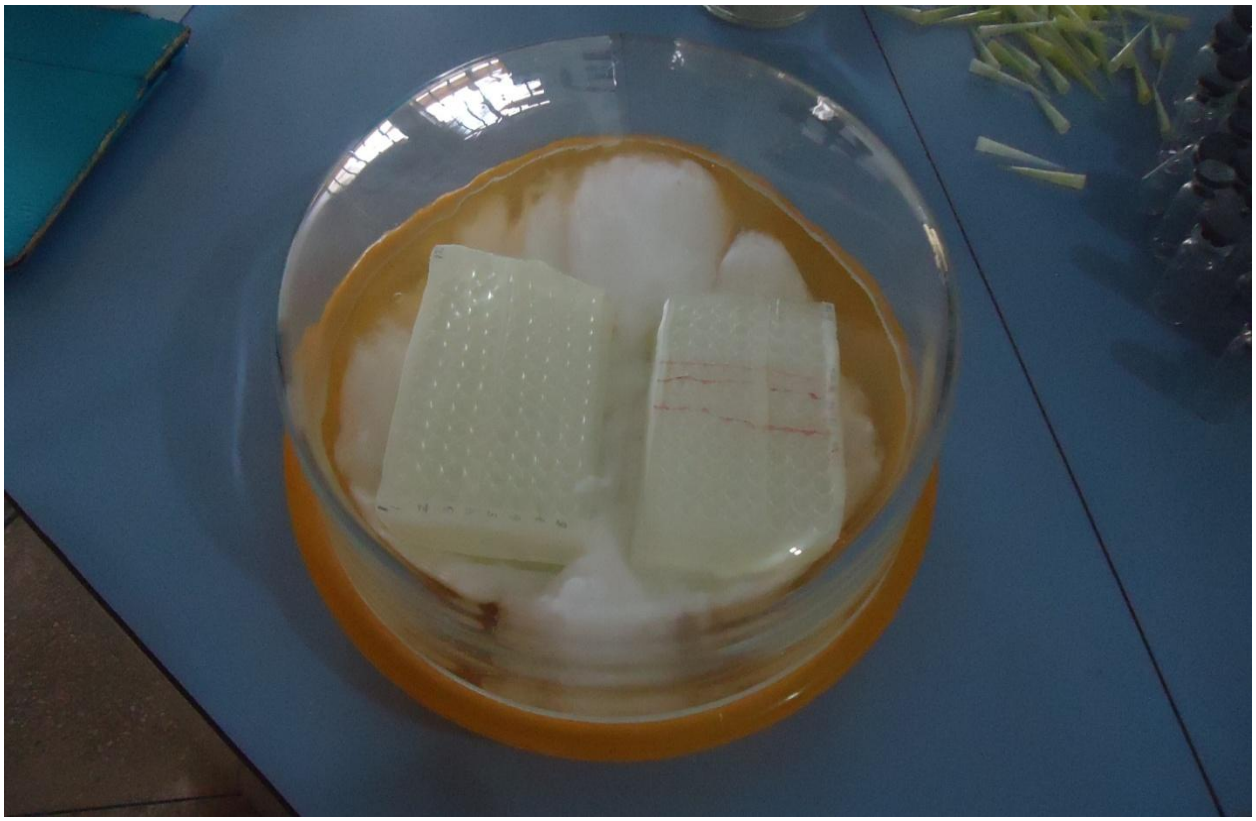
## APPENDICES

**Appendix I:** Flayed bovine foetus intended for human consumption in old Kano abattoir, Kano State.

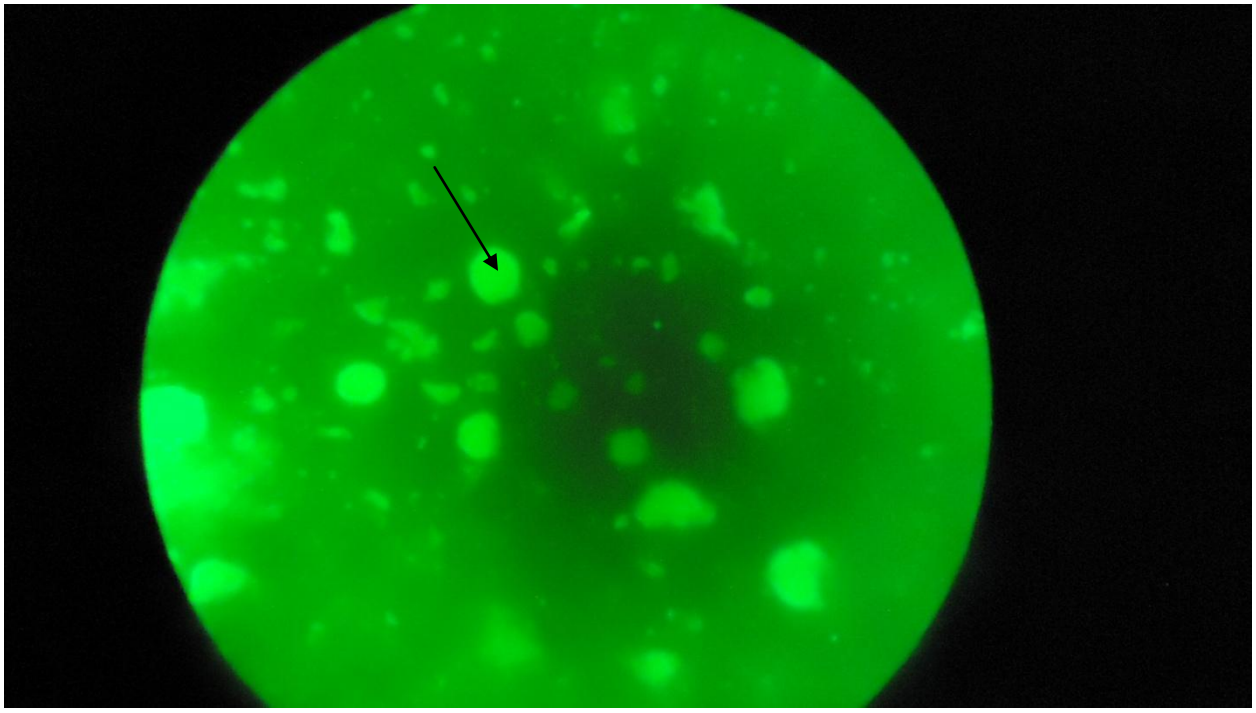



**Appendix II:** Tubes containing serovars of *Leptospira* spp; namely Icterohaemorrhagiae, Tarassovi, Pomona, Grippotyphosa, Australis, Canicola, Hardjo, and Mini.


**Appendix III:** Microplate titre plate with sealed serology plates containing serum and antigen.



**Appendix IV:** Plates placed in an improvised humid chamber



**Appendix V:** A positive serum-antigen agglutination reaction slide showing whitish clumping of the serum under Dark field Microscope using x200 magnification.

**Appendix VI:** ELISA Reagents Kit

**Appendix VII:** Enzyme Linked immunosorbent assay Optical Density (OD) readings of samples using ELISA reader at 450nm with a correction of 630nm

Plate 1

