# SEROPREVALENCE OF COXIELLA BURNETTI IN CATTLE AND ITS RISK FACTORS IN KADUNA METROPOLIS, NIGERIA

**BY**

**Habiba, Bello TUKUR**

**DEPARTMENT OF VETERINARY PUBLIC HEALTH**
**AND PREVENTIVE MEDICINE**
**FACULTY OF VETERINARY MEDICINE**
**AHMADU BELLO UNIVERSITY,**
**ZARIA, NIGERIA**

**DECEMBER, 2012**

# SEROPREVALENCE OF COXIELLA BURNETTI IN CATTLE AND ITS RISK FACTORS IN KADUNA METROPOLIS, NIGERIA

**BY**

**Habiba, Bello TUKUR**
**(DVM, UNIMAID, 1998)**
**M.Sc/VET-MED/04753/2008-2009**

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE**

**DEPARTMENT OF VETERINARY PUBLIC HEALTH**
**AND PREVENTIVE MEDICINE,**
**FACULTY OF VETERINARY MEDICINE,**
**AHMADU BELLO UNIVERSITY,**
**ZARIA, NIGERIA**

**DECEMBER, 2012**

## DECLARATION

I declare that the work in this thesis titled "Seroprevalence of *Coxiella burnetti* in cattle and its risk factors in Kaduna metropolis" has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. I. Ajogi, Dr. J. Kabir and Prof. J.U. Umoh.

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 at any university.

Habiba Bello TUKUR, ---------- ----------
Signature Date

## CERTIFICATION

This thesis entitled "SEROPREVALENCE OF *COXIELLA BURNETTI* IN CATTLE AND ITS RISK FACTORS IN KADUNA METROPOLIS" by Habiba Bello TUKUR (M.Sc/Vet-Med/04753/08-09) meets the regulations governing the award of the degree of Master of Science in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University, and is approved for the contribution to knowledge and literary presentation.

| | | |
|---|---|---|
| Professor I. Ajogi,<br>Chairman, Supervisory Committee. | ----------<br>Signature | ----------<br>Date |
| Dr. J. Kabir,<br>Member, Supervisory Committee. | ----------<br>Signature | ----------<br>Date |
| Professor J.U.Umoh,<br>Member, Supervisory Committee. | ----------<br>Signature | ----------<br>Date |
| Dr. J. Kabir,<br>Head of Department,<br>Veterinary Public Health<br>and Preventive Medicine. | ----------<br>Signature | ----------<br>Date |
| Professor A.A Joshua,<br>Dean, School of Postgraduate Studies,<br>Ahmadu Bello University,Zaria. | ----------<br>Signature | ----------<br>Date |

## DEDICATION

This work is dedicated to my parents; Late Alhaji Mohammed Bello Tukur and Hajiya Safiya Bello Tukur, who gave me everything.

## ACKNOWLEDGEMENTS

All praise be to Allah, the Beneficent, the Merciful, Who has made it possible for me to complete my masters degree programme.

I am grateful to my committee of supervisors, Professor I. Ajogi, Dr. J. Kabir and Professor J.U. Umoh for guiding me throughout the work and for their constructive criticisms.

My sincere appreciation goes to my husband Abdurrahman Dangaji for his loving support, my children Mohammad Sani, Habiba, Suleiman, Mohammad Awwal and Safiya. My mother Hajiya Safiya Tukur, my sisters Maryam, Amina, Safiya and Rahama and my Brother Mohammed Tukur, my entire family who are too numerous to mention, thank you for your encouragement.

A big thank you to Dr .E .Ella of the Biotechnology Centre, A.B.U Zaria, Dr.N. Obijiaku, Dr Emma, Dr. P. Ezibolu, Dr. J. Ado and all my friends and colleagues.

My eternal gratitude goes to Dr. U.Baba who assisted in the collection of samples.

## ABSTRACT

Coxiellosis (Q fever) is an infectious disease associated with reproductive disorders which is of animal and public health significance. This work was carried out to determine the prevalence of antibodies to *Coxiella burnetti* in cattle from farms in Kaduna metropolis, and to determine if management practices, ectoparasite control, abortion history, and presence of other animals on the farm are risk factors associated with *Coxiella burnetti* infection. Twenty percent of cattle were randomly sampled from each of 42 identified farms. A total of 539 blood samples from cattle of both sexes were collected. Animal record forms were used to collect information on each animal, and a questionnaire was administered to each farm to assess the possible risk factors associated with *Coxiella burnetti* infection. The samples were analyzed using a commercially available enzyme linked immunosorbent assay (ELISA) kit obtained from Innovative Diagnostics (ID Vet) Montpellier-France, for determination of *Coxiella burnetti* antibodies. Data analysis was performed using Statistical Package for Social Sciences (SPSS) Version 17.0. Odds ratio (OR) and 95% CI on OR were calculated to check for associations and statistical significance. Fisher's Exact Test was used to test for associations in cells with small numbers. Of the 539 serum samples tested, 78 (14.5%) were positive, and of the 42 sampled farms, 24 (57.1%) had positive cases. There was no significant difference in prevalence between females (14.5%) and males (14.1%). *Coxiella burnetti* infection was significantly higher in the local breed ($P<0.05$) than cross breeds while none of the exotic breeds sampled tested positive. Infection was more prevalent in cattle over 3 years than in cattle 3 years and below (OR=1.13, 95% CI, 0.69-

1.86). There was no statistically significant relationship between management system and *Coxiella burnetti* infection, but prevalence was higher in the intensive management system (66.7%). Prevalence of infection was higher in herds with no ectoparasite control (100%) than in herds with ectoparasite control (56.1%). Cleaning and disinfection of equipment after use was found to have a significantly protective effect (OR = 0.15, 95% CI, 0.03-0.79). The prevalence of infection was higher in herds with no history of abortions or stillbirths (70%) compared to herds with abortion history (45.5%) (OR=0.36, 95% CI 0.10-1.28). The presence of other animals on the farm was associated with infection (OR=1.35, 95% CI, 0.08-23.20). Ninety five percent of respondents had never heard of Q fever, only 31% of the respondents boil milk before drinking, this is of public health concern. Good hygienic practices and ectoparasite control are recommended.

## TABLE OF CONTENTS


Title Page - - - - - - - - - i

Declaration - - - - - - - - - ii

Certification - - - - - - - - - iii

Dedication - - - - - - - - - iv

Acknowledgements - - - - - - - - - v

Abstract - - - - - - - - - vi

Table of Contents - - - - - - - - - viii

List of Figures - - - - - - - - - - xii

List of Tables - - - - - - - - - - xiii

List of Appendices - - - - - - - - - xiv

**CHAPTER 1: INTRODUCTION**

1.1 Background - - - - - - 1

1.2 Statement of the Research Problem - - - - - 3

1.3 Justification of the Study - - - - - 4

1.4 Aim of the Study - - - - - - - 5

1.5 Objectives of the Study - - - - - - - 5

1.6 Research Questions - - - - - - - - 5

**CHAPTER 2: LITERATURE REVIEW**

2.1 History - - - - - - - - - 6

2.2 Taxonomy and Aetiology - - - - - - 7

2.3 Structure of *Coxiella burnetti* - - - - - 7

2.4 Bacteriology - - - - - - - - 8

2.5 Ecology - - - - - - - - 9

2.6 Epidemiology - - - - - - - - 9

2.6.1 Vectors - - - - - - - - 10

2.6.2 Reservoirs - - - - - - - - 11

2.6.3 Ruminants - - - - - - - - 11

2.6.4 Other animals - - - - - - - - 11

2.7 Routes of transmission - - - - - - 12

2.7.1 Inhalation - - - - - - - - 12

2.7.2 Oral route - - - - - - - - 13

2.7.3 Human to human transmission - - - - - 13

2.7.4 Sexual transmission - - - - - - - 13

2.7.5 Other routes - - - - - - - - 13

2.8 Pathogenesis - - - - - - - - 13

2.9 Pathology - - - - - - - - 15

2.10 Clinical manifestations in ruminants- - - - - - 15

2.11 Clinical manifestations in humans - - - - - 15

2.11.1 Acute Q fever - - - - - - - 16

2.11.2 Chronic Q fever - - - - - - - 16

2.12 Immunity - - - - - - - - 16

2.13 Diagnosis - - - - - - - - 17

2.13.1 Serology - - - - - - - - 17

2.13.1.1 Complement fixation test (CFT)- - - - - - 18

2.13.1.2 Enzyme linked immunosorbent assay (ELISA) - - - - 18

2.13.1.3 Immunofluorecence assay (IFA)- - - - - - 18

2.13.2 Colouration and direct visualization - - - - 19

2.13.3 Immunohistochemistry - - - - - - 19

2.13.4 Bacterial culture - - - - - - - 20

2.13.5 Polymerase chain reaction (PCR) - - - - - 20

2.14 Treatment - - - - - - - - 21

2.14.1 Treatment in humans - - - - - - 21

2.15 Prevention and Control - - - - - - 22

2.15.1 Vaccination - - - - - - - - 23

2.16 Bioterrorism - - - - - - - - 24

2.17 Worldwide distribution - - - - - 25

2.18 Distribution in Nigeria - - - - - - 26

2.19 Socio-economic implications of the disease - - - 27

**CHAPTER 3: MATERIALS AND METHODS**

3.1 Study Area - - - - - - - - 28

3.2 Study Design - - - - - - - - 30

3.3 Inclusion Criteria - - - - - - - 30

3.4 Sampling and Sample Size - - - - - - 30

3.5 Sample Collection - - - - - - - 31

3.6 Questionnaire Administration - - - - - - 31

3.7 Enzyme linked immmunosorbent assay (ELISA) Kits - - 31

3.8 Detection of *Coxiella burnetti* Antibodies - - - - 32

3.9 Validation - - - - - - - - 33

3.10 Interpretation - - - - - - - - 33

3.11 Statistical Analysis - - - - - - - 34

**CHAPTER 4: RESULTS**

4.1 Demographic characteristics - - - - - - - - - - 35

4.2 Prevalence of *Coxiella burnetti* in cattle from farms in Kaduna Metropolis - 35

4.3 Possible risk factors associated with *Coxiella burnetti* infection

in cattle from farms in Kaduna Metropolis - - - - - - - - 38

**CHAPTER 5: DISCUSSION**

Discussion - - - - - - - - - - 45

**CHAPTER 6: SUMMARY, CONCLUSION AND RECOMMENDATIONS**

6.1 Summary - - - - - - - - 50

6.2 Conclusion - - - - - - - - 50

6.3 Recommendations - - - - - - - 51

REFERENCES - - - - - - - - 52

APPENDICES - - - - - - - - 67

## LIST OF FIGURES

FIGURE

3.1 Map of Kaduna Metropolis showing the study areas - - - 29

4.1 Mode of consumption of milk by respondents - - - 40

## LIST OF TABLES


TABLE PAGE

4.1 Prevalence of *Coxiella burnetti* infection in relation to age, sex and breed of cattle in farms in Kaduna Metropolis - - - - 37

