# DETECTION OF *Chlamydophila abortus* ANTIBODIES IN ABORTED SHEEP AND GOATS IN NORTHERN AND CENTRAL PARTS OF TARABA STATE

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**MARCH, 2015**

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**M.Sc./Vet.Med/08980/2010-2011**

**A THESIS SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN THERIOGENOLOGY**

**DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION,**
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## DECLARATION

I declare that the work in this thesis titled "Detection of *Chlamydophila abortus* antibodies in aborted sheep and goats in Northern and Central parts of Taraba State" has been carried out by me in the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma in this or any other institution.

Abubakar MUKHTAR

| Name of Student | Signature | Date |
|---|---|---|

## CERTIFICATION

This thesis titled "DETECTION OF *CHLAMYDOPHILA ABORTUS* ANTIBODIES IN ABORTED SHEEP AND GOATS IN NORTHERN AND CENTRAL PARTS OF TARABA STATE" by Abubakar MUKHTAR meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

I dedicate this work to my late mother Hafsatu Ma'aji Bello, my late brother Umar Mukhtar Umar, and my father Malam Mukhtar Umar (Sarkin Shanun Muri).

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## ABSTRACT

A field study was conducted to determine serologically the aetiological role of *Chlamydophila abortus* in small ruminant abortion in northern and central areas of Taraba State, Nigeria. A total of 368 serum samples from aborted ewes and does were collected from 39 randomly selected villages in four Local Government Areas (LGAs) in the study area. The sera were tested for the presence of raised levels of *Chlamydophila abortus* antibodies using ELISA technique. *Brucella*-antibody test was also conducted as differential diagnosis to *Chlamydophila abortus* using the same technique. The prevalence of abortion in small ruminants in the study area was determined as a percent of breeding females in the sheep flocks and goat herds that were investigated. The result revealed an overall prevalence of 25.6% abortion in ewes and does over a two-year period. There was no significant ($P>0.05$) difference in the prevalence of abortion between sheep and goats. Fourteen animals tested positive for *Chlamydophila abortus* antibodies which gave a prevalence of 3.8%. *Chlamydophila abortus*-positive animals were detected in each of the four LGAs sampled. There was no significant ($P>0.05$) difference between sheep and goats in the prevalence of *Chlamydophila abortus* in this study. *Brucella*-antibodies were detected in nine animals in only one out of the four LGAs. The study has established for the first time, an evidence of chlamydial abortion in sheep and goats in northern and central areas of Taraba State. The higher prevalence and wider geographical spread of *Chlamydophila abortus*-related abortions compared to *Brucella*-related abortions indicated that *Chlamydophila abortus* may be a more important pathogen causing abortion in small ruminants in the study area than *Brucella* spp.

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## ABBREVIATIONS

| | |
|---|---|
| BCV | *Brucella*-Containing Vacuoles |
| BGM | Buffalo Green Monkey Cells |
| BHK | Baby Hamster Kidney Cells |
| CFSPH | Center for Food Security and Public Health, Iowa State University |
| CFT | Complement Fixation Test |
| CFU | Colony Forming Units |
| EAE | Enzootic Abortion of Ewes |
| EB | Elementary Body |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| FAO | Food and Agricultural Organisation |
| IDO | Indoleamine 2,3-Deoxygenase |
| IFN-γ | Gamma Interferon |
| IFT | Immunofluorescence Test |
| IgG | Immunoglobulin G |
| LGA | Local Government Area |
| LPS | Lipopolysaccharide |
| MOMP | Major Outer Membrane Protein |
| NADIS | National Animal Disease Information Service of the United Kingdom |
| NVRI | National Veterinary Research Institute |
| OEA | Ovine Enzootic Abortion |
| OIE | Organisation Internationale des Epizooties |
| PCR | Polymerase Chain Reaction |
| POMP | Polymorphic Outer Membrane Protein |
| RB | Reticulate Body |
| RBT | Rose Bengal Test |

| | |
|---|---|
| RER | Rough Endoplasmic Reticulum |
| SAT | Serum Agglutination Test |
| spp | Species |
| TNF-α | Tumor Necrosis Factor-alpha |
| %OD | Percent Optical Density |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND

*Chlamydophila abortus,* formally called *Chlamydia psittaci* serotype 1, is a Gram-negative, obligate intracellular parasite which belongs to the family *Chlamydiaceae* (Longbottom *et al.,* 2002). It is an economically important pathogen of small ruminants that has a specific predilection for the pregnant uterus (Smith, 2001). The organism is a common cause of reproductive disorder in both sheep and goats in almost every region of the world (Menzies, 2012), causing a disease known as enzootic abortion of ewes (EAE), ovine enzootic abortion (OEA), or ovine chlamydial abortion (Abd-El-Razik *et al.,* 2011). *Chlamydophila abortus* is generally introduced into immunologically naïve flocks by latently infected animals, which when become pregnant, abort and shed large amounts of the infectious chlamydiae in the foetal membranes and vaginal discharges (Gerber *et al.,* 2007). The excreted organisms contaminate the environment, which exposes susceptible ewes and does to the disease through ingestion or inhalation of *Chlamydophila abortus*-infected materials (Livingstone *et al.,* 2005). Following exposure, the organisms become localized in the pharyngeal lymphoid tissues and tonsils from where they systemically disseminate via blood or lymphatic circulation (Longbottom *et al.,* 2013). Infection of pregnant ewes/does from early to mid gestation results in abortion within the last two to three weeks of pregnancy (Buxton *et al.,* 1990), while in non-pregnant animals the infection becomes established in a latent form with no apparent clinical signs until the next pregnancy when the organisms multiply and invade

the placenta, eventually causing abortion (Entrican *et al.,* 2001). The aborted foetuses are usually well-developed without gross pathological lesions suggesting that placentitis is the key element in the pathogenesis of ovine enzootic abortion (Buxton *et al.,* 2002). The aborted ewes/does become carriers and continue to excrete the infectious organisms during oestrus and subsequent lambings/kiddings, thereby continuing to contaminate the environment and spread the infection (Papp *et al.,* 1994).

Due to the latent nature of *Chlamydophila abortus* infection, the organism cannot be detected either serologically or by direct detection of the pathogen until the time of abortion, when infectious organisms are excreted and maternal titres of antibody to the organisms rapidly increase. This coincides with the development of protective immunity (Livingstone *et al.,* 2005). As a result, diagnosis of enzootic abortion of ewes is made by serological demonstration of either raised or rising levels of *Chlamydophila abortus*-specific immunoglobulin G (IgG) antibodies between the acute and convalescent phases of infection (Griffiths *et al.,* 1996) using techniques such as complement fixation test (CFT), immunofluorescence test (IFT) and enzyme linked immunosorbent assay (ELISA) (Longbottom *et al.,* 2002).

*Chlamydophila abortus* is a zoonotic organism. Exposure of pregnant women to the organism results in its rapid growth in the unborn baby's placenta causing abortion within a week (Buxton, 1986). Most cases of *Chlamydophila abortus*-induced human abortions are due to direct association or exposure to contaminated sheep and goats (Entrican *et al.,* 2001). Transmission most probably occurs through contamination of food, smoking with unwashed hands, mouth-to-mouth resuscitation of weak lambs,

inhalation of contaminated air or contact with contaminated clothing (Longbottom and Coulter, 2003).

Ovine enzootic abortion has been reported in many regions of the world including Europe, North America, Middle East and Africa (Aitken, 1986; Papp *et al.,* 1993; Abassa, 1995; Mainar-Jaime *et al,* 1998; Gerber *et al,* 2007; Berri *et al,* 2009; Abd-El-Razik *et al.,* 2011). In the United Kingdom for instance, the disease accounts for about 50% of all diagnosed abortions, resulting in losses estimated to be in excess of £20 million each year (Longbottom *et al.,* 2002) and one in four flocks is estimated to be affected by the disease (Griffiths *et al.,* 1996). In other countries such as Switzerland, studies have shown that 39% of all diagnosed abortions in sheep and 23% in goats were caused by *Chlamydophila abortus* (Gerber *et al.,* 2007), while 46.8% of all ovine abortions in Canada were diagnosed to be caused by the organism (Papp *et al.,* 1993). The organism was also detected in 9.1% cases of abortion in small ruminants in Saudi Arabia (Abd-El-Razik *et al.,* 2011). In Africa, EAE has been reported in most ecological zones ranging from semi-arid regions of Chad and Tunisia, sub-humid zone of Nigeria to humid zone of Cote d'lvoire (Lefevre, 1979; Okoh, 1986; Armbrustrer, 1988; Abassa, 1995; Berri *et al.,* 2009).

In addition to EAE, brucellosis is an important and common abortion disease of small ruminants (Abd-El-Razik *et al.,* 2011) and has been recognized as a major cause of economic losses to the livestock industry worldwide through decreased productivity and restriction of international trade (Ocholi *et al.,* 2005; CFSPH, 2007). The main causative agent of brucellosis in sheep and goats is *Brucella melitensis* (Megid *et al.,* 2010). Sporadic infections of small ruminants by *Brucella abortus* and *Brucella suis* have also

been reported (OIE, 2009). Studies in various parts of Nigeria reported that the disease is prevalent in domestic ruminants, causing significant losses in form of abortion, infertility, low conception rate and low survival rate of neonates (Bertu *et al.,* 2010; Mbuk *et al.,* 2011). The role of *Brucella abortus* as a causative agent of abortion in sheep herded together with cattle has been established in some parts of the country (Okoh, 1980; Bale *et al*., 1982; Bawa *et al.,* 1987; Ocholi *et al.,* 2005). Brucellosis is therefore an important disease that needs to be differentiated from EAE due to their similarity in terms of clinical sign of abortion and its endemic nature in Nigeria.

## 1.2 STATEMENT OF RESEARCH PROBLEM

Enzootic abortion of ewes is one of the most common causes of reproductive failure in sheep and goats in most small ruminant-rearing countries (Menzies, 2012). The major consequences of the disease are abortion storms affecting 30 to 90% of pregnant ewes and does, stillbirth, birth of weak or moribund low birth weight lambs which fail to survive beyond 48 hours and occasionally foetal resorption and mummification. All these culminate into gross reduction in the expected number of kids and lamb crops born into a flock/herd each year, resulting in heavy financial losses by the affected farmers (Papp *et al.,* 1993; Papp and Shewen, 1996; Rodolakis, 2001; Smith, 2001; Mearns, 2007).

The first case of EAE in Nigeria was reported as an outbreak in a research farm (Okoh, 1986). Seven years later, serological evidence of the disease was reported in sheep and goats in Maiduguri metropolis (Amin and Wilsmore, 1993). Since then, there was no additional report concerning chlamydial abortion and the extent to which it contributes to reproductive losses in small rumunants in Nigeria. To the best of our

knowledge, the current status of chlamydial abortion and its effect on reproductive performance of sheep and goats in Nigeria is generally unknown.

## 1.3 JUSTIFICATION OF THE RESEARCH

Agriculture is central to Nigeria's socio-economic development and is crucial to the economic transformation of the rural sector. Livestock provides a suitable means for this rural transformation due to its national spread, contribution to rural economic growth and provision of all-year-round regular income (Oyedipe, 2011). Nigeria has an estimated livestock population of 199.6 million, which comprises 22.1 million sheep and 34.5 million goats (Bourn *et al.*, 1994). These and other animal resources have the potential to raise the income and livelihood of a large segment of the population thereby elevating them from poverty (Oyedipe, 2011). In contrast to cattle which are normally concentrated in the hands of restricted number of producers, almost every low-income rural household invariably owns small ruminants, which serve as source of food and financial security for the rural poor (Abassa, 1995). Abassa (1995) stated that small ruminants' annual population growth rate in Sub-Saharan Africa (Nigeria inclusive) was less than 1% which was the world's lowest growth rate over decades. Among the reasons that could be attributed to this poor performance are reproductive losses, which are widely recognized as the most important constraints to increased small ruminant production (Kelly, 1986). Such reproductive losses have generally been attributed to embryonic mortality, abortions and stillbirths. Despite the frequent occurrence of abortion and related causes of infertility, exhaustive investigations of the causative agents have not been undertaken in most African countries (Kasali *et al.,* 1989). As a result of this, reports on clinically

diagnosed diseases responsible for abortions in Sub-Saharan Africa are few in the literature (Abassa, 1995).