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.107 | 0.035 | 0.062 | 0.07 | 0.099 | 0.14 | 0.386 | 0.321 |
| 0.091 | 0.352 | 0.079 | 0.168 | 0.055 | 0.091 | 0.396 | 0.512 |
| 0.103 | 0.11 | 0.054 | 0.168 | 0.075 | 0.081 | 0.349 | 0.614 |
| 0.291 | 0.327 | 0.137 | 0.087 | 0.222 | 0.141 | 0.331 | 0.502 |
| 0.167 | 0.243 | 0.1 | 0.07 | 0.055 | 0.217 | 0.334 | 0.034 |
| 0.162 | 0.053 | 0.161 | 0.095 | 0.141 | 0.103 | 0.399 | 0.111 |
| 0.21 | 0.04 | 0.092 | 0.058 | 0.201 | 0.107 | 0.412 | 0.118 |
| 0.06 | 0.068 | 0.257 | 0.049 | 0.047 | 0.161 | 2.975 | 0.246 |
| 0.047 | 0.057 | 0.241 | 0.1 | 0.043 | 0.916 | 0.359 | 0.952 |
| 0.043 | 0.089 | 0.064 | 0.042 | 0.151 | 0.079 | 0.341 | 1.255 |
| 0.043 | 0.037 | 0.044 | 0.295 | 0.041 | 0.11 | 0.43 | 2.714 |
| 0.087 | 0.251 | 0.13 | 0.126 | 0.096 | 0.042 | 0.419 | 2.757 |