4.2 Prevalence of *Coxiella burnetti* infection in relation to management of cattle in farms in Kaduna Metropolis - - 41

4.3 Prevalence of *Coxiella burnetti* infection in relation to parasite and rodent control in cattle farms in Kaduna Metropolis - - 42

4.4 Prevalence of *Coxiella burnetti* infection in relation to biosecurity measures in cattle farms in Kaduna Metropolis - - - 43

4.5 Prevalence of *Coxiella burnetti* infection in relation to reproductive problems and presence of other animals in cattle farms in Kaduna Metropolis - - - - - - - 44

## LIST OF APPENDICES


| | | |
|---|---|---|
| Appendix I | Questionnaire on risk factors associated with Coxiellosis (Q fever) | 67 |
| Appendix II | Individual animal record form - - - - | 72 |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND

Q fever is an infectious disease caused by the bacterium *Coxiella burnetti*. The organism is a small obligate intracellular Gram negative bacterium and was reclassified from the order Rickettsiales to Legionellales and falls in the gamma group of Proteobacteria. The organism is enzootic in cattle, sheep and goats throughout most of the world (Lang, 1990; Kazar, 2005). Q fever was first described by Edward Holbrook Derrick in abattoir workers in Brisbane, Queensland, Australia. The "Q" stands for Query and was applied at a time when the causative agent was unknown (Derrick, 1937).

Q fever is a zoonosis which is endemic throughout the world with the exception of Antartica and New Zealand, occurring in diverse geographic regions and climate zones. *Coxiella burnetti* infects arthropods (mainly ticks), birds, domestic and wild mammals, as well as humans (Woldehiwet, 2004; Rodolakis, 2006). It is a highly infectious agent that is widespread among livestock around the world (Arricau-Bouvery and Rodolakis, 2005). Sheep, goats and cattle are the primary reservoirs of *Coxiella burnetti*, which is excreted in the urine, milk and faeces of infected animals. *Coxiella burnetti* is shed during parturition in high numbers in the amniotic fluids and the placenta, can survive for long periods in the environment and is resistant to heating, drying and many common disinfectants (Kim *et al.*, 2005; Guatteo *et al.*, 2006). Although over 40 tick species can be naturally infected with *C. burnetti*, they appear not to be important in the maintenance of infections in livestock or humans (Maurin and Raoult, 1999). The organism's resistance to chemical and physical agents is exceptional and enables it to maintain itself

in nature without an arthropod vector and makes airborne dissemination of infection possible (Little, 1983).

The main sources of environmental contamination and infection of humans are parturient ruminants, dogs and cats infected with *C. burnetti* (Woldehiwet, 2004). The main route of *C. burnetii* infection is by inhalation of contaminated aerosols or dusts containing the micro organism shed from infected animals. Persons in contact with farm animals can be infected by inhalation of contaminated aerosols from amniotic fluid or placenta or contaminated wool, but also at risk are laboratory personnel who work with infected animals (Johnson and Kadull, 1966).

Mammals also shed *C. burnetti* in milk and thus consumption of raw milk could be a source of infection (Maurin and Raoult, 1999). Person to person transmission is rare. A recent report describes sexual transmission in humans (Milazzo *et al*., 2001) and viable *C. burnetti* has been found in semen of bulls (Kruszewska and Tylewsk-Wierzbanowska, 1997).

Reproductive disorders such as abortion, still birth, and delivery of weak and unviable newborns have been reported in livestock infected with *C. burnetti* (Bildfell *et al*., 2000). However, lack of overt clinical signs exhibited among infected livestock make diagnosis a challenge (Cutler *et al*., 2007). Q fever is a polymorphic disease in humans with subclinical, acute and chronic forms. It is typically an acute febrile illness with non specific clinical signs, but hepatitis and atypical pneumonia are seen in severe cases, and a small percentage of infected people will develop chronic infection with endocarditis (Kazar, 2005).

Epidemiological studies are often based on serological investigations. These include complement fixation test (CFT), indirect fluorescent antibody test (IFAT), capillary agglutination (CA) test, indirect immunoflourescence assay (IFA), microagglutination test (MAT), enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

## 1.2 STATEMENT OF THE RESEARCH PROBLEM

*Coxiella burnetti* has been detected in a number of cases of abortions in different parts of the world (Waldham *et al*., 1978; Palmer *et al*., 1983; Raju *et al*., 1988; Zeman *et al*., 1989) making coxiellosis an economically important disease for domestic livestock.

Oral transmission, by ingestion of contaminated raw milk or dairy products could lead to infection and in few cases to Q fever (Cutler *et al.,* 2002; McQuiston *et al*., 2002; Rodolakis, 2006).

Past investigations confirmed that some infections occur frequently in farmers, where the contact with cattle and sheep is associated with Q fever (Coleman, 2000). The risk of Q fever on people who work with domestic animals is related to contact with farm environment rather than any specific animal exposure (Thomas *et al*., 1995).

The interest for Q fever is increasing worldwide as indicated by the rising number of reviews published (Arricau-Bouvery and Rodolakis., 2005; Cutler *et al*., 2002; EFSA., 2010a; Kovakova and Kazar, 2002) even in countries where its incidence is supposed to be very low. Indeed, the disease is considered as a re-emerging zoonosis in many countries. Because of the polymorphism of the clinical picture and because the diagnosis is based exclusively on serology, the prevalence of *C. burnetti* infection among animals is largely unknown (Rey *et al*., 2000).

From 1999 to 2004, there were 18 reported outbreaks of Q fever from 12 different countries involving 2 to 289 people. Six outbreaks involved sheep, three involved goats, one resulted from exposure to ovine manure, one involved exposure to wild animals, one involved exposure to cats and dogs, and in two outbreaks the source was unknown (Arricau-Bouvery and Rodolakis, 2005). In the Netherlands, since 2007, Q fever has become a public health problem with 2,357 human cases notified in 2010 (Van der Hoek *et al*., 2010).

Available studies on *Coxiella burnetti* in cattle in Nigeria indicate that Addo and Schnurenberger (1977) found a prevalence of 11% in slaughter cattle from Samaru, Zaria, and Kaduna abattoirs and from Bauchi meat processing plant, all in Northern Nigeria and Adesiyun *et al* (1984) reported a prevalence of 59.8% in some dairy cows and their suckling calves in Zaria. The findings of this study will provide the current situation of the disease in Kaduna, one of the major livestock producers in Nigeria.

## 1.2 JUSTIFICATION OF THE STUDY

The main source of animal protein is livestock such as cattle, sheep and goats. *Coxiella burnetti* infection leads mainly to reproductive disorders like abortions, stillbirths, weak calf delivery, mastitis, metritis and infertility, with associated economic impact for the herd (Arricau-Bouvery and Rodolakis, 2005) and a reduction in the available source of protein for human consumption. The major occupation of the people of Kaduna state is agriculture, mainly for food and cash crops and rearing livestock (KDSG, 2008). Kaduna state has an estimated population of 1,144,000 cattle, (KDSG, 2008). There is the need to investigate *Coxiella burnetti* infection within Kaduna where there are a large number of

cattle. The current study can be used for proper evaluation of the disease and possible control measures for Q fever in Kaduna and Nigeria as a whole.

## 1.3 AIM OF THE STUDY

To use serological test in determining the presence of antibodies and to administer questionnaires.

## 1. 4 OBJECTIVES OF THE STUDY

i. To determine the seroprevalence of *Coxiella burnetti* infection in cattle from farms in Kaduna Metropolis.

ii. To determine the effect of sex, breed and age on the occurrence of *Coxiella burnetti* infection in cattle from farms in Kaduna Metropolis.

iii. To determine the risk factors associated with *Coxiella burnetti* infection.

## 1.5 RESEARCH QUESTIONS

i. What is the prevalence of *Coxiella burnetti* infection in cattle from farms in Kaduna Metropolis?

ii. Is there a difference in prevalence between the breeds, sex and age groups?

iii. Are management practices, abortion history and presence of other animals on the farm, associated with *Coxiella burnetti* infection?

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 HISTORY

In 1935, the Q fever agent was first recognized by Edward Holbrook Derrick in febrile livestock handlers in Australia (Derrick, 1937). The nine mile agent later identified as the same organism as the Q-fever agent in Australia was isolated by a worker in 1936 in the United States at the Rocky Mountain Laboratory in Montana. In 1938 it was reported that transmission may occur via ticks (Rehacek and Tarasevich, 1988; McDade, 1990). This pathogen formerly referred to as *Rickettsia burnetti* was classified into a new genus by Philip (1948) known as *Coxiella*, named after Herald R. Cox who first isolated the organism in the United States. The definitive descriptions were published in the late 1930s as part of research into the cause of Q fever, by Edward Holbrook Derrick and Macfarlane Burnet in Australia, and Herald Rea Cox and Gordon Davis at the Rocky Mountain Laboratory (RML) in the United States (McDade and Joseph, 1990).

The RML team proposed the name *Rickettsia diaporica*, derived from the Greek word for having the ability to pass through filter pores. Around the same time, Derrick proposed the name *Rickettsia burnetti*, in recognition of Burnet's contribution in identifying the organism as a *Rickettsia*. As it became clear that the species differed significantly from other *Rickettsia* organisms, it was first elevated to a subgenus named after Cox, *Coxiella*, and then in 1948 to its own genus of that name, proposed by Cornelius B. Philip, another RML researcher (McDade and Joseph, 1990).

## 2.2 TAXONOMY AND AETIOLOGY

*Coxiella burnetti*, the causative agent of Q fever is of the scientific classification:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Legionelalles

Family: Coxiellaceae

Genus: Coxiella

Species: *Coxiella burnetti*

(Derrick, 1937; Philip, 1948)

The causal agent of Q fever is *Coxiella burnetti* an obligate intracellular, pleomorphic, Gram-negative bacterium of the Legionellales order, which was first observed as a rickettsia- like organism in the spleen and liver of mice inoculated with the urine of abattoir workers (Ransom and Huebner, 1951). Its predilected target cells are the macrophages located in body tissues (e.g. lymph nodes, spleen, lungs, and liver) and the monocytes circulating in the blood stream (Baca *et al.*, 1985).