Among the causes of abortion in small ruminants, EAE plays a significant role world wide. There was a case report of the disease in Nigeria in indigenous sheep (Okoh, 1986). Due to lack of documented information concerning the status of the disease in our domestic small ruminants over decades and the extent to which it contributes to reproductive losses and infertility, it is important to carryout studies to address such an information gap in different states of the Federation where sheep and goats contribute substantially to the household economies of the rural populations of such states. This study therefore intends to find the prevalence of enzootic abortion of ewes in aborted small ruminants in Taraba State, Nigeria. Information generated from the study could provide a baseline data which may be useful for further epidemiological investigations of this important production-limiting disease, and formulating preventive and control measures against it by both authorities and farmers, thereby enhancing the productivity of small ruminants and the economic benefits associated to their farming in our environment.

Since brucellosis has been extensively studied and largely recognized as the most important cause of abortion in livestock in Nigeria, its crucial role as a differential diagnosis of enzootic abortion of ewes should not be overlooked. It is therefore important to rule it out when trying to attribute abortion cases to other abortifacient organisms like *Chlamydophila abortus.* For this reason, serological test for brucellosis was conducted concurrent with the test for EAE in this research, in order to validate the role of *Chlamydophila abortus* as the causative agent of abortion in the animals being studied.

## 1.4 AIM OF THE STUDY

To investigate the presence of chlamydial abortion in ewes and does in Northern and Central parts of Taraba State, and differentiate it from *Brucella* abortion using serological technique.

## 1.5 OBJECTIVES

1. To detect the presence of *Chlamydophila abortus* antibodies in the sera of aborted sheep and goats in Northern and Central parts of Taraba State using ELISA technique.
2. To determine the prevalence of abortion in sheep flocks and goat herds in Northern and Central parts of Taraba State.
3. To differentiate chlamydial abortion from *Brucella* abortion in small ruminants in Northern and Central parts of Taraba State using ELISA technique.

## 1.6 RESEARCH QUESTION

What is the prevalence of *Chlamydophila abortus* infection in aborted sheep and goats in Northern and Central parts of Taraba State?

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 CHLAMYDIAL ABORTION

### 2.1.1 Introduction

Chlamydial abortion, also called ovine enzootic abortion (OEA) or enzootic abortion of ewes (EAE) is an infectious disease condition of small ruminants that causes serious reproductive wastage in sheep and goats worldwide, especially in flock/herds that are intensively managed over the parturient period (Aitken and Longbottom, 2007). The first published description of the disease was made in sheep by Greig (1936) who considered that it was due to nutritional deficiency, and named the condition "enzootic abortion". He however failed to experimentally confirm that dietary deficiency had any bearing on the disease (Longbottom and Coulter, 2003). Stamp *et al.* (1950) suggested that the disease was infectious following an observation that immunity developed after abortion. They reported experimentally-induced abortion in ewes inoculated with filtrates and suspensions of organs of aborted fetuses, obtained from farms with outbreaks of abortion. The group showed that the aborted foetal membranes possessed characteristic lesions that contained large numbers of small microorganisms that stained red with diluted Ziehl Neelson stain. Subsequently, Stamp (1951) reported that the organisms belong to the psittacosis lymphogranuloma venereum group of virus-like agents, and the following year Stamp *et al.* (1952) developed complement fixation test for field diagnosis of the disease. Later evidence established that the organisms were prokaryotic bacteria

that were fundamentally distinct from viruses, and were classified into the genus *Chlamydia* (Page, 1966).

### 2.1.2 Distribution

Chlamydial abortion is recognized as a major cause of reproductive failure in many sheep rearing areas of the world (Longbottom and Coulter, 2003). In countries of Northern Europe, the disease is the most common infectious cause of abortion in lowland flocks that are intensively managed (Stuen and Longbottom, 2011). In Southern Europe, however, *Brucella melitensis* infection is of greater prevalence (Aitken and Longbottom, 2007). Chlamydial abortion is reportedly absent in Australia and New Zealand (Menzies, 2012). In Africa, chlamydial abortion was diagnosed in the Western, Central and Southern sub-regions of the continent (Abassa, 1995; Ndou and Dlamini, 2012) and the causative agent was isolated and characterized in North Africa (Rekiki *et al.*, 2002). In Nigeria, chlamydial abortion was first diagnosed in an outbreak of abortion in a flock of sheep in a research farm in Vom, Plateau State (Okoh, 1986). Later on, evidence of exposure to *Chlamydophila abortus* was reported in sheep and goats in Maiduguri area (Amin and Wilsmore, 1993). Chlamydial abortion was also reported to be common in Middle East and the aetiological agent was isolated and characterized in Saudi Arabia and Palestine (Abd-El-Razik *et al.*, 2011; Manasrah, 2013). The disease is also common in North and South American continents affecting small ruminant flocks and herds (Jimenez-Estrada *et al.*, 2008). Evidence of exposure to the disease was recently reported on the Caribbean Islands (Stone *et al.*, 2012). Chlamydial abortion of goats is similar to sheep in severity but its geographical spread and economic impact are less defined because of lack of epidemiological data (Longbottom and Coulter, 2003).

### 2.1.3 Aetiology

Chlamydial abortion is caused by an obligate intracellular Gram-negative bacteria named *Chlamydophila abortus,* formerly called *Chlamydia psittaci* serotype 1 (Everett *et al.,* 1999). The organism belongs to a highly specialized bacterial family *Chlamydiaceae,* which undergoes a biphasic developmental cycle comprising two distinct developmental forms, the extracellular infectious elementary body (EB) and the intracellular non-infectious reticulate body (RB) (Stuen and Longbottom, 2011). All members of the family *Chlamydiaceae* are obligate intracellular bacteria and cause a wide range of disease conditions in man, other mammals and birds, which generally manifest in forms of abortion, infertility, enteritis, encephalomyelitis, conjunctivitis, polyarthritis or pneumonia (Longbottom and Coulter, 2003). *Chlamydophila abortus* is principally a pathogen of small ruminants that primarily affects the reproductive tract, causing late term abortion (Wilson *et al.,* 2009). The organism can survive and remain infective for weeks at low environmental temperatures and for years at -70$^{o}$C. However, its infectivity is destroyed at high temperature of 60$^{o}$C, or by treatment with ether or formalin (Aitken and Longbottom, 2007).

### 2.1.4 Taxonomy

*Chlamydophila abortus* belongs to the order *Chlamydiales*, family *Chlamydiaceae*, whose members are known to be the only exclusively intracellular parasites within the kingdom *Bacteria* (Everett *et al.,* 1999). Following the placement of all the members of the family *Chlamydiaceae* into a single genus *Chlamydia* (Page, 1968), the genus was later separated into two species, *Chlamydia trachomatis* and

*Chlamydia psittaci* on the basis of differences in the morphology of intracytoplasmic inclusions, presence or absence of glycogen in the inclusions, susceptibility to sodium sulphadiazine and natural host. Within each species, however, various serotypes could be distinguished on the basis of antigenic specificity of the components of the cell walls of the organisms (Page, 1968). The strain responsible for enzootic abortion of ewes is a member of *Chlamydia psittaci* serotype 1 group (Entrican *et al.*, 2001).

The development of DNA-based classification methods led to the designation of two *Chlamydia psittaci* strains into new species, *Chlamydia pneumoniae* (human respiratory pathogen) and *Chlamydia pecorum,* which is responsible for encephalomyelitis, pneumonia and polyarthritis in cattle and sheep (Grayston *et al.*, 1989; Fukushi and Hirai; 1992). In 1999, Everett *et al.* proposed the revision of classification of the family *Chlamydiaceae* based mainly on phylogenetic analysis of the 16s and 23s rRNA genes. One additional genus and five new species were then created. Thus, the family *Chlamydiaceae* has now been divided into two genera, *Chlamydia* and *Chlamydophila.* The genus *Chlamydia* contains three species, *Chlamydia trachomatis* (human strain), *Chlamydia suis* (porcine strain) and *Chlamydia muridarum* (mouse and hamster strain), while the new genus *Chlamydophila* was assigned six species namely *Chlamydophila psittaci* (avian strain), *Chlamydophila pneumoniae* (human strain), *Chlamydophila pecorum* (ruminant and swine strain) *Chlamydophila felis* (cat strain), *Chlamydophila caviae* (guinea pig strain) and *Chlamydophila abortus,* which is the classical serotype 1 responsible for OEA.

### 2.1.5 Host Range

Although *Chlamydophila abortus* is primarily associated with enzootic abortion in sheep and goats, the pathogen is a multi-host organism affecting a wide range of domestic and wild mammals as well as humans (Wilson *et al.,* 2009). *Chlamydophila abortus* is a recognized cause of abortion in cattle, but the incidences have been reported sporadically throughout the world (Wilson *et al.,* 2012). The pathogen has been isolated from abortion cases in horses, rabbits and llamas (CFSPH, 2005) and is a recognized cause of abortion in pigs (Longbottom and Livingstone, 2006; Wilson *et al.,* 2009). *Chlamydophila abortus* has been associated with ovarian hydrobursitis syndrome in camels (Ali *et al.,* 2012). Mice are used as laboratory models in the study of pathogenesis of abortion caused by *Chlamydophila abortus* infection (Rodolakis *et al.,* 1998). Few data are available on the prevalence and relevance of *Chlamydophila abortus* infection in wildlife hosts (Salinas *et al.,* 2008). The organism was isolated from an abortion case in springbok antelope in Paris zoo (Berri *et al.,* 2004). Serological evidence of infection with the organism was detected in a number of wild ruminants such as red deer, fallow deer, roe deer, alpine ibex, Iberian ibex and mouflon in Spain and Italy (Salinas *et al.,* 2008), as well as yaks in China (Chen *et al.,* 2014). Although human infection with *Chlamydophila abortus* is relatively uncommon, the biggest threat is to pregnant women because of the ability of the organism to colonise the human placenta. The infection in most cases is directly associated with exposure to infected sheep or goats and their aborted fetuses and offsprings. The outcome of such infection is acute influenza-like illness for some days, followed by spontaneous abortion in the first trimester of pregnancy, whereas later infection causes stillbirth or preterm labour (Longbottom and

Coulter, 2003). If left untreated, the infection in pregnant women may progress to septicaemia, hepatic and renal dysfunctions, pneumonia and disseminated intravascular coagulation, which may result in death (Buxton, 1986; CFSPH, 2005).

### 2.1.6 Life Cycle

*Chlamydophila abortus* undergoes a unique biphasic developmental cycle of replication characterized by two distinct morphological forms: the small extracellular, infectious, metabolically inactive elementary body (EB) (0.3 µm in diameter) and the larger intracellular, non-infectious, metabolically active reticulate body (RB) (0.5-1.6 µm in diameter) as well as by intermediate forms (Longbottom and Coulter, 2003). The developmental cycle is initiated by the attachment of an EB to the membrane of the host's epithelial cell where it is endocytosed into the cytoplasm and forms a membrane-bound vacuole called the inclusion. The EB then transforms back into metabolically active RB and replicates by binary fission (Moulder, 1991). As the RBs multiply, the inclusion rapidly fills and expands in size. After a period of 24-48 hours the RB progeny recondense back into metabolically inactive infectious EBs. The EBs are then released through host cell rupture or exocytosis, and go on to invade neighbouring cells (Moulder, 1991). The EB in contrast to RB, is characterized by resistance to both physical and chemical factors in the extracellular environment as well as lack of metabolic activity. This resistance is a consequence of the rigidity of the cell envelope which is both osmotically stable and poorly permeable, and also of the greatly reduced surface area of the EB compared to that of RB. Thus, the EB is adapted for prolonged extracellular survival which may mean many months outside the natural host (Longbottom and Coulter, 2003).