Plate 2

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.062 | 0.3 | 0.05 | 0.039 | 0.214 | 0.039 | 0.359 | 0.053 |
| 0.046 | 0.052 | 0.03 | 0.041 | 0.111 | 0.037 | 0.331 | 0.121 |
| 0.05 | 0.041 | 0.039 | 0.047 | 0.043 | 0.051 | 0.372 | 0.048 |
| 0.057 | 0.034 | 0.041 | 0.031 | 0.034 | 0.037 | 0.342 | 0.033 |
| 0.056 | 0.029 | 0.018 | 0.03 | 0.032 | 0.023 | 0.299 | 0.037 |
| 0.061 | 0.037 | 0.028 | 0.044 | 0.033 | 0.023 | 0.412 | 0.031 |
| 0.068 | 0.281 | 0.02 | 0.023 | 0.029 | 0.023 | 0.606 | 0.028 |
| 0.081 | 0.045 | 0.031 | 0.027 | 0.027 | 0.022 | 0.645 | 0.078 |
| 0.033 | 0.029 | 0.025 | 0.023 | 0.027 | 0.026 | 0.412 | 3.272 |
| 0.412 | 0.038 | 0.036 | 0.023 | 0.015 | 0.044 | 0.802 | 3.529 |
| 0.052 | 0.058 | 0.04 | 0.036 | 0.032 | 0.109 | 0.391 | 2.508 |
| 0.057 | 0.033 | 0.029 | 0.037 | 0.04 | 0.034 | 0.405 | 2.529 |

Plate 3

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.036 | 0.016 | 0.022 | 0.032 | 0.042 | 0.021 | 0.304 | 0.023 |
| 0.015 | 0.06 | 0.042 | 0.043 | 0.035 | 0.021 | 0.322 | 0.019 |
| 0.018 | 0.036 | 0.013 | 0.019 | 0.05 | 0.037 | 0.248 | 0.049 |
| 0.046 | 0.009 | 0.015 | 0.112 | 0.052 | 0.032 | 0.2 | 0.017 |
| 0.021 | -0.003 | 0.025 | 0.002 | 0.03 | 0.028 | 0.128 | 0.025 |
| 0.025 | 0.001 | 0.012 | 0.008 | 0.027 | 0.01 | 0.144 | 0.021 |
| 0.015 | 0.006 | 0.003 | 0.012 | 0.024 | 0.02 | 0.02 | 0.014 |
| 0.031 | 0.009 | 0.004 | 0.004 | 0.039 | 0.043 | 0.157 | 0.029 |
| 0.027 | 0.012 | 0.002 | 0.016 | 0.037 | 0.024 | 0.191 | 0.056 |
| 0.023 | 0.008 | 0.01 | 0.014 | 0.054 | 0.021 | 0.163 | 0.074 |
| 0.064 | 0.034 | 0.027 | 0.027 | 0.058 | 0.016 | 0.199 | 3.351 |
| 0.024 | 0.014 | 0.023 | 0.023 | 0.024 | 0.012 | 0.318 | 3.286 |

Plate 4

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.027 | 0.014 | 0.025 | 0.029 | 0.019 | 0.012 | 0.286 | 0.011 |
| 0.056 | 0.046 | 0.017 | 0.035 | 0.014 | 0.017 | 0.263 | 0.055 |
| 0.022 | 0.009 | 0.014 | 0.018 | 0.005 | 0.011 | 0.272 | 0.015 |
| 0.018 | 0.013 | 0.014 | 0.014 | 0.014 | 0.03 | 0.311 | 0.018 |
| 0.021 | 0.008 | 0.015 | 0.016 | 0.032 | 0.015 | 0.275 | 0.011 |
| 3.397 | 0.009 | 0.033 | 0.013 | 0.013 | 0.038 | 0.297 | 0.075 |
| 0.042 | 0.012 | 0.013 | 0.009 | 0.006 | 0.014 | 0.287 | 0.023 |
| 0.053 | 0.006 | 0.008 | 0.013 | 0.013 | 0.038 | 0.289 | 0.02 |
| 0.018 | 0.005 | 0.016 | 0.013 | 0.007 | 0.05 | 0.266 | 0.017 |
| 0.021 | 0.009 | 0.016 | 0.018 | 0.047 | 0.014 | 0.344 | 0.023 |
| 0.012 | 0.005 | 0.011 | 0.038 | 0.015 | 0.12 | 0.458 | 0.018 |
| 0.013 | 0.01 | 0.009 | 0.019 | 0.012 | 0.007 | 0.415 | 0.008 |

Plate 5

| | | | | |
|---|---|---|---|---|
| 0.188 | 0.074 | 0.091 | 0.216 | 0.709 |
| 0.153 | 0.126 | 0.063 | 0.211 | 0.568 |
| 0.104 | 0.085 | 0.086 | 0.252 | |
| 0.181 | 0.119 | 0.074 | 1.902 | |
| 0.102 | 0.682 | 0.215 | 0.31 | |
| 0.076 | 0.132 | 0.071 | 0.744 | |
| 0.251 | 0.144 | 0.216 | 0.128 | |
| 0.086 | 0.064 | 0.078 | 0.213 | |
| 0.358 | 0.922 | 0.949 | 1.023 | |
| 0.625 | 0.864 | 0.162 | 0.777 | |
| 0.48 | 0.92 | 0.309 | 0.148 | |
| 0.545 | 0.945 | 1.172 | 0.24 | |