## 2.3 STRUCTURE OF *COXIELLA BURNETTI*

*Coxiella* has a unique intracellular life with two distinct morphological forms, the large cell variant (LCV) and the small cell variant (SCV) (McCaul and Williams, 1981). They can be differentiated by size, morphology, peptidoglycan content and resistance to physical disruption. Small cell variants are the environment extracellular form of the organism. They are 0.2 to 0.5µm long, compact and typically rod-shaped with an electron-dense core bounded by cytoplasmic and outer membranes (McCaul and

Williams, 1981). They are metabolically inactive, and show a high degree of resistance to chemical agents and physical conditions, such as osmotic pressure and sonic disruption (McCaul and Williams, 1981).This confers the ability for the organism to survive for prolonged period in the environment. In contrast, LCVs resemble Gram-negative bacteria, they can exceed 1µm in length, but are more pleomorphic than the SCVs. They possess a thinner cell wall, with a more dispersed filamentous nucleoid region. They are metabolically active, and are the intracellular form of the organism. McCaul and Williams (1981) reported a 'spore-like particle' in the polar regions of some LCVs and thus they hypothesized that an endogenous spore was a part of the developmental cycle of *C. burnetti* ((McCaul and Williams, 1981). Further work showed that the spore like particle did not stain with spore stains, and was not detected by tests for dipicolinic acid, a traditional spore marker (McCaul, 1991)

## 2.4 BACTERIOLOGY

*Coxiella burnetti* exists in two antigenic phases, phase I and phase II forms. Phase variants display different lipopolysaccharide lengths (LPS). Lipopolysaccharide is the major structural component of the outer wall of all Gram negative bacteria and a potent activator of the immune system, with phase I organisms producing a full-length LPS with O antigen sugars, and phase II organisms producing a truncated LPS without O antigen (Schramek and Mayer, 1982). The phase I form is isolated from infected hosts, but not the phase II form. Only following serial passage in eggs or tissue culture can the phase II form be obtained (Fiset, 1957). The response to the phase II antigen is much higher during acute infection than chronic infection, whereas titers to phase I antigen are higher during chronic infection compared to acute infection (Olson *et al.*, 2006).

*Coxiella burnetti* enters cells passively (Baca *et al.*, 1993) and resides within a parasitophorous vacuole (PV), which has been described as a structure similar to a secondary lysosome. The PV is an acidic environment (PH 4.7-4.8). Morphogenesis from SCV to LVC occurs during an initial lag phase with no increase in bacterial number (Coleman *et al.*, 2004). Intracellular growth is relatively slow, with a doubling time of approximately 8-12 hours (Baca and Paretsky, 1983).

## 2.5 ECOLOGY

Human Q fever has been described in countries around the world with the exception of New Zealand (Hilbink *et al.*, 1993), where no case of Q fever has ever been reported. As it is not a notifiable disease in many countries, the geographical distribution of the organism is extrapolated from serological surveys and investigated outbreaks (Maurine and Raoult, 1999). Data obtained from public health laboratories or reference laboratories for bacterial diseases are also good source of information on the distribution of the disease.

## 2.6 EPIDEMIOLOGY

*Coxiella burnetti* infection of livestock is termed coxiellosis, a chronic but often symptomless disease. The uterus and mammary glands are sites of chronic infections in goats and sheep, and infertility in cattle (Palmer *et al.*, 1983; To *et al.*, 1998). *Coxiella burnetti* is shed from milk, urine and faeces. At birth, the placenta contains vast quantities of *Coxiella,* which are released into the environment. Farming practices such as transport of infected animals and the spreading of contaminated manure into fields can facilitate environmental spread (Enserink, 2010). This, together with the environmental stability of

the organism, poses a difficulty in containing outbreaks, as has been noted in the recent outbreak in the Netherlands (Enserink, 2010).

### 2.6.1 Vectors

A range of arthropods, including over 40 tick species are naturally infected with *C. burnetti*, can be colonized via ingestion of contaminated blood meals and release significant quantities of *Coxiella* in their faeces. While experimental transmission between guinea pigs has been achieved via tick bite (Smith, 1940; 1941), arthropod vectors are not considered essential to the natural cycle of infection in livestock that live closely together and likely contract the organism from close contact with other infected animals (Babudieri, 1959). In contrast, ticks may play a significant role in the transmission of coxiellosis among the wild vertebrates, especially in rodents, lagomorphs, and wild birds (Lang, 1990; Marrie *et al*., 1986; Stein and Raoult., 1999). The possibility of *C. burnetti* being transmitted to humans via tick bite has seldom been reported (Eklund *et al*., 1947) and human Q fever is rarely if ever an arthropod borne disease.

Some ticks involved in transmission include: *Rhipicephalus sanguineus* (Mantovani and Benazzi, 1953), *Ixodes holocyclus, Haemaphysalis bispinosa, Haemaphysalis humerosa* (Smith and Derrick, 1940), *Amblyomma triguttatum* (Pope *et al*.,1960), *Dermacentor andersoni, Ixodes ricinus, Dermacentor occidentalis, Amblyomma americanum, Haemophysalis leporis-palustris, Ixodes dentatus, Otobius magnini* (Cox, 1938; Cox, 1940; Davis, 1939).

### 2.6.2 Reservoirs

Q fever is a zoonosis with a worldwide distribution. The reservoir is large and includes many wild and domestic mammals, birds, and arthropods such as ticks. Babudieri (1959) in a review reported that *C. burnetti* was detected in virtually all the animal kingdoms. However, domestic ruminants represent the most frequent source of human infection with *C. burnetti* (Marrie, 1990). Animals are often chronically infected but do not experience symptoms of *C. burnetti* infection. The uterus and mammary glands are sites of chronic *C. burnetti* infection (Babudieri, 1959).

### 2.6.3 Ruminants

Cattle, goats and sheep are considered the primary reservoirs from which human contamination occurs. Infected mammals shed *C. burnetti* in their urine, faeces, milk and birth products, from which humans may be infected. Abortions in sheep and goats (Palmer and Young, 1982; Waldhalm *et al*., 1978) and lower birth weight and infertility in cattle have been associated with chronic *C. burnetti* infection (Ho *et al*., 1995; Schmeer *et al*., 1987). Epidemiological data indicate that cattle are more frequently chronically infected than sheep and thus may represent the most important source of human infection (Ho *et al*., 1995).

### 2.6.4 Other animals

Cats and dogs are reservoirs of *C. burnetti*. Dogs may be infected by tick bite (Mantovani and Benazzi, 1953), by consumption of placentas or milk from infected ruminants, and by the aerosol route. *Coxiella burnetti* infection in parturient dogs may lead to the early death of pups (Buhariwalla *et al.,* 1996). The possibility of human Q fever acquired from

infected dogs has been reported (Buhariwalla *et al*., 1996; Rauch *et al*., 1987). Human Q fever cases were described in Nova Scotia after contact with parturient cats (Kosatsky 1984; Marrie *et al*., 1988).

Anecdotal reports indicate that *C. burnetti* may be isolated from other arthropods including chiggers (Babudieri, 1959) lice (Giround and Jardin, 1954) and flies (Philip, 1948). The role of these arthropods in the natural cycle of *C. burnetti* remains unknown.

*Coxiella burnetti* infection has been reported less frequently in a number of other domestic or wild mammals, including horses, rabbits, swine, camels, water buffalo, rats and mice (Babudieri, 1959). Birds may also be infected, and *C. burnetii* was isolated from pigeons, chickens, ducks, geese, and turkeys (Babudieri, 1959). Humans may acquire Q fever from infected domestic poultry by consumption of raw eggs or inhalation of contaminated fomites. Anti *C. burnetti* antibodies have been found in snakes and tortoises in India, but *C. burnetti* has not been isolated from these animals (Babudieri, 1959).

## 2.7 ROUTES OF TRANSMISSION

### 2.7.1 Inhalation

This is the most common route of infection in both animals and human (Welsh *et al*., 1958; Russell-Lodrigue *et al.,* 2006). Under experimental conditions, inhalation of a single organism can produce infection and clinical disease in humans (Tigertt *et al*., 1961). However, similar studies have not been done in animals (EFSA 2010a). Infected animals contaminate the environment by shedding *C. burnetti* in milk, faeces, urine, saliva (Hirai and To, 1998; Guatteo *et al.,* 2006), and very importantly in vaginal secretions, placenta and amniotic fluids (Hirai and To, 1998). *Coxiella burnetti* also

spreads by aerosols causing infections at a distance from the initial source of bacteria (To *et al.*, 1996; Okimoto *et al.*, 2004).

### 2.7.2 Oral Route

In domestic ruminants, milk is the most frequent route of pathogen shedding (Rodolakis *et al.*, 2007) and thus consumption of raw milk is a source of infection (Maurin and Raoult, 1999).

### 2.7.3 Human to Human Transmission

Human to human transmission does not usually occur (OlE, 2005; Watanabe and Takahashi, 2008) however, it has been described following contact with parturient women (Deutch and Peterson, 1950; Raoult and Stein, 1994).

### 2.7.4 Sexual Transmission

Sexual transmission of Q fever has been reported in humans (Kruszewska *et al.*, 1996; Micelli *et al.*, 2010).

### 2.7.5 Other Routes

The risk of transmission through blood transfusion is considered negligible (Delsing and Kullberg, 2008). Transplacental transmission, intradermal inoculation, and postmortem examinations have been associated with sporadic cases of Q fever (Harman, 1949).

## 2.8 PATHOGENESIS

*Coxiella burnetti* infects a great number of cell types *in vivo* and *in vitro* including monocytes, macrophages, and a variety of transformed cells (L929, HELA or VERO cells) (Baca and Paretsky, 1983). This capacity to invade and subsequently to grow

within such eukaryotic cells is an important factor of virulence of *C. burnetti* that allows its propagation in different niches of the host (Arricau-Bouvery *et al.,* 2003; Bruneval *et al.,* 2001). *Coxiella burnetti* survives and divides in a phagolysosome, which is an acidified environment. The small and large forms express different genes that permit the bacterium to survive in the specific harsh compartment of each cell. For example, RpoS (Sigma S) is a sigma subunit that confers promoter specific transcriptional initiation by ribonucleic acid (RNA) polymerase to genes that are expressed during the stationary phase in main bacteria. The RpoS allows multi stress resistance and causes morphological and physiological alterations. Small cell variant does not contain significant RpoS while large cell variant express abundant RpoS (Sheshadri and Samuel, 2001).

The small forms are able to delay phagolysosome fusion perhaps to facilitate the transition from SCV to LCV that occurs at pH 5.5 in the endosome (Howe and Malavia, 2000). The *C. burnetti* secretion system is related to the *Legionella* secretion system (Zamboni *et al.,* 2003; Zusman *et al.,* 2003). This secretion system could play an important role in creating the specialized vacuole that supports *C. burnetti* replication.