### 2.1.7 Transmission

Although *Chlamydophila abortus* causes reproductive disease, the common route of transmission to susceptible animals is oro-nasal (Entrican and Wheelhouse, 2006). The primary sources of infection are considered to be diseased placentae and vaginal discharges from aborting sheep and goats as well as aborted foetuses (Papp *et al.,* 1994; Livingstone *et al.,* 2009). Large amounts of infectious chlamydiae are excreted in these materials which lead to contamination of lambing/kidding environment, feed and pasture. Susceptible ewes and does become infected through ingestion or inhalation of *Chlamydophila abortus*-infected materials (Livingstone *et al.,* 2005; Gerber *et al.,* 2007). It has been suggested that following contact with infectious chlamydiae, the EBs become localized in the pharyngeal lymphoid tissues and tonsils, from where they disseminate systemically via blood or lymphatic circulation to reach the uterus and other organs (Papp *et al.,* 1994; Longbottom *et al.,* 2013). Experimental infection of tonsillar mucosa with virulent chlamydial organisms resulted in abortion in susceptible pregnant sheep (Jones and Anderson, 1988). Experimental infection of vaginal mucosa of non-pregnant ewes prior to breeding resulted in pregnancy failure, whereas subcutaneous infection of non pregnant ewes with the same virulent chlamydial organisms failed to induce abortion, but instead, conferred immunity against abortion when the sheep were rechallenged during pregnancy (Papp and Shewen, 1996). However, infection of ewes by either subcutaneous or vaginal route during pregnancy resulted in abortion (Papp and Shewen, 1996). This indicated that mucosal site is more susceptible to *Chlamydophila abortus* infection than subcutaneous site, and therefore more likely to be the natural route of entry of the organisms (Entrican and Wheelhouse, 2006).

The observations that ewes that experienced *Chlamydophila abortus*-induced abortion become carriers and excrete the organisms from the reproductive tract during oestrus (Papp *et al.*, 1994), and that experimental vaginal infection during oestrus could cause abortion in naïve (previously unexposed) ewes (Papp and Shewen, 1996) suggested that venereal transmission or mechanical transfer of the organisms by rams during mating could play an important role in the spread and perpetuation of ovine enzootic abortion within a flock. However, recent study using a more specific, quantitative real-time PCR assay revealed that the amount of detectable *Chlamydophila abortus* genomes found in the vaginal swabs taken during oestrus was several fold lower than the experimental dose required to elicit abortion (Livingstone *et al.,* 2009). The authors therefore concluded that although this finding does not discount the possibility of chronic low level infection persisting in post-abortion ewes, the low level of chlamydial DNA detected does not support the idea of excretion of *Chlamydophila abortus* at oestrus as a major contributory factor in the transmission of ovine enzootic abortion via rams during mating. Thus, the products of abortion represent the major and principal source of transmission to naïve ewes and should be of greatest concern when taking management strategies to minimize or limit the spread of the disease within a flock.

### 2.1.8 Pathogenesis

#### *2.1.8.1 Latent Infection*

The disease ovine enzootic abortion typically presents itself in late gestation as a result of latent infection with *Chlamydophila abortus* acquired prior to pregnancy (Entrican *et al.,* 2012). These latently infected animals are very difficult to identify since

they do not typically exhibit clinical signs prior to abortion (Entrican *et al.,* 2012). The term latency could be defined as a static phase of the pathogen that is asymptomatic for the host (Rocchi *et al.,* 2009). The main recognized feature of *Chlamydophila abortus* infection in non-pregnant sheep/goat is the establishment of latency and subsequent recrudescence (emergence) to invade the placenta at a given time during pregnancy (Longbottom *et al.,* 2013). Study of the early phase of the pathogenesis of OEA showed that chlamydial antigens could be detected in epithelial cells and lymphocytes in some organs and lymph nodes, but attempt to isolate the organism from these tissues proved abortive (Amin and Wilsmore, 1995). Systemic dissemination of the organism to other organs was reported to be suppressed by the host immune response as early as 30 days post infection, thus forcing it into latency (Navarro *et al.,* 2004). However, the exact location where latency is established remains unknown (Navarro *et al.,* 2004; Entrican *et al.,* 2012). Similarly, the underlying mechanisms that control the series of events that lead to recrudescence of the organism from the site of latency and subsequent invasion of the placenta at a particular stage of gestation are poorly understood (Rocchi *et al.,* 2009; Longbottom *et al.,* 2013). The establishment of latency depends on the dose of chlamydial EBs taken by the susceptible host. Lower uptake results in insufficient immunological stimulation to induce protective immunity, thereby permitting a latent intracellular infection to persist and recrudesce in the next pregnancy (Longbottom *et al.,* 2013). In contrast, higher uptake appeared to invoke strong immune response that prevents the establishment of latency, thereby resulting in much lower abortion rates (Longbottom *et al.,* 2013). Recrudescence of latent infection is thought to occur as a result of immune modulation that allows chlamydial multiplication and intermittent low-

level chlamydaemia (Entrican *et al.,* 2001). This in turn initiates infection of the placenta which is particularly vulnerable to *Chlamydophila abortus* after 90 days gestation (Buxton *et al.,* 1990; Longbottom *et al.,* 2013). Susceptible animals that acquire the infection while pregnant will abort during the same pregnancy without developing latency (Navarro *et al.,* 2004).

*2.1.8.2 Placental Infection*

Following disruption of the state of latency and subsequent dissemination of the organisms to the gravid uterus, the chorionic epithelial cells (trophoblasts) are the first to be invaded by *Chlamydophila abortus* (Entrican *et al.,* 2001). Although reports indicated that the placental infection starts in the maternal epithelial cells of the placentomes, its dissemination on the maternal side is sufficiently controlled by the maternal immune system (Navarro *et al.,* 2004). It was suggested that special immunologic suppressor mechanisms operate at the materno-foetal interface of the placentomes to prevent the attack of the maternal immune system against the foetal placenta and permit the acceptance of the semi-allogeneic foetus (Hansen and Liu, 1996). This special environment favours the development of pathogens such as *Chlamydophila abortus* and make the foetus vulnerable to attack by such organisms (Entrican *et al.,* 2001). *Chlamydophila abortus* has special affinity to foetal trophoblast cells and these cells serve as the main targets for multiplication and dissemination of the organisms (Navarro *et al.,* 2004). It is not exactly known why the organisms grow preferentially in trophoblasts (Rocchi *et al.,* 2009). One hypothesis attributed this to non susceptibility of the trophoblast cells to the activity of pro-inflammatory cytokine gamma interferon (IFN-γ) which is a product of T-cell lymphocytes (Entrican *et al.,* 2002). Although IFN-γ is not

the only cytokine elicited by *Chlamydophila abortus*, it stands out as the strongest immunological correlate of protection against OEA that has been identified to date (Entrican *et al.,* 2012). The antichlamydial activity of IFN-γ has been reported to be directly associated with induction of an enzyme indoleamine 2,3-deoxygenase (IDO) which is a tryptophan-degrading enzyme (Brown *et al.,* 2001). *Chlamydophila abortus* lacks the ability to synthesize tryptophan and depends on the host's tryptophan for growth (Rocchi *et al.,* 2009). Experiments have shown that IFN-γ fails to induce expression of IDO in trophoblast cells so that an optimal tryptophan-rich environment is provided in these cells, which enables maximum multiplication of *Chlamydophila abortus* (Entrican *et al.,* 2002; Navarro *et al.,* 2004).

The average gestation period of sheep and goats is 147 days (Entrican and Wheelhouse, 2006). *Chlamydophila abortus* could be detected in these species from 60 days of gestation within the maternal haematomata in the hilus of the placentomes (Aitken and Longbottom, 2007). It is presumed that infection gains access to the maternal haematomata in latently infected animals via the blood circulation, and the direct contact of the foetal trophoblasts with the maternal blood in this region enables transfer of the organisms from mother to foetus (Entrican *et al.,* 2001). However, the placenta continues to be relatively resistant and no pathological changes appear until 90 days of gestation (Rodolakis *et al.,* 1998). Therefore, complex mechanisms are thought to operate at this stage to permit the release of *Chlamydophila abortus* from the site of suppression to enable colonization of foetal trophoblast cells (Entrican *et al.,* 2001). Following the establishment of the organisms in the foetal trophoblasts in the hilus of most of the placentomes, rapid exponential replication leads to local necrosis and contiguous spread

of the infection into surrounding intercotyledonary membranes and apposing endomerium, ultimately leading to abortion which occurs around 125 to 135 days of gestation (Buxton *et al.*, 1990).

*2.1.8.3 Placental Pathology*

The results of many investigations revealed that placentitis and subsequent necrosis of foetal trophoblasts and endometrial epithelium are the key elements in the pathogenesis of OEA (Buxton *et al.,* 1990; Buxton *et al.,* 2002; Navarro *et al.,* 2004; Rocchi *et al.,* 2009). Although infection starts on the maternal side of the placenta, pathological lesions mainly occur at the foetal chorioallantoic membrane (Navarro *et al.,* 2004). These pathological changes become more progressively severe at 110 days gestation, with the foetal portion of the placentome juxtaposing the maternal lesions presenting the most severe damage (Rocchi *et al.,* 2009). Rapid uncontrolled multiplication of *Chlamydophila abortus* in the trophoblast cells at this region is accompanied by chorioallantoic oedema, arteritis, thrombosis, and necrosis of the chorionic epithelial cells which give rise to the characteristic thickened placental membrane seen at the time of abortion (Entrican *et al.,* 2001; Rocchi *et al.,* 2009). The inflammatory cellular infiltrates to the area consist mainly of neutrophils and macrophages and a few number of lymphocytes (Buxton *et al.,* 2002; Navarro *et al.,* 2004).

*2.1.8.4 Mechanism of Abortion*

The specific mechanism responsible for abortion as a result of *Chlamydophila abortus* infection is unclear (Longbottom and Coulter, 2003). But the current knowledge

points to a combination of different factors which include damage to the placentomes, destruction of chorionic epithelium, chorioallantoic arteritis, vascular thrombosis (Entrican and Wheelhouse, 2006; Sammin *et al.*, 2009), as well as hormonal imbalance due to loss of endocrine function of the placenta (Leaver *et al*, 1989). The inflammatory process and subsequent destruction of chorionic epithelial cells at the foeto-maternal interface result in a reduced efficiency of exchange of oxygen, nutrients and waste products between the dam and foetus (Sammin *et al.*, 2009). Likewise vascular thrombosis and chorioallantoic arteritis impinge on placental blood flow and functional capacity which is detrimental to the viability of the foetus (Sammin *et al.*, 2009). Vascular thrombosis is a consequence of placental cellular infiltration and excessive expression of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) which is incompatible with successful pregnancy (Buxton *et al.*, 2002; Entrican and Wheelhouse, 2006). The expression of TNF-α is most likely induced by chlamydial lipopolysaccharide (LPS), since LPS has been shown to experimentally induce the production of TNF-α by inflammatory mononuclear cells (Aitken and Longbottom, 2007). TNF-α mRNA is also readily expressed in the mononuclear cells within the infected arterioles and arteries as well as in the inflammatory exudates associated with the chorionic epithelium (Longbottom and Coulter, 2003; Aitken and Longbottom, 2007). The high concentration of TNF-α at the materno-foetal interface is incompatible with pregnancy and therefore its production, which is not found in uninfected ovine placentas, has been suggested to cause damage to placenta thereby contributing to abortion or premature birth (Longbottom and Coulter, 2003).

Progesterone is necessary in the maintenance of pregnancy by inhibiting myometrial contraction throughout pregnancy (Noakes, 2001). This hormone is produced by chorionic epithelial cells in sufficient concentration to maintain pregnancy from 55 days gestation in sheep (Allen, 1975) and interacts with oestradiol and prostaglandin to control the onset of parturition (Entrican *et al.,* 2001). Rapid destruction of chorionic epithelium as a result of chlamydial infection can lead to disruption of secretion of progesterone and subsequent loss of balance of pregnancy maintaining hormones which may trigger premature labour and abortion (Entrican *et al.,* 2001; Entrican *et al.,* 2002; Rocchi *et al.,* 2009). Chlamydial infection in sheep has been shown to induce premature decline in plasma progesterone level and premature rise in oestradiol 17β and prostaglandin $E_2$ concentrations, which is thought to contribute to the initiation of premature labour and subsequent foetal expulsion (Leaver *et al.*, 1989).