**Appendix VIII**: Distribution of *Leptospira* MAT antibody titres in bovine foetuses sampled from old Kano Abattoir.

| | Antibody titre | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Serovars** | **1:20** | **1:40** | **1:80** | **1:160** | **1:320** | **1:640** | **1:1280** | **1:2560** | **Total** |
| **L.Icterohaemorrhagiae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **L.Tarassovi** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **L.Pomona** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **L. Grippotyphosa** | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| **L. Australis** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **L. Canicola** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| **L. Hardjo** | 4 | 4 | 3 | 3 | 0 | 0 | 0 | 0 | 14 |
| **L. Mini** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | 8 | 6 | 4 | 3 | 0 | 0 | 0 | 0 | 21 |

**Appendix IX**: Statistical analysis of association between foetal wstage and season.

**SEASON * FOETAL WASTAGE Cross tabulation**

Count

| | | DISEASE | | Total |
|---|---|---|---|---|
| | | POSITIVE | NEGATIVE | |
| SEASON | WET SEASON | 7013 | 239165 | 246178 |
| | DRY SEASON | 6550 | 268077 | 274627 |
| Total | | 13563 | 507242 | 520805 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 110.044[a] | 1 | .000 | | |
| Continuity Correction[b] | 109.862 | 1 | .000 | | |
| Likelihood Ratio | 109.839 | 1 | .000 | | |
| Fisher's Exact Test | | | | .000 | .000 |
| Linear-by-Linear Association | 110.044 | 1 | .000 | | |
| N of Valid Cases | 520805 | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6411.06.

b. Computed only for a 2x2 table

**Appendix X**: **Statistical analysis of association between foetal wastage and years**

**YEAR * FOETAL WASTAGE Cross tabulation**

Count

| | | DISEASES | | Total |
|---|---|---|---|---|
| | | POSITIVE | NEGATIVE | |
| YEAR | 2005-2009 | 4715 | 235518 | 240233 |
| | 2010-2014 | 8848 | 271724 | 280572 |
| Total | | 13563 | 507242 | 520805 |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 723.631[a] | 1 | .000 | | |
| Continuity Correction[b] | 723.161 | 1 | .000 | | |
| Likelihood Ratio | 738.610 | 1 | .000 | | |
| Fisher's Exact Test | | | | .000 | .000 |
| Linear-by-Linear Association | 723.629 | 1 | .000 | | |
| N of Valid Cases | 520805 | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6256.24.

b. Computed only for a 2x2 table

**Appendix XI:** Statistical analysis of association between seropositive samples and years using ELISA.

**Age * Disease (ELISA) Cross tabulation**

Count

| | | Disease | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Age | 1-3 months | 0 | 32 | 32 |
| | 4-6 months | 13 | 243 | 256 |
| | 7-9 months | 8 | 76 | 84 |
| Total | | 21 | 351 | 372 |

**Chi-Square Tests**

| | Value | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) | Point Probability |
|---|---|---|---|---|---|---|
| Pearson Chi-Square | 4.442[a] | 2 | .109 | .110 | | |
| Likelihood Ratio | 5.866 | 2 | .053 | .062 | | |
| Fisher's Exact Test | 3.902 | | | .126 | | |
| Linear-by-Linear Association | 4.417[b] | 1 | .036 | .040 | .029 | .018 |
| N of Valid Cases | 372 | | | | | |

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.81.

b. The standardized statistic is -2.102.

**Appendix XII**: Statistical analysis of association between seropositive samples and years using MAT.

**Age * Disease (MAT) Cross tabulation**

Count

| | | Disease | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Age | 1-3monts | 0 | 32 | 32 |
| | 4-6monts | 43 | 213 | 256 |
| | 7-9monts | 9 | 75 | 84 |
| Total | | 52 | 320 | 372 |

**Chi-Square Tests**

| | Value | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) | Point Probability |
|---|---|---|---|---|---|---|
| Pearson Chi-Square | 7.635[a] | 2 | .022 | .020 | | |
| Likelihood Ratio | 12.041 | 2 | .002 | .004 | | |
| Fisher's Exact Test | 8.797 | | | .011 | | |
| Linear-by-Linear Association | .229[b] | 1 | .633 | .679 | .366 | .098 |
| N of Valid Cases | 372 | | | | | |

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.47.

b. The standardized statistic is -.478.