The uptake of virulent *C. burnetti* depends on toll like receptor 4 (TLR4) which detect lipopolysaccharide from Gram negative bacteria (Dellacasagrande *et al.,* 2000). TLR4 controls the early events of *C. burnetti* infection including macrophage phagocytosis, granuloma formation and cytokine production (Honstettre *et al.,* 2004). This receptor is not specific for humans and can also be found in insects (Janssens and Beyaert, 2003; Vasselon and Detmers, 2002). Thus, *C. burnetti* has developed a cell uptake strategy that

allows it to invade a broad range of hosts and has developed a unique tactic to multiply in the harsh phagolysosome environment of cells.

## 2.9 PATHOLOGY

In the acute phase, the presence of *C. burnetti* may be demonstrated in the blood, lungs, spleen and liver. Mice injected intranasally or intraperitoneally with *C. burnetti* usually remain asymptomatic (Lang, 1990). However, these animals develop granulomatous lesions with mononuclear cells in the spleen, liver, kidneys, and adrenals (Burnet and Freeman 1937; Perrin and Bengtson, 1942). Placental necrosis and bronchopneumonia were also significantly associated with the presence of C*oxiella burnetti* in the trophoblasts (Bildfell *et al.*, 2000).

## 2.10 CLINICAL MANIFESTATIONS IN RUMINANTS

In ruminants, well- known manifestations of Q fever are abortion, stillbirth, premature delivery, and delivery of weak offspring's (Angelakis and Raoult, 2010). However these clinical manifestations are generally only expressed in sheep and goats. In cattle, Q fever is frequently asymptomatic. Clinically infected cows develop infertility, metritis, and mastitis (To *et al.,* 1998). In addition, *C. burnetti* was found to be significantly associated with placentitis (Bildfell *et al.,* 2000). Unlike humans and experimentally infected cows, naturally infected ruminants rarely present respiratory or cardiac signs (Plommet *et al.*, 1973).

## 2.11 CLINICAL MANIFESTATIONS IN HUMANS

Q fever is a polymorphic disease in humans with subclinical, acute and chronic forms. Infections can be totally asymptomatic in 50 to 60% of cases or cause a self limiting illness associated with fever, fatigue, headache and myalgia (Influenza-like syndrome).

### 2.11.1 Acute Q Fever

Acute Q fever is frequently accompanied by atypical pneumonia and/or hepatitis. Pneumonia is an important manifestation of Q fever in humans (Derrick, 1937; Babudieri, 1959). In pregnant women, Q fever can lead to spontaneous abortion, intrauterine fetal death, premature delivery, or intrauterine growth retardation. Transplacental infection of the fetus inutero has also been reported (Raoult and Stein, 1994; Raoult *et al.,* 2002)

### 2.11.2 Chronic Q Fever

Chronic Q fever is associated with the persistence of infection for more than 6 months. It occurs in 5% of infected individuals (Fournier *et al.,* 1998). Most commonly, endocarditis is observed in 60-70% of cases of chronic infections (Fenollar *et al.,* 2004), but chronic hepatitis, osteomyelitis, septic arthritis, interstitial lung disease, chronic fatigue syndrome, or infection of aneurysm and vascular grafts can also occur (Derrick, 1937; Benenson and Tigertt, 1956). Meningitis, encephalitis, polyradiculoneuritis, peripheral neuropathy, cranial nerve deficiency, optic neuritis, and paralysis of the oculo motor nerve have been reported in 3.5% of infected patients. The prognosis of chronic infections is less favorable than for acute infections and the course of the disease is

usually long, with mortality rates that can reach more than 50% (Watanabe and Takahashi, 2008).

## 2.12 IMMUNITY

Cell- mediated immune responses are important to controlling Q fever, but the role of humoral responses is not clear. For a range of intracellular pathogens, it has been shown that both cell- mediated and humoral responses are required to defend against intracellular pathogens (Casadevall and Pirofski, 2006). During acute Q fever, antibodies are important during the bacteraemic phase of infection, but are potentially detrimental during chronic infection, where immune complexes can cause pathology (Raoult, 1990). Passive transfer of antibody can protect animals against subsequent challenge with the organism (Burnet and Freeman, 1983). No protection was seen if antibody was transferred to severe combined immunodeficiency (SCID) mice, a strain of mice lacking in lymphocytes and immunoglobulins, showing the importance of the cell-mediated response (Zhang *et al.,* 2007). Opsonization resulted in increased uptake *in vitro* by macrophages and dendritic cells, but did not subsequently impact on growth intracellularly (Shannon *et al.,* 2009).

## 2.13 DIAGNOSIS

Q fever diagnosis is based on serological methods because culture and molecular biology techniques are available only in reference laboratories (Fournier *et al*., 1998).

### 2.13.1 Serology

Due to the fact that clinical diagnosis of this disease is difficult, serology has been relied upon for diagnosis. Various methods employed include microagglutination (Fisset *et al*.,

1969), complement fixation (Herr *et al*., 1985), radioimmunoassay (Doller *et al*., 1984), indirect immunofluorescence antibody tests (Field *et al*., 1983), indirect haemolysis test (Tokarevich *et al*., 1990), enzyme linked immunosorbent fluorescence assay (Kovacova *et al*., 1987; Schemer *et al*., 1988), dot immunoblotting, and western immunoblotting (Blondeau *et al*., 1990). The techniques most commonly used include complement fixation, indirect immunofluorescence, enzyme linked immunosorbent assay, and microagglutination.

#### 2.13.1.1 *Complement Fixation Test*

In veterinary medicine, complement fixation test (CFT) was the method of reference for serological diagnosis according to OIE (AFSSA, 2004; Herr *et al*., 1985). Complement fixation test usually utilizes phase II antigens only (Kit, 2003; AFSSA, 2004). It is capable of detecting approximately 65% of infected subjects during the second week after initial clinical signs and 90% during the fourth week (OlE, 2005). Complement fixation test is more laborious, less specific, and less sensitive than indirect immunofluorescence assay (IFA) or ELISA (Fournier *et al*., 1998). Rousset *et al*. (2007) advised not to use CFT for serological animal screening because of its low sensitivity.

#### 2.13.1.2 *Enzyme - linked immunosorbent assay*

Another method of diagnosis for human and animal cases is enzyme-linked immunosorbent assay (ELISA). This method is more sensitive and easier to perform, and to standardize than CFT (Fournier *et al*., 1998). Commercially available ELISA kits are frequently coated by phase I and phase II antigens. The antigens present are of two possible origins, antigens of the American Nine Mile strain of *Coxiella burnetti* isolated from an endogenous tick or antigens of a strain originating from infected European

domestic ruminants. Enzyme-linked immunosorbent assay kits coated by the latter antigens are more sensitive and are advised for serological diagnosis (ACERSA, 2006).

#### 2.13.1.3 *Immunofluorescence assay*

In human medicine, immunofluorescence assay (IFA) is commonly considered the reference diagnostic test and is the most frequently used worldwide. It is accurate, highly sensitive, and specific (Fournier *et al*., 1998). The study by Rousset *et al*. (2007) on goats, demonstrated an overall good agreement between IFA and ELISA. Also, IFA is capable of detecting the two antigenic variants of *Coxiella burnetti* (Phase 1 and Phase II).

### 2.13.2 Coloration and Direct Visualization

Direct visualization of *Coxiella burnetti* on smears or frozen tissue is a method of Q fever diagnosis. Smears are taken from the placenta of aborted ruminants, from the foetus stomach content, or from other body tissues. *Coxiella burnetti* does not stain reliably with Gram stain, and Gimenez stain is preferentially used (Rousset *et al*., 2007). A stamp-Macchivello coloration, commonly called Macc staining, or routine Giemsa stain can also be performed. The specificity and sensitivity of direct visualization by bacterioscopic examination is poor, due to possible confusion with other pathogens such as *Brucella spp*, *Chlamydophilia spp* or *Chlamydia spp* (Guatteo *et al*., 2006).

### 2.13.3 Immunohistochemistry

Immunohistochemistry (IHC) has been performed for the diagnosis of chronic cases of Q fever. It can be utilized for detection of *Coxiella burnetti* in tissues fixed in paraffin

(Raoult *et al*., 1994). Dilbeck and McElwain, (1994), developed an avidin-biotin peroxides complex IHC staining method for diagnosis and routine screening of ovine and caprine placenta tissues after abortion. This technique is rapid and does not necessitate live bacteria or fresh tissues for diagnosis. Furthermore, it renders retrospective studies on stored samples possible. However, IHC is not useful for large- scale epidemiological studies.

### 2.13.4 Bacterial Culture

*In vitro* cell culture of the bacteria is the gold standard for diagnosis of bacterial infections. *Coxiella burnetti* can be cultured efficiently in the yolk sac of chicken embryos and also on diverse cellular specimens, such as human embryonic lung fibroblasts, mosquito cells, green monkey kidney (Vero) cells, L cells, tick tissue cultures, and so forth (Baca and Paretsky, 1983). However, technically, culture of *Coxiella burnetti* remains a difficult process and sensitivity of this diagnostic method is low. Another practical limitation to bacterial isolation is that it requires a biosafety level 3 laboratory because of its high infectivity. As a result, culture is rarely performed, especially in veterinary medicine (Fournier *et al*., 1998).

*Coxiella burnetti* can also be grown outside a host cell in a cell-free laboratory medium (Omsland *et al.*, 2009). This medium has a composition that strictly corresponds to the organisms metabolic requirements in the phagolysosome (*in vivo Coxiella* is strictly intracellular). This finding is revolutionary and will permit further studies on *Coxiella burnetti.*

### 2.13.5 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) offers substantial benefits for the identification of *Coxiella burnetti* compared to other laboratory techniques, especially in the early stages of the illness (Frangoulidis *et al*., 2009). It has been successful in detecting *Coxiella burnetti* DNA in various samples, including cell cultures, biopsy samples, blood, arthropods, and serum samples (Fenollar *et al*., 2004; Ughetto *et al*., 2009). Its sensitivity and specificity are high. However, the usefulness of conventional PCR is limited by its inability to quantify the bacteria present. The development of real - time quantitative PCR (RTq PCR) not only renders PCR a rapid diagnostic tool but also provides quantifiable information. The RTq PCR can be automated and thus can be used in large- scale studies. The qualities of PCR make it very useful for early diagnosis of infection during the period when antibodies are not yet present (Fournier and Raoult, 2003). Several primers are available for diagnosis (Frangoulidis *et al.,* 2009*).* A primer originating from the frequently repeated DNA sequence IS 1111 (7 to 120 copies per genome), is commonly used and has proven to allow very sensitive testing (Frangoulidis *et al*., 2009; Schneebergel *et al*., 2010). RT-PCR assays are now recognized as the most suitable tool for diagnosing abortion due to *C. burnetti* and for identifying animals shedding the organism (Guatteo *et al.,* 2006).