### 2.1.9 Clinical Signs

Ovine enzootic abortion is clinically characterized by abortion which mostly occurs in the last three weeks of gestation (Aitken, 1986). In addition to abortion, stillbirth and birth of weak or moribund low birth-weight lambs/kids that fail to survive beyond 48 hours are also encountered (Papp *et al.,* 1994; Rodolakis, 2001). This death of premature liveborn lambs and kids contribute to the sum of reproductive wastage caused by chlamydial infection (Aitken and Longbottom, 2007). With the exception of short-lived rise in rectal temperature after experimental inoculation, late term abortion is the only clinical outcome of chlamydial infection in sheep and goats (Papp *et al.,* 1994; Papp and Shewen, 1996; Gutierrez *et al.,* 2011; Longbottom *et al.,* 2013). Sometimes ewes and does may show a sign of vaginal discharge 24 to 48 hours before the onset of abortion,

but otherwise appear healthy until abortion ensues (Rodolakis *et al.,* 1984; Rodolakis, 2001; Entrican *et al.,* 2001). After abortion, affected animals recover rapidly or continue to shed dirty brown infectious exudates from vagina for further 7 to 10 days (Appleyard *et al.,* 1983). Occasionally, the placenta is retained and an associated metritis develops, leading to loss of condition and death as a result of secondary bacterial infection (Aitken and Longbottom, 2007). There is an increased frequency of placental retention, endometritis and vaginitis in goats compared to sheep (Rodolakis *et al.,* 1984; Rodolakis *et al.,* 1998). The aborted fetuses appear normal without gross anatomical lesions (Entrican *et al.,* 2001), or show a degree of subcutaneous oedema giving rise to a "pot bellied" appearance (Longbottom and Coulter, 2003). The absence of autolytic change indicates that death *in utero* has been fairly recent. The fleece of the aborted foetus may be covered with, or discoloured by flecks of pink-brown material originating from placental exudates (Aitken and Longbottom, 2007). Some infected ewes may give birth to healthy lambs and it is not uncommon for an infected ewe to deliver one dead and one weak or healthy lamb (Longbottom and Coulter, 2003). The placenta shows extensive necrosis and thickening of intercotyledonary areas. The thickened areas are covered with dirty brownish exudates over the surface which is a characteristic feature of *Chlamydophila abortus*-infected placenta (Smith, 2001).

In newly infected flocks, OEA occurs in form of abortion storm involving 30% or more of pregnant sheep (Mearns, 2007). The outbreak may sometimes be very severe in goat herds in which 90% of pregnant does will abort (Rodolakis, 2001). After the outbreak flocks become chronically infected where 5 to 10% of pregnant females abort every year (Aitken, 1986). After the first abortion, ewes and does develop protective

immunity and breed successfully in subsequent pregnancies with no evidence of chlamydiae in the vaginal discharges, placental or neonatal samples (Rodolakis *et al.,* 1998; Smith, 2001). The disease can take a cyclic nature in a flock/herd where after five years of 5 to 10% prevalence of abortion, a repeat episode of abortion storm occurs, where all the yearlings in a flock/herd will abort (Rodolakis *et al.,* 1998; Rodolakis, 2001).

### 2.1.10 Diagnosis

Diagnosis of OEA depends on isolation and identification of the causative agent from abortion materials (placenta, foetus and vaginal discharge) combined with serology, or detection of the nucleic acid of the agent using polymerase chain reaction (OIE, 2012). Where clinical signs and gross lesions of the aborted placenta suggest chlamydial abortion, diagnosis can be made by detection of *Chlamydophila abortus* in smear of the affected area of fresh placenta using modified Ziehl Neelson technique as described by Stamp *et al.* (1950). Positive cases will reveal numerous chlamydial EBs that appear as minute (0.3 µm) red staining bodies against blue background cellular debris under high power microscopy. If placenta is not available smears can be made from vaginal swabs taken within 24 hours of abortion or swab of moist coat of freshly aborted foetus or stillborn lamb, but such smears contain far fewer organisms than placental smears (Aitken, 1986). Interpretation of results of smears requires experienced personnel because *Chlamydophila abortus* well resembles *Coxiella burnetii* and *Brucella* species in terms of morphology and staining characteristics (Rodolakis, 2001). However, the three organisms can be differentiated serologically (Aitken, 1986). For this reason, examination of placental smear should be combined with serology especially in cases of abortion that

lack good evidence of chlamydia-induced placental pathology (Mearns, 2007; OIE, 2012).

Serology depends on demonstration of raised or rising levels of *Chlamydophila abortus*-specific immunoglobulin G (IgG) antibodies in the animal sera between the acute and convalescent phases of infection (Griffiths *et al.,* 1996). The serological techniques employed in diagnosis of OEA are complement fixation test (CFT), immunofluorescence test and enzyme linked immunosorbent assay (ELISA) (Longbottom *et al.*, 2002). CFT is the most commonly used assay for diagnosis and screening of sheep and goats for ovine enzootic abortion (OIE, 2012). However, the test lacks specificity because it detects genus-specific LPS antigen which cross reacts with other members of the genus *Chlamydophila* such as *Chlamydophila pecorum* (Vretou *et al.,* 2007; Wilson *et al.,* 2009). A number of serological assays such as indirect immunofluorescence test and ELISA using purified LPS have been developed in an attempt to produce more specific techniques that could distinguish between *Chlamydophila pecorum* and *Chlamydophila abortus* infections, but most of them were not sufficiently sensitive and specific (Rodolakis, 2001; Longbottom *et al.,* 2002; Wilson *et al,* 2009). More specific indirect ELISAs based on recombinant antigen preparations such as major outer membrane protein (MOMP) and polymorphic outer membrane protein (POMP) that express a part of a protein at 80-90 kDa have been developed and shown to be more sensitive and specific than CFT in differentiating animals infected with *Chlamydophila abortus* from those infected with *Chlamydophila pecorum* (Rodolakis, 2001; Longbottom *et al.,* 2002; Vretou *et al.,* 2007; OIE, 2012). However, most of these tests are mainly used as in-house

laboratory research tools and few of them have been commercialized (Vretou *et al.,* 2007; OIE, 2012).

*Chlamydophila abortus* can only be isolated in living cells such as embryonated chicken eggs and cell culture (OIE, 2012). The success of this procedure depends on the acquisition of good quality, well-preserved diagnostic tissue samples and the availability of specialist culturing facilities and expertise (Longbottom *et al.,* 2002). Isolation in cell culture can be carried out in a variety of cell types such as McCoy cells, buffalo green monkey (BGM) cells and baby hamster kidney (BHK) cells (OIE, 2012). Due to the zoonotic nature of *Chlamydophila abortus*, isolation and identification procedures must be carried out under biosafety level 2 conditions (OIE, 2012).

Molecular methods such as PCR have been developed for identifying chlamydial DNA in clinical samples and have demonstrated high sensitivity and specificity (Berri *et al.,* 2009). Amplification of *Chlamydophila abortus* DNA by real-time PCR is considered to be the most sensitive and rapid diagnostic method available for diagnosis of OEA and has been made available in commercial kit form for veterinary application (Sachse *et al.,* 2009; OIE, 2012).

### 2.1.11 Control and Preventive Measures

During an outbreak of OEA, the primary aim is to limit the spread of infection to other naïve animals (Stuen and Longbottom, 2011). Controlling an outbreak of abortion in a flock requires strict sanitation measures and isolation of aborting animals until definitive diagnosis is established (Smith, 2001). Prompt removal and destruction of aborted fetuses and placentae through burial or burning should be given priority as these

materials and uterine discharges are the main sources of infection to susceptible in-contact animals (Aitken, 1986; Smith, 2001). Lambing or kidding pens should be cleaned and disinfected. Contaminated beddings should be removed and disposed of through burning. Aborted females should continue to be isolated until their uterine discharges have dried off and such animals should not be used as foster mothers (Aitken, 1986; Smith, 2001; Mearns, 2007).

Since OEA has bacterial aetiology, treatment with antibiotic is possible and should be considered an option when diagnosis is made of the disease or when active chlamydial infection is thought to be present in a flock/herd of pregnant animals (Entrican *et al.,* 2001). Tetracycline suppresses replication of *Chlamydophila abortus* and is used in pregnant flocks/herds to reduce the incidence of lamb/kid losses when signs of OEA are first observed or when there is perceived threat of the disease (Entrican *et al.,* 2012). Intramuscular injection of long-acting oxytetracycline at a dose of 20 mg/kg body weight to pregnant sheep reduces the overall incidence of abortion and increases the number of viable lambs born to infected flocks (Aitken and Longbottom, 2007; Mearns, 2007). To obtain best result, treatment should be given as soon after 95 days of gestation as possible when placental infection may have commenced, and repeated at 10 to 14 days intervals until lambing (Smith, 2001). However, this treatment will only reduce the number of chlamydial organisms shed, but will not eliminate the infection nor can it reverse the pathological damage already done to a heavily infected placenta (Stuen and Longbottom, 2011). Thus, some abortions and stillbirths will occur despite treatment (Aitken and Longbottom, 2007). Due to this limitation, antibiotic treatment is not considered a sustainable strategy for control and prevention of OEA. The better approach

is to use a combination of flock/herd management and vaccination (Entrican *et al.*, 2001; Longbottom and Coulter, 2003; Entrican *et al.*, 2012).

Flock/herd management is aimed to create a flock/herd free of enzootic abortion. This is achieved by keeping a flock/herd "closed" through breeding own replacement animals, or buying replacement stock from farms known to be free of EAE (Stuen and Longbottom, 2011). For example, in the United Kingdom, the Premium Health Scheme run by the Scottish Agricultural Colleges' Veterinary Services accredits EAE free flocks and such flocks are regarded as safe sources for farmers to purchase replacement stocks (Entrican *et al.*, 2001; Stuen and Longbottom, 2011). In situations where this strategy is impracticable, the best approach is protection by vaccination. Non-pregnant healthy animals can be vaccinated at any time until four weeks before breeding (Entrican *et al.*, 2001). All breeding females should be vaccinated in the first year the disease is first diagnosed in a flock/herd and this should be repeated after three years or sooner if the flock/herd is heavily infected. All new entrants into the breeding flock/herd for the first time should also be vaccinated (Entrican *et al.*, 2001). Both live attenuated and inactivated vaccines are currently commercially available. The live attenuated vaccine is a temperature-sensitive mutant of *Chlamydophila abortus* strain 1B (Longbottom and Livingstone, 2006; Wheelhouse *et al.*, 2010; Entrican *et al.*, 2012). Two forms of this vaccine are currently licensed and marketed by two commercial companies in the United Kingdom and other European countries. These include Enzovac® (Intervet Animal Health Ltd, UK) and CEVAC Chlamydia® (CEVA Animal Health Ltd, UK) (Wheelhouse *et al.*, 2010; Entrican *et al.*, 2012). The inactivated vaccine is a whole organism killed vaccine manufactured and marketed under the trade name of Mydiavac®

by Novartis Animal Health Ltd, UK (Longbottom and Livingstone, 2006). Both vaccines offer good protection against OEA and significantly reduce the shedding of infective organisms, a factor that is important in limiting the spread of infection to other animals (Longbottom and Livingstone, 2006).

## 2.2 BRUCELLOSIS IN SHEEP AND GOATS

### 2.2.1 Aetiology

Brucellosis is one of the most important causes of infectious abortion and reproductive disorders in domestic animals (Blasco, 2010). The disease is also called contagious abortion, infectious abortion or epizootic abortion (Megid *et al.,* 2010). Ovine and caprine brucellosis is primarily caused by *Brucella melitensis* which contains three biovars (biovars 1, 2 and 3). Sporadic cases of brucellosis caused by *Brucella abortus* and *Brucella suis* have also been observed in small ruminants but such cases are rare (OIE, 2009). Another species that primarily infects sheep is *Brucella ovis* which causes contagious epididymitis in rams and occasionally abortion in ewes, but this is less frequent than abortion due to *Brucella melitensis* infection (Megid *et al.,* 2010).