## 2.14 TREATMENT

Antibiotic treatment, consisting of two injections of Oxytetracycline (20mg/kg), in the last month of gestation is often used to reduce the number of abortions and the quantity of *C. burnetti* shed at parturition. The efficacy of this treatment has never been accurately

assessed, but it has been shown that it does not entirely prevent abortion (Berri *et al*., 2005) or the shedding of *C. burnetti* at lambing (Arricau - Bouvery and Rodolakis, 2005).

### 2.14.1 Treatment in Humans

Acute Q fever in humans resolves most often spontaneously and when Q fever is diagnosed by serology the patient is convalescent. When antibiotics are used, tetracycline and doxycline are the most used to treat acute Q fever (Kovacova and Kazar, 2002). A regimen of doxycline at 200 mg daily (100 mg bid) for 15 - 21 days is commonly prescribed. Quinolone compounds should be considered in Q fever meningoencephalitis as these compounds penetrate the cerebro-spinal fluid. Chronic Q fever is treated with doxycycline in combination with hydroxychloroquine for a period of eighteen months to three years (Maurin and Raoult, 1999).

## 2.15 PREVENTION AND CONTROL

Adding 0.5% of Calcium Cyanamid to contaminated dung is an effective disinfectant (Arricau- Bouvery *et al*., 2001).

In the UK, Health Protection Agency guidelines suggest the use of 2% formaldehyde, 1% Lysol, 5% Hydrogen peroxide, 70% ethanol, or 5% chloroform for decontamination of surfaces.

Spills of contaminated material should be dealt with immediately using hypochlorite (500 ppm), 5% peroxide, or phenol- based solutions.

High risk material (Contaminated bedding, placenta, and aborted fetuses) should be buried with lime or incinerated.

Treatment of manure with lime or calcium cyanide is also recommended before spreading, and spreading must be performed on a calm day, to reduce airborne transmission.

Prevention of exposure to animals or wearing gloves and masks during manipulation of animals or their litter is advised (AFSSA, 2004; Dorko *et al.*, 2008).

Tick control and good hygiene procedures can reduce the incidence in livestock (Angelakis and Raoult, 2010).

Separating infected animals from the herd and restricting animal movements from infected areas may also help to reduce spread.

Antibiotic treatment of infected animals does not stop shedding and as such is ineffective in controlling the disease (Astobiza *et al*., 2010)

The Q fever outbreak in the Netherlands has shown how difficult it is to contain an outbreak in livestock due to airborne transmission (Enserink, 2010). Control measures during the outbreak included culling of infected animals and pregnant livestock in infected areas.

### 2.15.1 Vaccination

Vaccination of naive animals does not completely prevent infection, but does reduce abortion rates, which curtails the spread of the organism. A widespread programme of cattle vaccination in Slovakia significantly reduced the local incidence of Q fever (Kovacova and Kazar, 2002).

Since *Coxiella burnetti* infection has been associated with abortions (sheep and goats) and infertility (Cattle) (Ho *et al.*, 1995; Waldham *et al*., 1978), prevention of Q fever infection may have an economic impact. The first vaccines available were composed of

inactivated whole *C. burnetti* cells, whereas chloroform-methanol residue of *C. burnetti* cells have been proposed more recently (Schmeer *et al*., 1987; Williams and Cantrell, 1982). Chloroform-methanol residue vaccines were shown to be better tolerated in animals than *C. burnetti* whole-cell vaccines (Elliott *et al*., 1998; Williams *et al*., 1993). Q fever vaccines vary in their composition, including the strain and phase of *C. burnetti* used. Vaccines prepared from phase I *C. burnetti* organisms were more protective than those prepared from phase II bacteria, whereas cross protection among various *C. burnetti* strains was found in vaccinated guinea pigs (Ormsbee *et al*., 1964). Q fever vaccination was also shown to protect cattle against abortion (Behymer *et al*., 1976) low fetal weight (Brooks *et al*., 1986) and chronic infertility (Schmeer *et al*., 1987). In contrast, vaccination did not prevent *C. burnetti* shedding in animals naturally infected prior to vaccination and shedding of the organism persisted unchanged (Schmeer *et al*., 1987).

Q fever vaccination of domestic animals (mainly cattle, sheep and goats) is currently not widely used because it is protective and safe only in animals that are uninfected at the time of vaccination.

Q fever vaccines used in humans consist of killed, purified phase I *C. burnetti* whole cells, which contain LPS- protein complex antigens (Kazar and Rehacek, 1987; Williams *et al*., 1993). A formalin - inactivated Q fever vaccine (Q - vax., Common Wealth serum laboratories), prepared from phase I *C. burnetti* Henzerling strain, was approved for the general market in Australia in March 1989. Although highly immunogenic, this type of vaccine may induce adverse effects, especially when administered in previously infected populations (Bell *et al*., 1964). Immunity with this vaccine lasts at least 5 years

(Sonenshine, 1993). Since Q fever in humans is often an occupational hazard, vaccination should be considered primarily in high risk populations (Ormsbee and Marmion, 1990). Thus, Q fever vaccine should be recommended for livestock handlers, processors of animal products (including abattoir workers), persons in contact with dairy products, veterinarians and laboratory personnel working with *C. burnetti* infected animals.

## 2.16 BIOTERRORISM

*Coxiella burnetti* has been identified by organizations such as the World Health Organization (WHO), the United Nations and the Australia Group as an agent of concern (WHO, 2004). Because of its airborne transmission, low infective dose, easily accessible sources, resistance to environmental degradation and ability to cause debilitating diseases in large groups of people, *C. burnetti* has been considered as a potential weapon for bioterrorism (Cutler *et al.,* 2002; McQuiston *et al*., 2002). The infectious dose is as low as 1-10 colony forming unit (C.F.U) (Tigertt *et al*., 1961).

## 2.17 WORLDWIDE DISTRIBUTION

Q fever is a zoonotic disease which is endemic throughout the world with the exception of Antarctica and New Zealand, occurring in diverse geographic regions and climate zones and infects arthropods, birds, domestic and wild mammals, as well as humans (Woldehiwet, 2004; Rodolakis, 2006). Indeed, the disease is considered as a re- emerging zoonosis in many countries.

From 1999 to 2004, there were 18 reported outbreaks of Q fever from 12 different countries involving 2 to 289 people (Arricau - Bouvery and Rodolakis, 2005).

In the Netherlands, since 2007, Q fever has become a public health problem with 2,357 human cases notified in 2010 (Van der Hoek *et al*, 2010).

In Europe, there are several studies reporting the seroprevalence of Q fever using different diagnostic methods. In Italy, Masala *et al*. (2004) using ELISA, found that 38% of sheep and 47% of goats were seropositive. Parisi *et al*. (2006) using PCR identified *C. burnetti* in 11.6% of cattle and 21.5% of sheep and goats.

In America, studies with concern to Q fever are summarized by McQuiston and Childs (2002) and reported as 41.6% in goats, 16.5% in sheep and 3.4% in cattle.

In Asia, To *et al.* (1996) reported a prevalence of 60.4% in cattle with reproductive disorders in Japan.

In Africa, Reinthaler *et al*. (1988) using MAT, found that 62.5% of sheep, 5.39% of goats and 40.4% of cattle were seropositive. In Zimbabwe, Kelly *et al*. (1993) using IFAT, detected seropositivity to *C. burnetti* in 39% of cattle, 10% of goats. In Chad, Schelling *et al*. (2003) using ELISA, found that 80% of camels, 13% goats, 11% sheep and 4% cattle were seropositive. Nakoune *et al*. (2004) using IFAT, found that 14.3% of cattle were positive in Central African Republic. In addition, Cekani *et al*. (2008) investigated Q fever seroprevalence using ELISA technique and found 9.8% in sheep and goats and 7.9% in cattle in Algeria.

## 2.18 DISTRIBUTION IN NIGERIA

In Nigeria, Addo and Schnurenberger (1977) using CAT, found that 11 % cattle, 16.5% sheep and 8.8% goats were seropositive in Kaduna and Zaria abattoirs. Addo, (1980) using CAT, identified the presence of *Coxiella burnetti* in 12% of single- humped tropical camels in Kano, Sokoto and Zaria abattoirs. Addo and James (1981) also found

that 28.8% of dogs around Zaria were seropositive. Adesiyun *et al.* (1984) using CAT, got a seroprevalence of 59.8% in some dairy cows in Zaria.

In humans, a study of evidence of Q fever infection using CFT in Ibadan by Collard and Udeozo (1959), revealed 5.4% positive reaction among sera from donors and 0.8% in pregnant women. In another study in Katsina province among the Habe and Fulani tribes, 5.5% and 2.7% rates of infection were reported respectively (Collard, 1962). Addo *et al*. (1977) reported a seroprevelence of 20.5% in a survey of 507 patients at the Ahmadu Bello University Teaching Hospital, Zaria.

## 2.19 SOCIO-ECONOMIC IMPLICATIONS OF THE DISEASE

Until recently, *Coxiella burnetti* has been regarded as economically unimportant for domestic livestock. However, *C. burnetti* has been detected in a number of cases of abortions in different parts of the world (Waldham *et al*., 1989). *Coxiella burnetti* infection leads mainly to reproductive disorders like abortions, stillbirths, weak calf delivery, mastitis, metritis and infertility, with associated economic impact for the herd (Arricau-Bouvery and Rodolakis, 2005). Abortions constitute an important economic burden for the livestock industry due to deaths and decreased milk production. The economic impact of abortions depends on direct costs and value of foetuses lost. Indirect costs include those associated with establishing the diagnosis, re-breeding cows that aborted, possible loss of milk yield, cost of veterinary intervention and medication and replacement costs if cows that aborted are culled.

Q fever is considered as a re-emerging zoonosis in many countries and in humans it is typically an acute febrile illness, with hepatitis and atypical pneumonia seen in severe

cases. A small percentage of infected people will develop chronic infection with endocarditis (Kazar, 2005).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 STUDY AREA

The study area was Kaduna metropolis, Kaduna State. Kaduna metropolis consists of two local government areas, Kaduna North and Kaduna South and parts of two other Local Government Areas, Chikun and Igabi. The metropolis may be demarcated by the intersection of longitude $7^0$50E and latitude $10^0$50N at an altitude of about 643m. Kaduna is nestled within the gently undulating terrain of the central plains of Northern Nigeria. Climatic type is characterised by a rainy season (about 6 months), followed by a dry and dusty harmattan season lasting from November to February. The rainy season is preceded by a short hot dry spell with mean maximum daily temperature of between $35^0$C and $40^0$C. Annual total rainfall is about 1,272mm and rain falls mostly from May to October (KDSG, 2008).