### 2.2.2 Taxonomy

Members of the genus *Brucella* are facultative intracellular, coccobacillus, Gram-negative bacteria that belong to the family α2-proteobacteriaceae (Xavier *et al.,* 2010). *Brucella* organisms are capable of causing disease in a variety of animal species, and classification of the genus into species is mainly based on the organisms' host preference (Al-Dahouk *et al.,* 2003a). Six classical species were identified based on this method which include *Brucella melitensis* (goat and sheep), *Brucella abortus* (cattle), *Brucella suis* (pig), *Brucella canis* (dog), *Brucella neotome* (desert wood rat) and *Brucella ovis* (causes epididymitis in rams) (Osterman and Moriyon, 2006). In addition, two new species of *Brucella* were isolated from marine animals namely *Brucella ceti* from cetaceans and *Brucella pinnipedialis* from seals (Foster *et al.,* 2007). Other newer

species, *Brucella microti* and *Brucella inopinata* were isolated from common vole (*Microtus arvalis*) and human breast implant infection, respectively (Scholz *et al.,* 2008; Scholz *et al.*, 2010).

### 2.2.3 Transmission

The major sources of transmission of brucellosis in small ruminants are the excretions from reproductive tract of infected animals and milk (Alton, 1990; Blasco, 2010). Introduction of the organisms into naïve flocks/herds occurs through introduction of infected animals that persistently excrete brucellae through these routes (Xavier *et al.*, 2009; Blasco, 2010). The routes of infection for both adult and young sheep and goats are the mucous membranes of oropharynx, conjunctiva or broken skin (CFSPH, 2007). Infected placenta, foetal fluids and uterine discharges serve as major sources of infection to susceptible animals (Mustafa *et al.,* 2011). Infected does and ewes shed large numbers of brucellae to the environment through their uterine discharges and placentae when they abort or have full term parturition (Blasco, 2010; Megid *et al.,* 2010). This leads to contamination of pasture, soil, water and other objects around the kidding/lambing environment (OIE, 2009; Megid *et al.,* 2010).

The majority of natural *Brucella melitensis* infection in goats during pregnancy lead to infection of the mammary gland and excretion of the organisms in the milk in subsequent lactations (Xavier *et al.,* 2010). This is as a result of persistent infection of the mammary alveoli and supramammary lymph nodes in chronically-infected animals (Meador *et al.,* 1989). This persistence of infection is facilitated the ability of *Brucella* organisms to resist lysosomal degradation by phagocytic leucocytes associated with

mammary glands and supramammary lymph nodes (Harmon *et al.,* 1988). Aborted females, despite having subsequent normal deliveries, will continue shedding the bacteria through the placenta, vaginal fluids and milk (Megid *et al.,* 2010). Hence vertical transmission from dam to foetus can occur *in utero* or through ingestion of colostrum or milk (Grillo *et al.,* 1997). Such infections will continue to persist in latent form until the offsprings reach sexual maturity when they abort the first pregnancy. This is a major means of transmission and perpetuation of *Brucella melitensis* in a herd or flock (Grillo *et al.,* 1997).

### 2.2.4 Pathogenesis

*Brucella* organisms are capable of invading and surviving intracellularly within phagocytic and non-phagocytic mammalian host cells (Poester *et al.,* 2013). The major target cells for *Brucella* include trophoblasts, macrophages and dendritic cells (Celli, 2006; Salcedo *et al.,* 2008; Xavier *et al.,* 2010). In order to reach its target cells, *Brucella* needs to cross mucosal barriers of the respiratory, digestive or urogenital tract (Xavier *et al.,* 2010), where it undergoes phagocytosis by resident macrophages and dendritic cells (Celli, 2006; Salcedo *et al.,* 2008) and establish a replication niche within these cells (Salcedo *et al.,* 2008). Once internalized, *Brucella* are found in intracellular compartments called *Brucella*-containing vacuoles (BCV) (Hamer *et al.,* 2014). The BCV interfere with the intracellular trafficking pathway in order to escape lysosomal degradation, thereby ensuring their survival and replication (Hamer *et al.,* 2014). This is achieved by preventing the fusion of BCV with lysosomes and directing the vacuole towards the rough endoplasmic reticulum (RER) associated compartments (Poester *et al.,* 2013), which is the optimal site for intracellular replication of *Brucella* (Anderson *et al.,*

1986b; Pizarro-Cerda *et al.,* 1998). The escape from the lysosomal pathway and fusion with the RER interfere with the ability of the host cells to mount an efficient immune response against *Brucella* (Salcedo *et al.,* 2008). This enables the organism to avoid recognition by the host immune system and disseminate systemically to lymphoid and reproductive organs (Xavier *et al.,* 2010). The organisms reach the regional lymph nodes via the lymphatic vessels draining the area (Megid *et al.,* 2010). Bacteraemia ensues, resulting in a generalized infection affecting target organs such as uterus and udder and their associated lymph nodes (Megid *et al.,* 2010; Poester *et al.,* 2013).

*Brucella* organisms have marked predilection for the ruminant placenta and mammary gland (Anderson *et al.,* 1986a), and its localization within the reproductive tract accounts for the most common clinical sign of abortion seen in *Brucella*-infected females (Poester *et al.,* 2013). The chorioallantoic trophoblasts of the gravid uterus of ruminants are the key target cells of *Brucella* infection and replication during the mid to late phase of gestation (Anderson *et al.,* 1986a; Meador and Deyoe, 1989; Xavier *et al.,* 2009). The preferential growth of *Brucella* organisms within the trophoblast cells has been associated with, and apparently enhanced by the presence of high concentration of erythritol and progesterone (Anderson *et al.,* 1986a; Xavier *et al.,* 2010). Both erythritol and progesterone are synthesized in high concentration by the trophoblast cells of the ruminant placenta and are capable of stimulating the growth of *Brucella* organisms *in vitro* (Anderson *et al.,* 1986a; Samartino and Enright, 1996). In addition, only the host species in which erythritol was present in their placentae and foetal fluids were susceptible to acute placentitis when challenged with *Brucella* spp (Sammin *et al.,* 2009). The ability of *Brucella* spp to utilize erythritol was therefore suggested to be a

determinant factor for the organism's tropism to the ruminant placenta and foetus (Poester *et al.,* 2013).

During maternal bacteraemia, *Brucella* first enters and replicates within the erythrophagocytic trophoblasts in the placentomes of the gravid uterus (Anderson *et al.,* 1986b). Thereafter, the organisms spread to the trophoblast cells of the adjacent chorioallantoic membrane and replicate in massive number within the rough endoplasmic reticulum (Anderson *et al.,* 1986b). This induces hypertrophy of the rough endoplasmic reticulum and subsequent release of the *Brucella* organisms into the uterine lumen (Poester *et al.,* 2013). Necrosis of the trophoblasts leads to ulceration of the chorioallantoic membrane and vasculitis in both maternal and foetal placental tissues (Anderson *et al.,* 1986b). This event leads to separation of foetal trophoblsts from the maternal epithelium, resulting in foetal death and subsequent abortion (Anderson *et al.,* 1986b; Poester *et al.,* 2013). It has also been suggested that a decrease in progesterone level associated with trophoblast damage results in endometrial production of prostaglandin F2α which initiates premature delivery process and subsequent expulsion of the foetus (Xavier *et al.,* 2010).

### 2.2.5 Clinical Signs

The main clinical manifestation of acute brucellosis in naturally infected goats and sheep is reproductive failure in form of abortion, stillbirth and the birth of weak offspring (Xavier *et al.,* 2009; Blasco, 2010; Mustafa *et al.,* 2011). Abortion generally occurs in the last two months of pregnancy and is accompanied by retention of placenta in some cases (Alton, 1990). Animals usually abort only once, but reinvasion of the

uterus and shedding of the organisms in foetal membranes and vaginal fluids can occur in subsequent pregnancies (CFSPH, 2007). Acute infection in goats leads to infection of the udder in most cases (Blasco, 2010). Persistent infection of the mammary gland and supramammary lymph node is accompanied by constant intermittent shedding of the organisms in the milk in subsequent lactations (Alton, 1990; Xavier *et al.,* 2009). Inflammatory changes in the infected mammary glands reduce milk production, but clinical signs of mastitis are uncommon in naturally infected goats (Alton, 1990).

Newly infected flocks/herds experience abortion storms followed by a period of flock resistance during which abortions seldom occur (Radostits *et al.*, 2000). Abortion is much lower in flocks/herds where the disease is enzootic because animals abort during the gestation when they are first infected (CFSPH, 2007). Although systemic signs of fever, depression and loss of condition accompanied by arthritis and hygroma have been observed in experimental infections in goats, these signs are rare in natural disease and their occurrence in the experimental disease was a reflection of a massive challenge dose (Alton, 1990).

The aborted placentae show oedema and necrosis of the cotyledons that appear dull gray in colour and in some cases thickening of intercotyledonary areas (Megid *et al.*, 2010). The foetus may appear normal or autolysed, or have an excess blood-stained fluid in the body cavity with enlarged spleen and liver. However, these lesions are not pathognomonic for brucellosis (CFSPH, 2007). Heavy colonization of the udder may result in acute mastitis with production of clotted and watery milk and reduction in milk yield (Megid *et al.*, 2010).

### 2.2.6 Diagnosis

Diagnostic tests for brucellosis are applied in livestock to achieve confirmatory diagnosis, screening/prevalence studies, certification or surveillance in countries where brucellosis has been eradicated (Mainar-Jaime *et al.,* 2005; OIE, 2009; Godfroid *et al.,* 2010). These tests include direct tests such as microbiological analysis and DNA detection by PCR-based techniques and indirect tests such as serology (Godfroid *et al.,* 2010).

Bacteriological diagnosis of *Brucella* organisms can be made by microscopic examination of stained smears from vaginal swabs, aborted fetuses or placentae using modified Ziehl Neelson technique (Stamp's method). However, the organisms can be confused with *Chlamydophila abortus* and *Coxiella burnetii* which appear morphologically similar when stained using this technique. Hence isolation on appropriate culture media is recommended for accurate diagnosis (Al-Garadi *et al.,* 2011). Because isolation of *Brucella* organisms on culture media is time consuming, insensitive and hazardous, clinicians rely on indirect proof of infection (serology) for diagnosis in most cases (Al-Dahouk *et al.,* 2003a).

Serology relies on detection of high or rising titres of *Brucella*-specific antibodies in serum. It requires a combination of at least two techniques to avoid false negative results (Al-Dahouk *et al.,* 2003a). The CFT, Rose Bengal test (RBT) and serum agglutination test (SAT) are the most common tests applied for routine diagnosis of brucellosis in small ruminants (Baum *et al.*, 1995). Because of its high sensitivity and specificity, CFT is considered the most reliable test and is recommended for confirmatory

diagnosis (Baum *et al.,* 1995; OIE, 2009; Al-Dahouk *et al.,* 2003b). The RBT is recommended as a screening test in the field and is comparable to CFT in terms of sensitivity and specificity (OIE, 2009). Both tests identify immunoglobulin G1 (IgG1) subtypes antibodies which are predominant in the later phase of infection (Baum *et al.,* 1995; Al-Dahouk *et al.,* 2003b). The SAT is less sensitive and specific than CFT and RBT, nevertheless it is still being used in several countries as a surveillance method (Baum *et al.,* 1995). Because of the delicate and cumbersome nature of CFT procedure, it is gradually being replaced by the newer technique of ELISA (Godfroid *et al.,* 2010). Most ELISA tests detect IgG or IgG subclasses and their main advantage is higher sensitivity than CFT and RBT (Al-Dahouk *et al.,* 2003b; Godfroid *et al.,* 2010).

Molecular methods such as PCR that allow detection of *Brucella* DNA in clinical samples have been developed (Godfroid *et al.,* 2010). The PCR technique has proven to be more sensitive and more specific than bacteriological culture and serological tests (Queipo-Ortuno *et al.,* 1997; Al-Dahouk *et al.,* 2003a). It allows the earliest detection of *Brucella* spp at ten days post inoculation (Al-Dahouk *et al.,* 2003a). Thus, it has become a promising alternative to the conventional culture and identification method of diagnosis (Al-Dahouk *et al.,* 2003a).

### 2.2.7 Control

In general, control of small ruminant brucellosis through antimicrobial therapy has not been fully successful because of the intracellular localization of the organisms within the phagocytic cells of the reticuloendothelial system (Radwan *et al.,* 1992). In addition, the high cost of therapy and long duration of treatment reported in experimental

trials has made it economically ineffective to be adopted for routine application in livestock (Radwan *et al.,* 1992). As a result, control measures are based on strict hygiene and vaccination (Kolar, 1984; Samadi *et al.,* 2010). Control through hygiene is achieved by the use of separate pens for lambing/kidding ewes and does, thorough disinfection of such pens and disposal of all aborted foetuses and placentae through burial (Radostits *et al.,* 2000).

Vaccination is applied as a measure to reduce the prevalence of the disease to a level where eradication by test and slaughter can be implemented (Samadi *et al.,* 2010). It is also applied to protect non exposed flocks/herds against the disease (Blasco, 1997; Blasco, 2010) and protect females from abortion in regions where the disease is endemic (Samadi *et al.,* 2010). The live attenuated *Brucella melitensis* Rev 1 vaccine has proven to be superior to all other vaccines for prophylaxis of brucellosis in small ruminants and its extensive use is a milestone in brucellosis control in many countries (Kolar, 1984; Blasco, 1997). It is currently the only licensed vaccine available for control of *Brucella melitensis* in small ruminants (Menzies, 2012). It can be administered through conjunctival or subcutaneous route (Kolar, 1984; Menzies, 2012), at a dose of $1\text{-}2 \times 10^9$ colony forming units (CFU) per animal (Blasco, 2010). A whole flock, mass vaccination of all sheep and goats, including males and females is the simplest and the most reasonable control strategy to be applied in high prevalence situations and extensive management systems (Blasco, 2010). But the major drawback of Rev 1 vaccine is induction of abortion when administered to pregnant ewes and does by subcutaneous route (Zundel *et al.,* 1992; Blasco, 1997). This side effect can be reduced, but not completely eliminated, by administering the vaccine conjunctivally (Blasco, 2010).

Therefore care must be taken as much as possible to avoid vaccinating ewes and does in mid to late gestation, which is the main critical period during which the animals can experience abortion (Blasco, 2010). The alternative strategy is to initially vaccinate all animals on the farm, followed in subsequent years by annual vaccination of only replacements and new comers into the farm (Menzies, 2012). It is recommended to perform serological test of animals randomly selected two to three weeks post vaccination using Rose-Bengal test to ensure that the vaccination has been effective (Menzies, 2012).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 STUDY AREA

The study was conducted in Taraba State, situated in North Eastern Nigeria between latitudes $6^{o}$ 55´ to $9^{o}$60´N and longitudes $9^{o}$40´ to 11 $^{o}$ 90´E. The state has a land size of 60,291.82 square kilometers, consisting of 16 Local Government Areas (LGAs), which are grouped into three Senatorial Districts, namely Northern, Central and Southern Senatorial Districts (Anonymous, 2010). Taraba State is bounded on the northern border by Bauchi and Gombe States, on the west by Nassarawa and Plateau States, on the southwest by Benue State and on the eastern border by Adamawa State and Cameroun Republic (Anonymous, 2004). The state lies within the northern and southern guinea savannah vegetation zones of Nigeria. Agriculture is the major occupation of the people in the state. The state is among the leading producers of livestock in Nigeria, with an estimated population of 1,040,000 sheep and 3,195,000 goats according to FAO 2005 global census data (Wint and Robinson, 2007).

## 3.2 SAMPLE SIZE

The sample size was determined using the formula $N = Z^2pq \,/\, d^2$ (Thrusfield, 1997).

Where:

N = sample size

Z = standard deviation at 95% confidence interval = 1.96

p = prevalence rate from previous study

q = 1-p

d = desired precision = 0.05 (95% confidence interval)

The prevalence of enzootic abortion of ewes in Nigeria is unknown. Therefore the conventional prevalence of 50% (Putt *et al.,* 1988) was used to determine the sample size for this study.

$$N = \frac{1.96^2 \times 0.5 \times (1-0.5)}{0.05^2} = 384.16 \qquad (384 \text{ animals})$$

### 3.3 SAMPLING PROCEDURE

The study was conducted in northern and central parts of Taraba State which form the sampling area, from May to November 2013. The southern part of the state was omitted from the study due to frequent ethnic clashes that occur in the area, which usually lead to loss of lives and property. The sampling area was divided into two districts consisting of a total of eleven LGAs. Each district was regarded as a stratum. Two LGAs were randomly selected from each stratum, making a total of four LGAs. These include Bali and Gashaka LGAs from central senatorial district, and Ardo-Kola and Lau LGAs from northern senatorial district (Plate 1). Samples were collected from sheep flocks and goat herds in 39 villages and 265 households in the selected LGAs. Within each flock/herd, the total number of breeding ewes/does was determined, out of which blood samples were collected from the ewes and does that had had abortion within the previous two years. The sampling covered 1439 breeding ewes and does, out of which serum samples were collected from 368 animals with history of abortion.

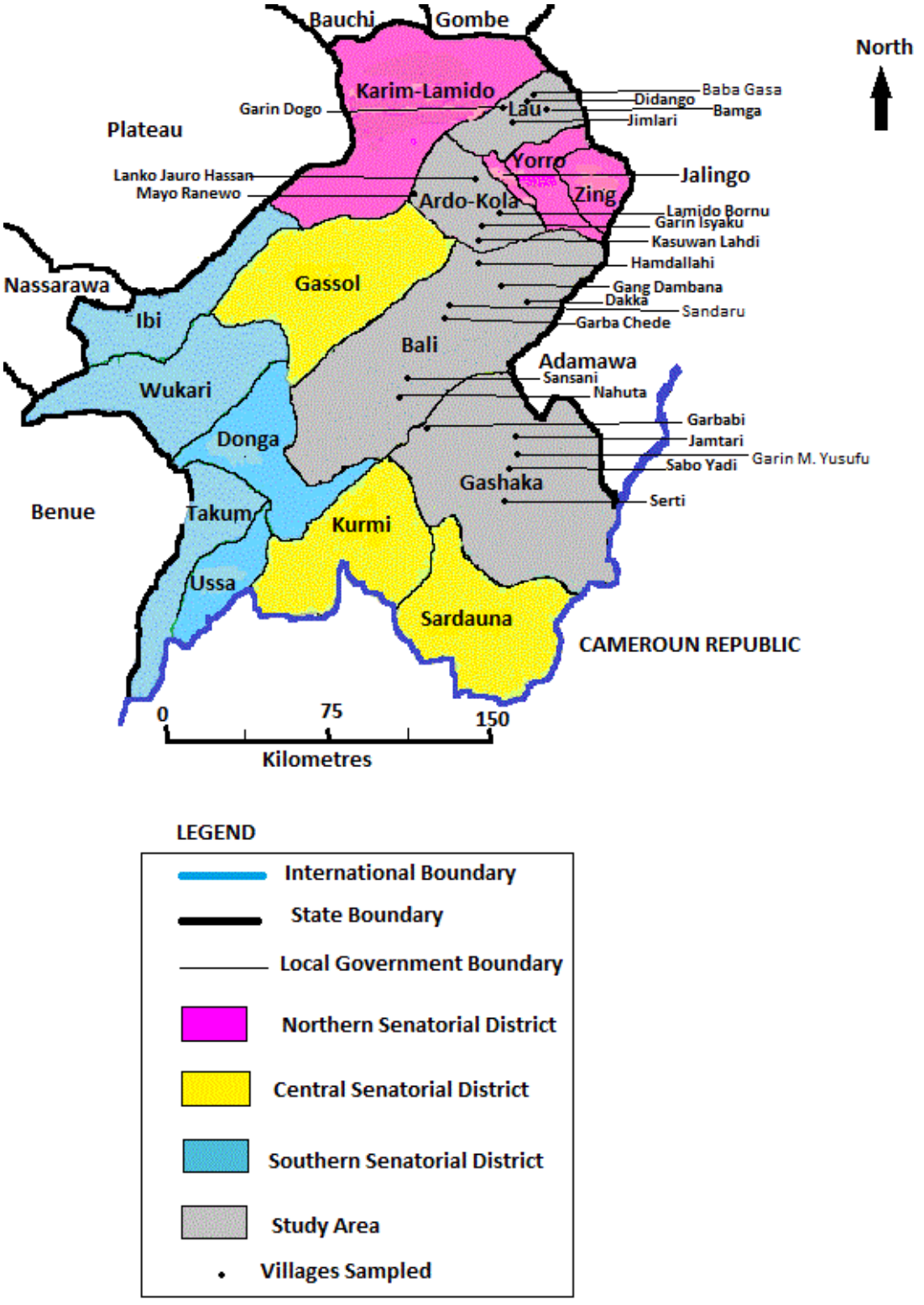


**Plate 1:** Map of Taraba State showing the locations of some of the villages sampled in the study area
Source: Adapted and modified from Anonymous, (2004)

## 3.4 SAMPLE COLLECTION

Five ml of blood was asceptically collected from each aborted ewe/doe via the jugular vein using sterile 5 ml syringe and 21 gauge needle and transferred to sterile test tube containing no anticoagulant. The blood samples were allowed to stand at ambient temperature for 30 minutes. The samples were transported on ice pack to National Veterinary Research Institute (NVRI) Jalingo out-station laboratory. The samples were centrifuged at 1000 revolutions per minute for 10 minutes to properly separate the sera from the clotted red blood cells. Using sterile syringe and needle, the sera were carefully aspirated and transferred to sterile test tubes. Each test tube-containing serum was labeled and stored at -20$^{o}$C until use.

## 3.5 LABORATORY ANALYSIS

After collecting the required sample size, the serum samples were transported on ice pack to the Animal Reproduction Research Programme Laboratory, National Animal Production Research Institute, Ahmadu Bello University, Zaria for analysis. Each serum sample was subjected to two ELISA tests to detect the presence of IgG antibodies against *Chlamydophila abortus* and *Brucella* organisms. The ID Screen® indirect multi-species ELISA kits for *Chlamydophila abortus* and Brucellosis were obtained from IDVET Innovative Diagnostics, France, and used according to the manufacturer's instructions as described below:

**a. *Chlamydophila abortus***

i. All the reagents were allowed to come to room temperature and homogenized by vortex.

ii. 90 µl of dilution buffer 13 was added to each microwell that was coated with *Chlamydophila abortus*-specific antigen, major outer membrane protein (MOMP).

iii. 10 µl of negative control was added to wells A1 and B1, and 10 µl of positive control to wells C1 and D1.

iv. 10 µl each of the samples to be tested was added to the remaining wells and incubated at 25$^{o}$C for 45 minutes.

v. The wells were then emptied and washed three times each with 300 µl of wash solution.

vi. Multi-species peroxidase conjugate was prepared by diluting the concentrated conjugate to 1:10 in dilution buffer solution.

vii. After washing, 100 µl of the multi-species peroxidase conjugate was added to each well and incubated at 25$^{o}$C for 30 minutes to fix the antibodies.

viii. The wells were emptied again and washed three times each with 300 µl of wash solution to eliminate the excess conjugate.

ix. 100 µl of substrate solution was added to each well and incubated at 25$^{o}$C in the dark for 15 minutes.

x. 100 µl of stop solution was added to each well to stop the reaction.

xi. The optical density was read at 450 nm.

**b. Brucellosis**

i. All the reagents were allowed to come to room temperature and homogenized by vortex.

ii. 190 µl of dilution buffer 2 was added to each microwell that was coated with purified *Brucella abortus* LPS antigen.

iii. 10 µl of negative control was added to wells A1 and B1, and 10 µl of positive control to wells C1 and D1.

iv. 10 µl each of the samples to be tested was added to the remaining wells and incubated at 25$^{o}$C for 45 minutes.

v. The wells were then emptied and washed three times each with 300 µl of wash solution.

vi. Multi-species peroxidase conjugate was prepared by diluting the concentrated conjugate to 1:10 in dilution buffer solution.

vii. After washing, 100 µl of the multi-species peroxidase conjugate was added to each well and incubated at 25$^{o}$C for 30 minutes to fix the antibodies.

viii. The wells were emptied again and washed three times each with 300 µl of wash solution to eliminate the excess conjugate.

ix. 100 µl of substrate solution was added to each well and incubated at 25$^{o}$C in the dark for 15 minutes.

x. 100 µl of stop solution was added to each well to stop the reaction.

xi. The optical density was read at 450 nm.