Kaduna state has a human population of over 6,066,562 people according to the 2006 census figures (KDSG, 2008). Agriculture is the main stay of the economy of Kaduna state with about 80% of the people actively engaged in animal and crop farming.

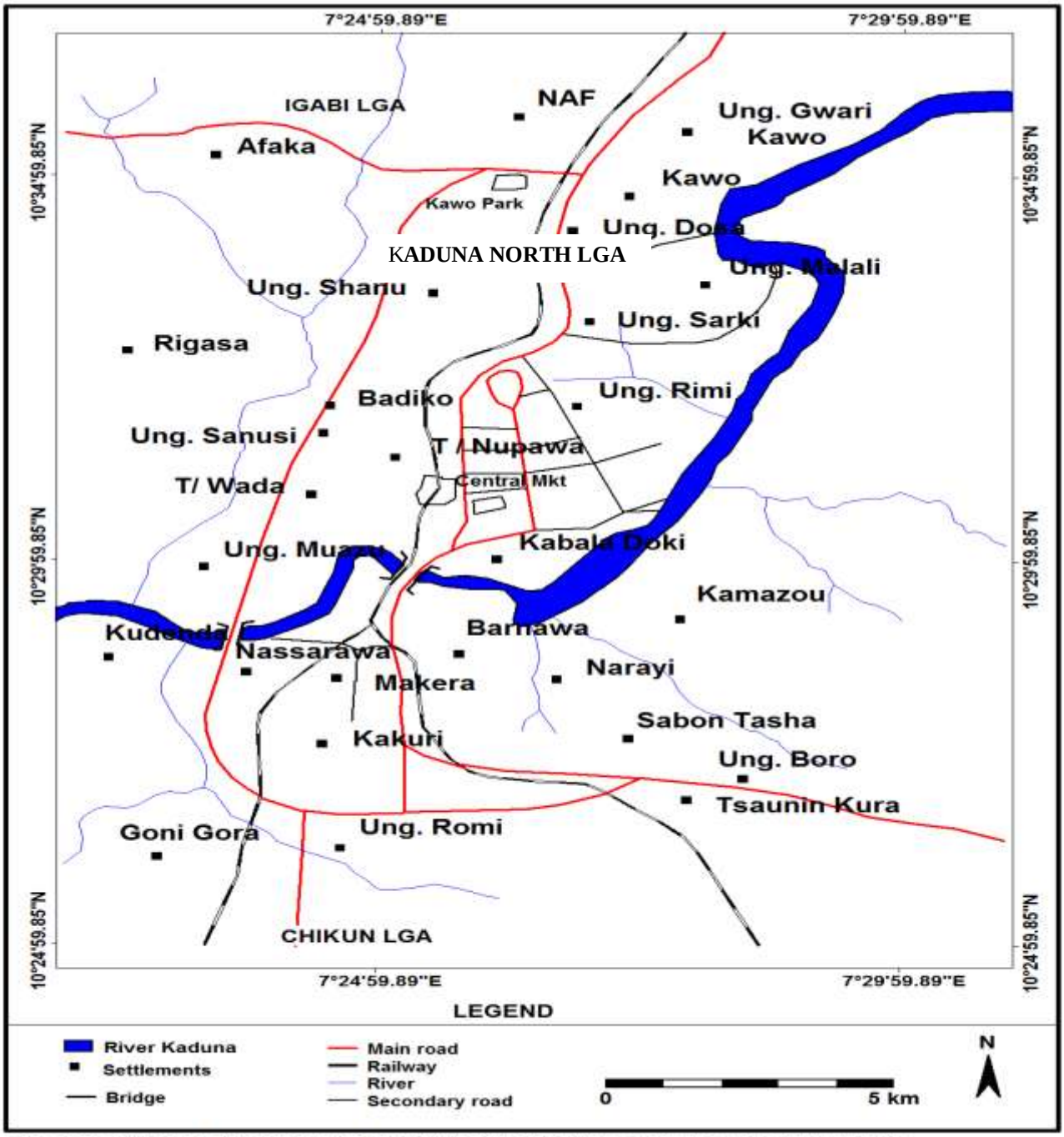



FIG. 3.1: KADUNA METROPILIS SHOWING PARTS OF IGABI AND CHIKUN LGA's

Source : Modified from Kaduna street guide map

## 3.2 STUDY DESIGN

A cross sectional approach was used. The target populations were cattle from semi settled Fulani herds, private and commercial farms. Sampling frame was drawn from a list of cattle farms compiled from data from animal health workers and private veterinary practitioners.

## 3.3 INCLUSION CRITERIA

i. Only farms whose owners consented were included.

ii. Only farms that had 20 or more cattle were included.

iii. Only cattle farms around Kaduna Metropolis were included.

Those that did not meet the above criteria were excluded from the study.

## 3.4 SAMPLING AND SAMPLE SIZE

Some cattle farms around Kaduna metropolis were identified and approached seeking consent of the owners for inclusion in this study. The localities identified to have cattle farms were Sabon Gayan and Babban Saura from Chikun LGA, Rigachikun, Barakallahu, and Tollgate from Igabi LGA, Kinkinau, Kaduna express and Gonin Gora from Kaduna South LGA, Kabala and Rafinguza from Kaduna North LGA. To increase the chances of getting positive results, twenty percent of cattle were sampled from each of 42 identified farms and a sample size of 539 was obtained. Selection of animals was done using systematic random technique, in farms that have crushes, while in farms that used ear tags, sampling was done by balloting without replacement. On the other farms, animals that could be restrained were sampled.

## 3.5 SAMPLE COLLECTION

About five milliliters of blood was aseptically collected from the jugular vein using a 10ml syringe and 18G needle following adequate restraint. The blood was transferred into clean sample bottles without anticoagulant and labeled with information on age, sex and breed of each animal. These were transported in ice packed coolers to the Bacterial Zoonoses Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria. Blood samples were centrifuged at 1000g for 10 minutes. The resultant sera was transferred into clean labeled serum bottles and stored in the freezer until analyzed.

## 3.6 QUESTIONNAIRE ADMINISTRATION

A structured questionnaire (Appendix I) and individual animal record forms (Appendix II) were used to collect information on age, sex, breed, management system, handling of sick animals, ectoparasite control, type of breeding, type of milking, waste disposal, biosecurity, frequency of barn cleaning, abortion history, other animals on the farm, milk consumption etc. The questionnaire was administered to each of the farms to assess the risk factors associated with *Coxiella burnetti* infection.

## 3.7 ENZYME LINKED IMMUNOSORBENT ASSAY KITS

The commercial ELISA kits, ID Screen ® Q fever indirect test kits were obtained from ID Vet Innovative Diagnostics, Montpellier – France. This test uses a *Coxiella burnetti* phase I and phase II antigen strain isolated in France from an aborted bovine placenta.

### 3.8 DETECTION OF *COXIELLA BURNETTI* ANTIBODIES

All samples were analyzed according to the manufacturer's instructions. Reagents were provided ready for use including the negative and positive controls. The wash solution (1x) was prepared by diluting the wash concentrate (20x) 20-fold in distilled water. The conjugate (1x) was prepared by diluting the concentrated conjugate (10x) 10-fold in dilution buffer 3. One hundred microlitres of dilution buffer 2 was added to each microwell, 10µl of the positive control was added to wells A2 and A3 leaving A1 as blank. Ten microlitres of the negative control was added to wells A4 and A5. Ten microlitres of each sample was added to the remaining wells. Six 96-well plates containing the test and control specimens were prepared before transferring them into an ELISA microplate using a multichannel pipette. This was done to avoid differences in incubation times between specimens. The plates were incubated for 45minutes at room temperature. After incubation, the contents of the wells were emptied and the wells were washed 3 times with approximately 300µl of the wash solution. The inverted microtitre plate was tapped against a sheet of clean absorbent tissue paper so as to remove all the liquid. The conjugate (a peroxidase-labelled anti-multi species IgG) was added at 100µl per well and incubated for 30 minutes at room temperature.

The plates were rinsed three times with washing solution, tapped against a sheet of clean absorbent tissue paper and 100µl of the substrate solution (tetramethylbenzidine, TMB) was added to each well. The plates were incubated for 15 minutes at room temperature in the dark. The stop solution (0.5M, sulphuric acid) was added at 100µ1 to each well to stop the reaction.

The optical density (OD) of the wells was read and recorded at an absorbance of 450nm using an ELISA reader (GF – M3000, B. Bran Scientific and Instrument company England) soon after addition of the stop solution. Optical density for each sample was compared against the positive control optical density to give a sample to positive control ratio (SP%).

## 3.9 VALIDATION

The test is validated if:

i. The mean value of the positive control O.D ($OD_{PC}$) is greater than 0.350

ii. The ratio of the mean O.D values of the positive and negative controls ($OD_{PC}$ and $OD_{NC}$) is greater than 3 (Manufacturer's guideline).

Mean $OD_{PC} = \frac{1.691+1.887}{2} = 1.789$

1.789>0.350 (validated)

Mean $OD_{NC} = \frac{0.197+0.190}{2} = 0.1935$

$\frac{1.789}{0.1935} = 9.2$ 9.2 > 3 (validated)

## 3.10 INTERPRETATION

For each sample, the S/P percentage (S/P%) was calculated using the formula provided by the manufacturer.

$$S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

| Result | Status |
|---|---|
| S/P ≤ 40% | Negative |
| 40% < S/P ≤ 50% | Doubtful |
| 50% < S/P ≤ 80% | Positive |
| S/P > 80% | Strong positive |

For the purpose of analysis, results that were doubtful were classified as negative.

### 3.11 STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 17.0. Statistical methods used included descriptive statistics employing frequencies and percentages. Odds ratio and 95% CI on OR were calculated to check for associations and statistical significance. Odds ratio values greater than unity (one) indicates an association, values less than one indicate that the factor may be protective. A 95% confidence interval which does not pass through one indicates statistical significance at $\alpha = 0.05$. Because of the small numbers in some cells, Fisher's Exact Test was used to test for association between a categorical risk factor and the presence of *Coxiella burnetti* antibody.

# CHAPTER 4

# RESULTS

## 4.1 DEMOGRAPHIC CHARACTERISTICS

About 71.4% of the farms were located in Kaduna South and Chikun Local Government Areas whereas 28.6% were located in Kaduna North and Igabi Local Government Areas. About 47.6% of the respondents were farm managers and 52.4% were farm owners. Based on educational qualification, 47.6% of the respondents had secondary education, 23.8% had tertiary education, 26.2% had primary education whereas 2.4% said they had no formal education.

About 23.8% of the respondents purchased their livestock from the market, 2.4% purchased from other farms and 73.8% from a combination of different sources. Thirty three percent keep the animals as a source of family income, 42.9% as source of milk and 23.8% as a source of meat.

## 4.2 PREVALENCE OF *COXIELLA BURNETTI* IN CATTLE FROM FARMS IN KADUNA METROPOLIS

Out of the 539 serum samples tested for the presence of *Coxiella burnetti* antibodies by enzyme-linked immunosorbent assay (ELISA), 78 (14.5%) were positive. Out of the 42 sampled herds, 24 were positive giving a herd prevalence of 57.1%.

There was no statistically significant difference between female and male cattle (14.5% and 14.1% respectively). (OR = 1.03, 95% CI, 0.54 – 1.97).

*Coxiella burnetti* antibodies were more prevalent in cattle >3 years old than in cattle aged 3 years and below (OR = 1.13, 95% CI, 0.69-1.86). Age was however not significantly associated with infection.

The prevalence of *Coxiella burnetti* infection was significantly higher ($P<0.05$) in the local breed of cattle than in the cross breeds (Table 4.1) while none of the exotic breeds sampled had infection.

**Table 4.1 Prevalence of *Coxiella burnetti* infection in relation to sex, age and breed of cattle from farms in Kaduna metropolis**

| Risk factor | Positive (%) | Negative (%) | Total (%) | OR | 95% CI on OR | FE |
|---|---|---|---|---|---|---|
| **Sex** | | | | | | |
| Male | 13(14.1) | 79(85.9) | 92(100) | 1 | | |
| Female | 65(14.5) | 382(85.5) | 447(100) | 1.03 | 0.54-1.97 | |
| **Total** | 78(14.5) | 461(85.5) | 539(100) | | | |
| **Age** (years) | | | | | | |
| ≤3 | 29(13.6) | 184(86.4) | 213(100) | 1 | | |
| >3 | 49(15.1) | 275(84.9) | 324(100) | 1.13 | 0.69-1.86 | |
| **Total** | 78(14.5) | 459(85.5) | 539(100) | | | |
| **Breed** | | | | | | |
| Local | 77(17.1) | 373(82.9) | 450(100) | | | P=0.00 |
| Exotic | 0(0) | 10(100) | 10(100) | | | |
| Cross | 1(1.3) | 78(98.7) | 79(100) | | | |
| **Total** | 78(14.5) | 461(85.5) | 539(100) | | | |

**Key**

OR = Odds ratio

CI = Confidence interval on Odds ratio

FE = Fisher's Exact Test

## 4.3 POSSIBLE RISK FACTORS ASSOCIATED WITH *COXIELLA BURNETTI* INFECTION IN CATTLE FROM FARMS IN KADUNA METROPOLIS

The rate of detection of *Coxiella burnetti* antibodies was higher in the intensive management system (66.7%) compared to that in the semi intensive system (55.6%) (Table 4.2). However, there was no statistically significant relationship between the management systems and *Coxiella burnetti* infection. (OR = 0.63, 95% CI, 0.10 – 3.86).

A higher rate of infection was observed from herds supplemented with commercial feed (62.5%) than herds fed on locally compounded feed (53.8%). There was no significant relationship between mode of supplementary feeding and prevalence of infection (Table 4.2).

There was no association between herd infection and type of housing (OR = 1.00) (Table 4.2). Prevalence of infection was higher in herds with no ectoparasite control (100%) compared to herds with ectoparasite control (56.1%) (Table 4.3).

Deworming routinely was associated with a lower prevalence but the association was not significant (P>0.05). (Table 4.3).The use of foot bath was associated with a lower prevalence rate but the association was not significant (P>0.05) (Table 4.4). Cleaning and disinfection of equipment after use was found to have a significantly protective effect (OR = 0.15, 95% CI, 0.03-0.79) (Table 4.4). Quarantine of newly purchased animals and rodent control were associated with lower prevalence (OR = 0.63, 95%CI, 0.18-2.20) and (OR= 0.46, 95%CI, 0.07-3.06) respectively. (Table 4.4).However, the associations were not statistically significant.

The prevalence of infection was higher in herds with no history of abortions or stillbirths (70%) compared to herds with abortion history (45.5%). (Table 4.5) This was however not statistically significant (OR = 0.36, 95% CI, 0.10-1.28).

Farms that burn after- birth materials had a higher rate of infection (100%) (Table 4.5), than farms that bury after- birth materials (55%).

The presence of other animals like sheep, goats, dogs and cats on the farm was associated with infection (Table 4.5) but the association was not statistically significant (OR = 1.35, 95% CI 0.08 – 23.20).

Questionnaire analysis showed that 2.4% of the respondents don't drink milk, 50% consume fermented milk, 16.7% consume raw milk and 31% boil milk before drinking (Figure 4.1). Ninety five percent of the respondents had never heard of Q fever.

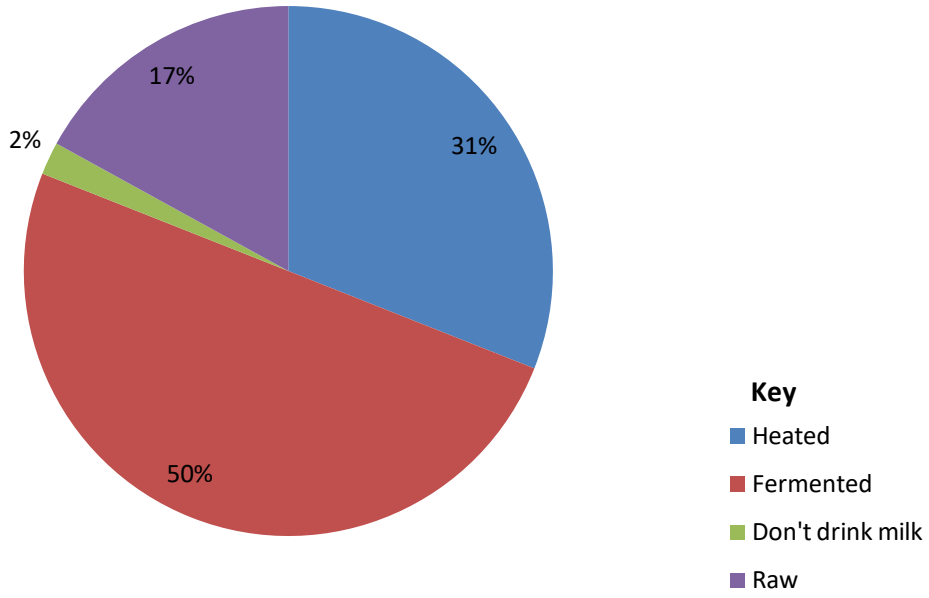



**Figure 4.1 Mode of consumption of milk by respondents**

**Table 4.2 Prevalence of *Coxiella burnetti* infection in relation to management of cattle from farms in Kaduna metropolis.**

| Risk factor | Positive (%) | Negative (%) | Total (%) | OR | 95% CI on OR |
|---|---|---|---|---|---|
| **Management system** | | | | | |
| Intensive | 4(66.7) | 2(33.3) | 6(100) | 1 | |
| Semi-intensive | 20(55.6) | 16(44.4) | 36(100) | 0.63 | 0.10-3.86 |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | |
| **Supplementary feeding** | | | | | |
| Commercial feed | 10(62.5) | 6(37.5) | 16(100) | 1 | |
| Locally compounded | 14(53.8) | 12(46.2) | 26(100) | 0.70 | 0.20-2.50 |
| **Total** | 24)57.1) | 18(42.9) | 42(100) | | |
| **Type of housing** | | | | | |
| Complete housing | 4(57.1) | 3(42.9) | 7(100) | 1.00 | |
| Fenced area | 20(57.1) | 15(42.9) | 35(100) | 1.00 | 0.19-5.15 |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | |

**Key**

OR = Odds ratio

CI = Confidence interval on Odds ratio

**Table 4.3 Prevalence of *Coxiella burnetti* infection in relation to parasite and rodent control in cattle farms in Kaduna metropolis.**

| Risk factor | Positive (%) | Negative (%) | Total (%) | OR | 95% CI on OR | FE |
|---|---|---|---|---|---|---|
| **Ectoparasite control** | | | | | | |
| Yes | 23(56.1) | 18(43.9) | 41(100) | | | P=1.00 |
| No | 1(100) | 0(0) | 1(100) | | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Routine deworming** | | | | | | |
| Yes | 21(53.8) | 18(46.2) | 39(100) | | | P=0.25 |
| No | 3(100) | 0(0) | 3(100) | | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Rodent control** | | | | | | |
| Yes | 2(40) | 3(60) | 5(100) | 0.46 | 0.07 – 3.06 | |
| No | 22(59.5) | 15(40.5) | 37(100) | 1 | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |

**Key**

OR = Odds ratio

CI = Confidence interval on Odds ratio

FE = Fisher's Exact Test

**Table 4.4 Prevalence of *Coxiella burnetti* infection in relation to biosecurity measures in cattle farms in Kaduna metropolis.**

| Biosecurity measures | Positive (%) | Negative (%) | Total (%) | OR | 95% CI on OR | FE |
|---|---|---|---|---|---|---|
| **Foot bath** | | | | | | |
| Yes | 0(0) | 2(100) | 2(100) | | | P=0.18 |
| No | 24(60) | 16(40) | 40(100) | | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Cleaning and disinfection of equipment after use** | | | | | | |
| Yes | 13(44.8) | 16(55.2) | 29(100) | 0.15 | 0.03 – 0.79 | |
| No | 11(84.6) | 2(15.4) | 13(100) | 1 | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Quarantine of newly purchased animals** | | | | | | |
| Yes | 8(50.0) | 8(50) | 16(100) | 0.63 | 0.18 – 2.20 | |
| No | 16(61.5) | 10(38.5) | 26(100) | 1 | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |

**Key**

OR = Odds ratio

CI = Confidence interval on Odds ratio

FE = Fisher's Exact Test

**Table 4.5 Prevalence of *Coxiella burnetti* infection in relation to reproductive problems and presence of other animals in cattle farms in Kaduna metropolis.**

| Risk factor | Positive (%) | Negative (%) | Total (%) | OR | 95% CI on OR | FE |
|---|---|---|---|---|---|---|
| **Abortion or stillbirth history** | | | | | | |
| Yes | 10(45.5) | 12(54.5) | 22(100) | 0.36 | 0.10-1.28 | |
| No | 14(70) | 6(30) | 20(100) | 1 | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Disposal of afterbirth materials** | | | | | | |
| Burn | 2(100) | 0(0) | 2(100) | | | P=0.50 |
| Bury | 22(55) | 18(45) | 40(100) | | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |
| **Presence of other animals** | | | | | | |
| Yes | 23(57.5) | 17(42.5) | 40(100) | 1.35 | 0.08- 23.20 | |
| No | 1(50) | 1(50) | 2(100) | 1 | | |
| **Total** | 24(57.1) | 18(42.9) | 42(100) | | | |

**Key:**

OR = Odds ratio

CI = Confidence interval on Odds ratio

FE = Fisher's Exact Test

# CHAPTER 5

## 5.0 DISCUSSION

A prevalence of 14.5% to *Coxiella burnetti* antibodies was obtained in this study. This indicates that cattle in Kaduna metropolis have been exposed to *Coxiella burnetti* infection and this may be responsible for some of the reproductive disorders among infected cattle. Infected cattle are important reservoirs of *Coxiella burnetti* and are a major source of infection to humans (Woldehiwet, 2004). The high prevalence recorded may be due to local management practices, environmental and climatic conditions and method of screening of samples. Seroepidemiological studies available for cattle have indicated that *Coxiella burnetti* antibody prevalence in these animals is higher than 20 or 30 years ago (Lang, 1990). Geographical variations in the prevalence of infections have been reported in a number of studies. In Nigeria, Addo and Schnurenberger (1977) reported a prevalence of 11% in cattle. In USA, Italy, Southern Sudan, Zimbabwe, Chad, Central African Republic and Algeria, prevalences reported were 3.4% (McQuiston and Childs, 2002), 11.6% (Parisi *et al*., 2006), 40.4% (Reinthaler *et al*., 1988), 39% (Kelly *et al*., 1993), 4% (Schelling *et al*., 2003), 14.3% (Nakoune *et al*., 2004) and 7.9% (Cekani *et al*., 2008) respectively.