### 3.6 DATA ANALYSIS

Chi-square test was conducted to measure the association between positively tested sera and species using SPSS version 17 software.

# CHAPTER 4

# RESULTS

## 4.1 SHEEP AND GOAT OWNERSHIP IN THE STUDY AREA

The holding sizes of sheep and goats in the 265 households sampled in the study area were shown in Figure 1. The great majority of the households (88.9%) possessed a few number of breeding ewes and does within the range of 1 to 10 per household. Less than 10% of the households possessed 11 to 20 and only 2.2% possessed greater than 20 breeding ewes and does in their flocks/herds. The pattern of ownership of small ruminants, as shown in Figure 2, indicated that the greater proportion (81.6%) of the households in the study area possessed goats, while only 18.4% of the households possessed sheep. The most common breed of goat reared by farmers in the study area was Kano brown, while the breed of sheep in the area was predominantly Yankasa.

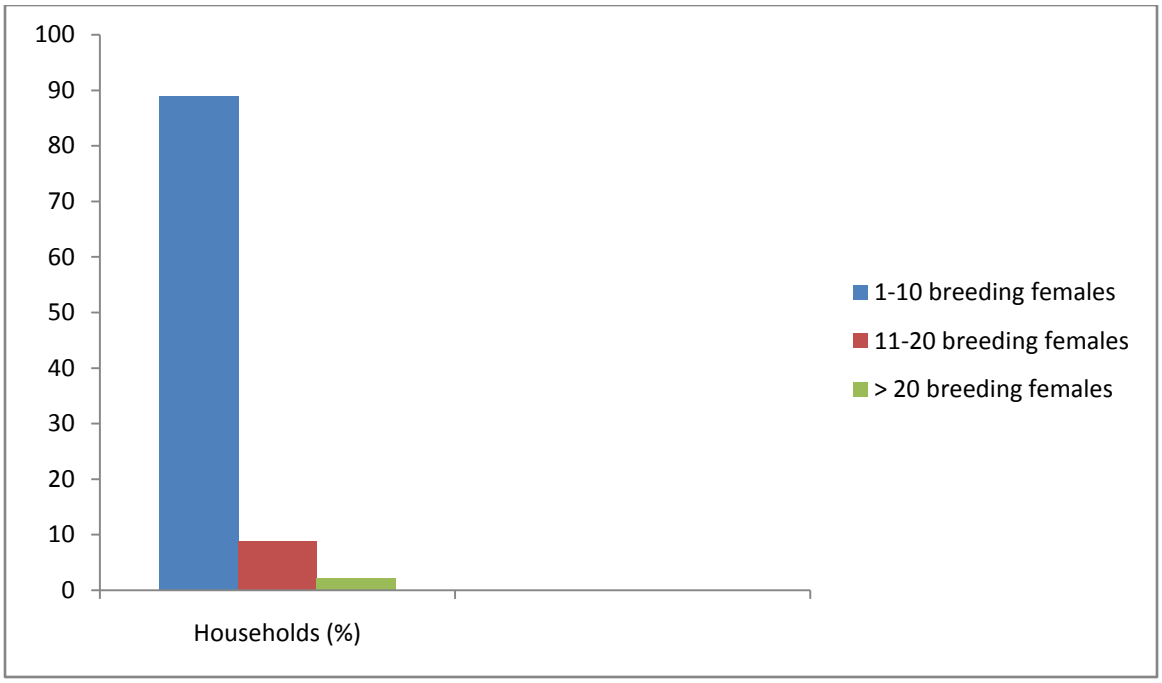


**Figure 1.** Holding sizes of breeding ewes and does in the study area

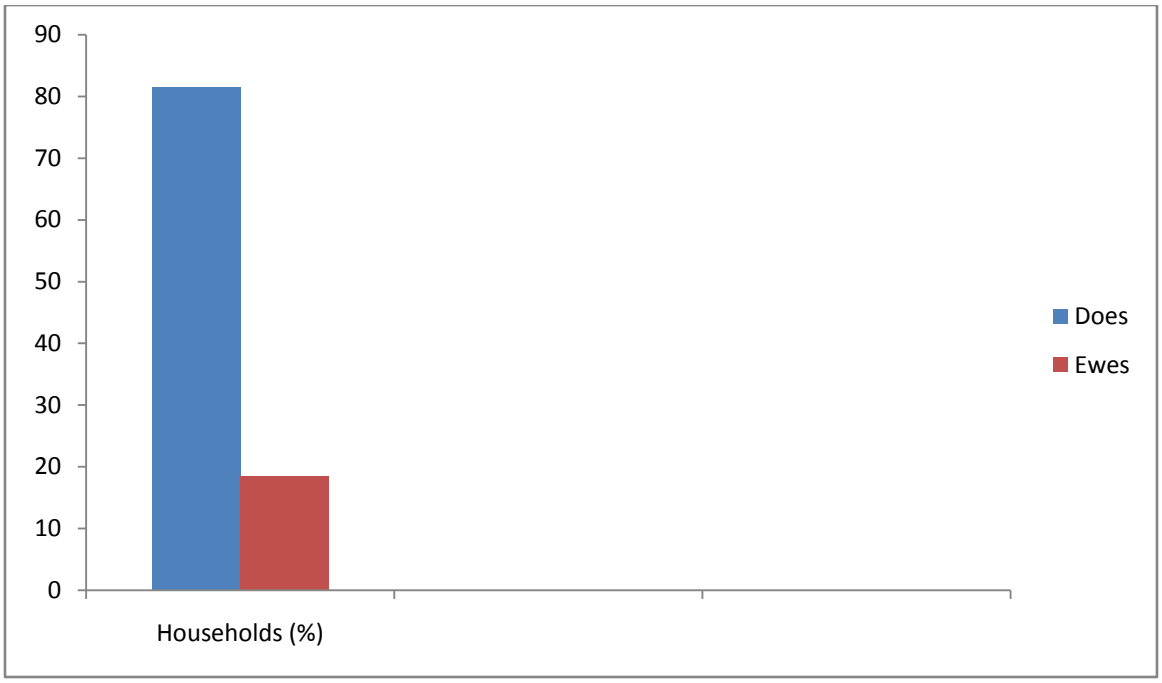


**Figure 2.** Pattern of ownership of breeding ewes and does in the study area

## 4.2 PREVALENCE OF ABORTION IN SMALL RUMINANTS IN THE STUDY AREA

The prevalence rates of abortion in small ruminants in the study area are presented in Table 1. The overall prevalence of abortion in ewes and does was 25.6%. In sheep, the total prevalence was 23.1%, while the total prevalence in goats was 26.3%. Chi-square test revealed no significant ($P>0.05$) difference between sheep and goats in the prevalence of abortion in the four LGAs investigated.

**Table 1:** Prevalence of abortion in small ruminants in northern and central areas of Taraba State

| LGA | Breeding Females | | | Aborted Females | | | Prevalence (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ewes | Does | Total | Ewes | Does | Total | Ewes | Does | Total |
| Bali | 66 | 340 | 406 | 20 | 89 | 109 | 30.3 | 26.2 | 26.9 |
| Lau | 111 | 440 | 551 | 19 | 102 | 121 | 17.1 | 23.2 | 22.0 |
| Ardo-Kola | 49 | 226 | 275 | 10 | 66 | 76 | 20.4 | 29.2 | 29.6 |
| Gashaka | 107 | 100 | 207 | 28 | 34 | 62 | 26.2 | 34.0 | 30.0 |
| Total | 333 | 1106 | 1439 | 77 | 291 | 368 | 23.1 | 26.3 | 25.6 |

$X^2 = 12.000$; $P > 0.05$

## 4.3 ELISA ASSAY

Of the 368 sera that were subjected to ELISA test, 23 positive samples were detected, which gave an overall percentage seropositivity of 6.3% (Table 2). Out of the 23 positive sera, 14 were found to be positive for *Chlamydophila abortus* antibodies, while 9 were positive for *Brucella* antibodies. This gave the seroprevalence of 3.8% for *Chlamydophila abortus* and 2.5% for brucellosis (Table 2). Out of the 14 *Chlamydophila abortus*-positive animals, 12 were does, while the remaining two were ewes, which gave the prevalence of 4.1% in does and 2.3% in ewes (Table 3). There was no significant ($P>0.05$) difference between sheep and goats in the prevalence of *Chlamydophila abortus* in the study area. Only goats in Lau LGA were positive to brucellosis (Table 4) with percent optical density (% OD) greater than 120%. None of the sheep tested in this study was positive to antibodies against brucellosis. Likewise no *Brucella*-positive animal was detected in Ardo-Kola, Bali and Gashaka LGAs.

**Table 2:** Overall prevalences of *Chlamydophila abortus* and *Brucella* antibodies in aborted small ruminants in northern and central parts of Taraba State

| **LGA** | **Number of Aborted Animals** | **Number of Positive Animals** | | | **Prevalence (%)** | | |
|---|---|---|---|---|---|---|---|
| | | *Ch.abortus* | *Brucella* | Total | *Ch.abortus* | *Brucella* | Total |
| Bali | 109 | 3 | 0 | 3 | 2.8 | 0.0 | 2.8 |
| Lau | 121 | 4 | 9 | 13 | 3.3 | 7.4 | 10.7 |
| Ardo-Kola | 76 | 3 | 0 | 3 | 4.0 | 0.0 | 4.0 |
| Gashaka | 62 | 4 | 0 | 4 | 6.5 | 0.0 | 6.5 |
| Total | 368 | 14 | 9 | 23 | 3.8 | 2.5 | 6.3 |

**Table 3:** Prevalence of *Chlamydophila abortus* antibodies in aborted ewes and does in northern and central parts of Taraba State

| LGA | Number of Aborted Animals | | Number of Positive Animals | | Prevalence (%) | |
|---|---|---|---|---|---|---|
| | Ewes | Does | Ewes | Does | Ewes | Does |
| Bali | 20 | 89 | 1 | 2 | 5.0 | 2.3 |
| Lau | 19 | 102 | 1 | 3 | 5.3 | 2.9 |
| Ardo-Kola | 10 | 66 | 0 | 3 | 0.0 | 4.6 |
| Gashaka | 28 | 34 | 0 | 4 | 0.0 | 11.8 |
| Total | 77 | 291 | 2 | 12 | 2.3 | 4.1 |

$X^2 = 8.000$; $P > 0.05$

**Table 4:** Prevalence of *Brucella* antibodies in aborted ewes and does in northern and central parts of Taraba State

| LGA | Number of Aborted Animals | | Number of Positive Animals | | Prevalence (%) | |
|---|---|---|---|---|---|---|
| | Ewes | Does | Ewes | Does | Ewes | Does |
| Bali | 20 | 89 | 0 | 0 | 0 | 0.0 |
| Lau | 19 | 102 | 0 | 9 | 0 | 8.8 |
| Ardo-Kola | 10 | 66 | 0 | 0 | 0 | 0.0 |
| Gashaka | 28 | 34 | 0 | 0 | 0 | 0.0 |
| Total | 77 | 291 | 0 | 9 | 0 | 3.1 |

# CHAPTER 5

# DISCUSSION

The holding size of small ruminants in the study area showed that small ruminant production in northern and central areas of Taraba State is predominantly subsistent in nature, where animals are kept in small numbers by individuals or families whose primary occupation is crop farming. This result corresponds to earlier report of von Kaufman and Francis (1989) who reported that small ruminant rearing in the humid zone of Nigeria was mainly secondary to the main business of farming, where animals were kept in small flocks of about two to six heads per household. Ademosun (1993) also reported that the main characteristic feature of sheep and goat production in tropical Africa is that stock owners were mostly crop farmers for whom livestock keeping is of secondary importance, and thus keep only a few number of animals to meet some specific objectives. Otchere (1986) reported that in the sub-humid zone of northern Nigeria, the majority of small ruminants were owned by individuals and families in rural areas with a mean flock size of 12.6 sheep or herd size of 13.0 goats, and that only 54.2 to 56.9% of the flocks or herds constitute breeding females. Similarly, Ajala *et al.* (2008) reported that small ruminant farmers in northern guinea savannah zone of Nigeria are smallholders who owned a mean flock size of 8.4 sheep and herd size of 10.1 goats and concluded that the practice in the area is typically a peasant farming culture where few animals are kept to augment family income. From these reports and the present study, it could be deduced that small ruminant production in Nigeria has over decades failed to develop to commercial size and remained as smallholder family activity whose main objective is to

keep animals as a form of savings that could be sold to generate cash to meet some compelling or emergency financial demands.

The pattern of ownership of sheep and goats indicated that goats are preferably kept by farmers in the study area than sheep. This pattern of ownership is similar to what was reported in other parts of Nigeria and sub-Saharan African countries. von Kaufman and Francis (1989) observed that goat population outnumbered sheep by three to one in the humid zone of south western Nigeria. Aphunu and Okojie (2011) reported that 75% of households in Delta State keep goats in preference to sheep. Tologbonse *et al.,* (2011) in a survey of all the agro-ecological zones of northern and southern Nigeria reported that greater percentage of farmers rear goats than sheep in all the regions. The preference of small ruminant producers to goats than sheep could be attributed to the fact that goats are hardier and more effective at grazing on more vegetation types than sheep (Wilson, 1991; Baah *et al.,* 2012). They are also very good browsers, thus they walk longer distances in search of food and utilize poor quality forage to meet their nutritional requirements better than sheep (Wilson, 1991; Rege, 1994). Furthermore, goats are generally regarded to have higher chances of twin pregnancies and more prolific than sheep (Mwaba, 2011; Baah *et al.,* 2012). Therefore, goats require little capital investment and less care, which made them more popular to the poor resource rural farmers (Wilson, 1991; Rege, 1994; Baah *et al.,* 2012).

The present study reported a high prevalence of abortion in small ruminants in the study area. The overall prevalence of 25.6% was higher than the acceptable limit of 5% reported for sheep and goats under modern husbandry system (Navarro *et al.,* 2009; Menzies, 2011; NADIS, 2014) or 10% reported for small ruminants reared under

traditional extensive management system (Sharma *et al.*, 2008). This indicated a serious negative impact on the reproductive performance of small ruminants in the area. The high rate of abortion reported here could possibly be due to non observance of biosecurity measures by farmers in the study area when cases of abortion occur in their flocks/herds. From the interviews conducted during sample collection, there was general lack of knowledge among the small ruminant owners on the need to isolate aborted animals and disinfect the premises where abortions occurred. Aborted fetuses and placentae were usually disposed of at the backyard without burial or left on the field for dogs to come and eat, and dogs can carry such fetuses and placentae far distant places. These practices are among the factors that could promote contamination of the environment with abortifacient organisms, and thus, may have a bearing on the relatively high prevalence of abortion obtained in this study.

In this study, the prevalence of *Chlamydophila abortus* antibodies was higher in does than ewes, but this was not statistically significant. The slight difference could be due to higher number of samples collected from goats than sheep, and this was unavoidable because the goat population was higher than the sheep population in the study area. The sample collection in this study was conducted from early rainy season to the onset of dry season (May to November, 2013). The rationale for choosing this period was to enable easy access to sheep and goats, because in the study area, small ruminants are generally tethered during rainy season to avoid crop damage and released immediately after harvest to roam freely and feed on crop residues on farmlands. This practice caused limitation to this study as the calculated sample size of 384 was unable to be achieved within the time interval available for sampling. However, the 368 samples

collected were sufficient to enable detection of *Chlamydophila abortus*-positive animals in the present study.

*Chlamydophila abortus*-positive animals were detected in all the four LGAs investigated. This showed that chlamydial abortion was prevalent in small ruminants in the study area. Seroprevalence of *Chlamydophila abortus* in small ruminants varies widely from one country to another, ranging from 2.9% in China (Zhuo *et al.,* 2012) to 31.1% in Mexico (Jimenez-Estrada *et al.,* 2008). Even within the same country, regional variations have been reported, for example in Turkey (Otlu *et al.*, 2007; Gokce *et al.,* 2007) and Saudi Arabia (Abd-El-Razik *et al.*, 2011; Aljumaah and Hussein, 2012). There are few published reports on the prevalence of *Chlamydophila abortus* in Africa. These reports have indicated prevalences of 3.2% in Botswana (Sharma *et al.,* 2003), 8% in Namibia (Samkange *et al.,* 2010) and 30.4% in South Africa (Ndou and Dlamini, 2012). The seroprevalence of 3.8% reported in this study was similar to 3.2% reported by Sharma *et al.* (2003) in does in Botswana. The slight difference between the present study and Sharma *et al.* could be because of the CFT used in the earlier study, which has lower specificity than ELISA used in the present study (Jones *et al.,* 1997; OIE, 2012). However, in both studies, the animals sampled had history of reproductive failure. This could maximize the chance of detecting *Chlamydophila abortus*-specific antibodies in the aborted animals that were sampled, because abortion has to occur before the antibody titre rises to the level that could be detected by the currently available serological techniques (Entrican *et al.,* 2001; Livingstone *et al.,* 2005; Entrican *et al.,* 2012). A lag time of three weeks normally exists between abortion and a detectable rise in serum antibody titre in *Chlamydophila abortus*-infected animals (Kennedy *et al.,* 2001).

Samkange *et al.* (2010) and Ndou and Dlamini (2012) reported higher prevalences than what was obtained in this study. This could be attributed to differences in sample size and sampling methods between the previous studies and the present study. Samkange *et al.* (2010) used a sample size of 1076 which was higher than the sample size of 368 used in the present study. However, in the previous study, the animals were randomly selected irrespective of whether they had a history of reproductive failure or not. Ndou and Dlamini (2012) reported higher prevalence (30.4%) in goats with history of abortion in South Africa. It could thus be postulated from these reports that the level of exposure of small ruminants to *Chlamydophila abortus* in Southern African countries was higher than the level of exposure in Nigeria.

The present study is the first detailed study that investigated the distribution of *Chlamydophila abortus* infection in aborted small ruminants on a wider scope in Nigeria. The earlier information on the disease was reported in an outbreak of abortion in sheep in a research farm in Vom, Plateau State (Okoh, 1986). Later on, Amin and Wilsmore (1993) detected antibodies against the organism in sheep and goats in Maiduguri metropolis. The seroprevalence of 3.3% in sheep and 3.6% in goats reported by Amin and Wilsmore (1993) were comparable to 3.8% reported in this study. However, these prevalences reported in the present and earlier study in Nigeria were lower than the prevalences reported in many other countries such as Saudi Arabia (Abd-El-Razik *et al.,* 2011; Aljumaah and Hussein, 2012), Turkey (Otlu *et al.,* 2007; Gokce *et al.*. 2007), Brazil (Santos *et al.,* 2012), Lithuania (Bagdonas *et al.,* 2007), Jordan (Al-Qudah *et al.,* 2004), Caribbean Island of Grenada (Stone *et al.,* 2012) and Mexico (Jimenez-Estrada *et al.,* 2008), but similar to the prevalence reported by Zhuo *et al.* (2012) in China. The low

level of exposure to *Chlamydophila abortus* in Nigeria was possibly due to the traditional practice of extensive management system of small ruminant production, which is also the most popular practice in northern and central areas of Taraba State. Chlamydial abortion is known to be more common in intensively managed flocks than extensively managed flocks (Longbottom and Coulter, 2003; Aitken and Longbottom, 2007; Stuen and Longbottom, 2011). Intensive management offers greater chance of heavy environmental contamination by infectious chlamydial elementary bodies due to massive use of the same lambing/kidding field or pen, either outdoors or indoors. Exposure of susceptible females to such heavy infection is a major component in the transmission of the disease (Aitken and Longbottom, 2007).

*Chlamydophila abortus* antibodies were detected in both sheep and goats in this study without significant difference in terms of prevalence of the antibodies between the two species. This is in agreement with the report of Amin and Wilsmore (1993) in which seroprevalence of *Chlamydia psittaci* serotype 1 in sheep and goats did not differ significantly in Maiduguri area. Although most of the work on chlamydial infection of small ruminants was conducted in ewes (Rodolakis, 2001), there was no evidence that indicated higher susceptibility of ewes to the organisms than does or vice versa. While some workers reported higher prevalence in sheep than goats (Tsakos *et al.,* 2001; Al-Qudah *et al.,* 2004; Cislakova *et al.,* 2007), others on the other hand reported lower prevalence in sheep than goats (Travnicek *et al.,* 2002; Sharma *et al.,* 2008; Aljumaah and Hussein, 2012).

*Chlamydophila abortus* is considered the second most important cause of infectious abortion after brucellosis in many countries, and the main cause in most of the

countries where brucellosis has been controlled (Rodolakis, 2001). In Nigeria, several studies over the past four decades have shown that brucellosis was endemic in all parts of the country, causing serious reproductive losses in many livestock species (Cadmus *et al.*, 2006). It is on this basis that serological test for brucellosis was conducted in this study since it is a very important differential as far as diagnosis of chlamydial abortion is concerned in small ruminants in Nigeria. The test revealed a total of nine seropositive animals all of which were caprine species. No mixed infection was detected between *Chlamydophila abortus* and *Brucella* spp antibodies in any of the positive animals, indicating that the abortions in the animals were not of mixed aetiology. It is however, possible that other abortifacient organisms such as *Coxiella burnetti, Campylobacter fetus, Toxoplasma gondii, Listeria monocytogenes* or *Salmonella abortusovis* may play a role in the abortions that occurred in the animals that were sampled in this study. The overall prevalence of brucellosis was 2.45%. This was lower than the prevalence of *Chlamydophila abortus* reported in the same study. This finding is therefore suggesting that chlamydiosis most likely plays a more important role in small ruminant abortion than brucellosis in the study area. Furthermore, *Brucella* antibodies were detected in does in only one out of the four LGAs that were sampled, and all, except one, were from a single village in the study area. This is in contrast to chlamydial abortion in which evidence of existence of the disease was detected in all the sampling areas. Earlier report of brucellosis in Taraba State showed prevalences of 11.1% and 20% in sheep and goats, respectively (Zubairu *et al.,* 2014), which contradicts the finding of the present study. However, this could be attributed to the differences in sensitivity and specificity between the RBT used in the earlier study and the ELISA used in the present study. Previous

studies using different serological techniques showed that RBT detected higher number of seropositive animals than the other serological techniques that were used simultaneously (Bertu *et al.*, 2010; Kaltungo *et al.*, 2013). The RBT has also been described as not completely specific but only adequate as a screening test to detect infected flocks or to guarantee the absence of infection in brucellosis-free flocks (OIE, 2009). At the same time, ELISA has been described to have advantage over RBT in terms of sensitivity and specificity (OIE, 2009). Thus, it is not surprising that higher seroprevalence of brucellosis was detected using RBT than ELISA in the same study area.

# CHAPTER 6

# CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

This study has established a high prevalence of abortion in sheep flocks and goat herds in northern and central areas of Taraba State, Nigeria. Evidence of chlamydial abortion was detected in the area by demonstration of *Chlamydophila abortus*-specific Ig G in the sera of ewes and does that aborted. Serological evidence of brucellosis was also detected but with lower prevalence and very narrow geographical distribution than chlamydial abortion. The study has thus provided preliminary evidence that *Chlamydophila abortus* is likely more important pathogen causing abortion in small ruminants than *Brucella* spp in northern and central parts of Taraba State, Nigeria. This is also the first time serological exposure to *Chlamydophila abortus* was detected in ewes and does with history of abortion in northern and central parts of Taraba State, Nigeria.

## 6.2 RECOMMENDATION

Further work needs to be carried out to isolate and characterize *Chlamydophila abortus* from placentae, foetuses and vaginal discharges of aborting ewes and does in order to confirm its definitive role in causing abortion in small ruminants in the study area.

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