This study has shown a herd prevalence of 57.1%. This high prevalence could be due to the semi intensive management system that allows different herds with different infection status to mix together while grazing or drinking water. This favors the spread of infection from one herd to the other.

There was no significant association between prevalence of infection and sex of cattle. Rate of infection was slightly higher among females than males and this is probably because, the organism has a high affinity for the placenta, fetal membranes and mammary glands (Babudieri, 1959) and is found in large numbers in these tissues. This agrees with the study by Cetinkaya *et al*. (2000) where no significant association between seroprevalence of coxiellosis and sex of cattle was found in the east of Turkey.

Local breed of cattle had a significantly higher rate of infection than the cross breeds while none of the exotic breeds sampled had infection. Exotic breeds of cattle are priced animals and they tend to be better managed in terms of disease and tick control. This probably accounts for the lack of seropositivity seen. However, in the study by Cetinkaya *et al*. (2000), no significant association was seen between seropositivity and breed of cattle.

Cattle over 3 years had a higher prevalence of infection than cattle 3 years and below. However age was not significantly associated with infection. Cattle older than 3 years are more likely to be breeding and shedding the organism, especially during parturition. Cattle less than 3 years are less likely to shed the organism in high numbers. Also, it may be due to a higher probability of contact with the organism with increasing age. In a study by Cetinkaya *et al.* (2000), no significant association between age and seroprevalence in cattle was seen. On the other hand, Ruiz-Fons *et al*. (2010) reported an age associated prevalence with infection rates higher in adult cattle than in heifers.

Cattle reared under the intensive management system had a higher rate of infection compared to those under semi-intensive management system. This is probably due to the fact that contact between animals is greater in the intensive management system thereby increasing the likelihood of exposure to infection. Capuano *et al*. (2001) reported that different management systems showed different degree of exposure to infection. In this study, management system was not significantly associated with infection. Ruiz-Fons *et al*. (2010) reported a higher prevalence in the extensive management system and attributed it to the movement of animals thus reducing contact rate between cattle. There was no association between herd infection and type of housing. The lack of association may be attributable to the similar conditions seen in complete housing and fenced areas, in terms of restricting movement and reducing the likelihood of contact with ticks, as opposed to animals that are left to continuously roam about, which was not reported on any farm in this study.

Herds with ectoparasite control had a lower rate of infection compared to herds with no ectoparasite control. The significance of ticks in transmitting the disease in ruminants has been documented (Lang, 1990), and therefore tick control can reduce the incidence of *Coxiella burnetti* infection in livestock (Angelakis and Raoult, 2010).This agrees with the study by Cetinkaya, *et al*. (2000), who reported a prevalence which was higher in herds in which no tick control measures were taken than in herds with tick control. In another study, Cantas *et al.* (2011) reported the presence of ticks on animals as a significant risk factor associated with *Coxiella burnetti* abortions. On the other hand, Gazyagci *et al.* (2011) reported no evidence of association between seropositivity and tick presence in cattle.

Routine deworming was found to be associated with a lower prevalence. This is probably because deworming routinely makes animals less susceptible to infections by boosting their immunity.

Biosecurity measures such as the use of foot bath and quarantine of newly purchased animals were associated with a lower rate of infection, which was not significant. Biosecurity measures help in reducing infectious agents, and also reduce the risk of their transmission. Farms with rodent control had a lower frequency of infection compared to farms with no rodent control. However, rodent control was not significantly associated with infection. Rodents are a source of infection as previous studies have reported the occurrence of *Coxiella burnetti* in rodents (Lang, 1990; Marrie *et al.*, 1986). Cantas *et al*. (2011) found a significant association between the presence of rodents on farms and *Coxiella burnetti* infection.

Cleaning and disinfection of equipment after use was found to have a significantly protective effect. Good hygienic practices are an important way of reducing the risk of spread of infectious diseases. Good hygienic procedures can reduce the incidence of *Coxiella burnetti* infection in livestock (Angelakis and Raoult, 2010). Studies have indicated that poor hygiene could be an exacerbating factor in the spread of *Coxiella burnetti* (Luoto, 1960; Lyytikainen *et al*., 1998).

Abortion history was not significantly associated with infection and frequency of infection was higher in herds with no history of abortions or stillbirths compared to herds

with abortion history. This may be due to the lack of sincerity on the part of some respondents as they do not like divulging information on losses due to abortions or other causes. In cattle, Q fever is frequently asymptomatic (To *et al.,* 1998) and in some reports, infection with *Coxiella burnetti* did not result in abortion suggesting that infection sometimes can pass unnoticed (Paiba *et al*., 1999). Coxiellosis in cattle should be a cause for concern as it is also associated with other reproductive problems like infertility, mastitis, metritis and weak calf delivery, which this study did not investigate. As with this study, Ruiz-Fons *et al.* (2010) found that *Coxiella burnetti* antibody prevalence did not differ statistically in relation to herd abortion history. On the other hand, significant associations between seroprevalence and abortion have been reported for cattle in several studies (Cabassi *et al.*, 2006; Cetinkaya *et al.*, 2000).

The presence of other animals like sheep, goats, cats and dogs was associated with infection, but the association was not significant. Farms that reared other animals together with cattle had a higher prevalence rate. Domestic ruminants like sheep and goats are reservoirs of *Coxiella burnetti* which is excreted in the urine, milk and faeces of infected animals and domestic pets such as dogs and cats are a source of infection (Lang, 1990; Woldehiwet, 2004). However, in a study by Cantas *et al.* (2011), the presence of carnivores like dogs and cats on the farm was significantly associated with *Coxiella burnetti* infection.

# CHAPTER 6

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 6.1 SUMMARY

This study was designed to determine the seroprevalence of *Coxiella burnetti* infection in cattle from farms in Kaduna metropolis. The objectives of the study were to determine the seroprevalence of *Coxiella burnetti* infection in cattle, to determine the effect of sex, breed and age on the occurrence of *Coxiella burnetti* infection and to determine if management practices, abortion history, ectoparasite control and presence of other animals on the farm are risk factors associated with *Coxiella burnetti* infection in cattle.

A total of 539 blood samples were collected from 42 different cattle farms. The serum samples were analyzed using a commercial enzyme-linked immunosorbent assay (ELlSA) test kit, for the detection of *Coxiella burnetti* antibodies. Animal record forms were administered on each sampled animal and questionnaires were administered to the farms.

An individual animal prevalence of 14.5% and a herd prevalence of 57.1% for *Coxiella burnetti* infection in cattle were obtained. Abortion was not significantly associated with infection. Ectoparasite control was associated with a lower prevalence to *Coxiella burnetti* infection.

## 6.2 CONCLUSION

This study reports for the first time to the best of our knowledge the prevalence and risk factors associated with coxiellosis in Kaduna metropolis. The high prevalence obtained

necessitates that people at risk should be informed and preventive measures taken. Improved sanitation on farms through good hygienic practices, parasite and rodent control can reduce the rate of infection.

## 6.3 RECOMMENDATIONS

i. Further studies using molecular techniques like PCR should be conducted to diagnose the infection in cattle, sheep and goats.

ii. A serological survey in sheep and goats and in risk groups such as veterinarians, cattle herdsmen, farmers ,butchers and milk sellers should be carried out to assess the occurrence of Q fever.

iii. Practices such as consumption of raw milk should be discouraged, and manure should be disposed of routinely and properly.

iv. Discourage the rearing of more than one species of animals together.

## REFERENCES


ACERSA (French Certification Association for Animal Health) (2006). "*Diagnostic d'un élevage cliniquement atteint de fièvre Q*," pp 1–11.

Addo, P. B. (1980). A serological survey for evidence of Q fever in camels in Nigeria. *British Veterinary Journal,* 136: 519- 521

Addo, P.B., Greenwood, B.M and Schnurenberger, P.R. (1977). A serological investigation of Q fever in clinical patients. *Journal of Tropical Medicine and Hygiene,* 80: 197-199.

Addo, P.B. and Schnurenberger, P. R. (1977). Q fever antibodies in food animals of Nigeria: a serological survey of cattle, sheep and goats. *Revue de Elevage et de Medicine Veterinaire des Pays Tropicaux*, 30(4): 359 - 362.

Addo,P.B.and James,O.B.(1981). A serological survey for Q fever in dogs around Zaria. *Nigerian Veterinary Journal, 10(2):25-26.*

Adesiyun, A.A., Jagun, A.G. and Tekdek, L.B. (1984). *Coxiella burnetti* antibodies in some Nigerian dairy cows and their suckling calves. *International Journal of Zoonoses*, 11: 155-160.

Agence Francaise de Securite Sanitaire (AFSSA) (2004). Fièvre Q : rapport sur l'évaluation des risques pour la santé publique et des outils de gestion des risques en élevage de ruminants. pp 1–88.

Angelakis, E. and Raoult, D. (2010). Q fever. *Veterinary Microbiology,* 140(3-4): 297–309.

Arricau - Bouvery, N. and Rodolakis, A. (2005). Is Q fever an emerging or re-emerging zoonosis? *Veterinary Research,* 36: 327-349.

Arricau-Bouvery, N., Souriau, A., Moutoussamy, A., Ladenise, K. and Rodolakis, A. (2001). Study of *Coxiella burnetti* excretion in an experimental goat model and decontamination of dung with calcium cyanamid. *Rencontres Autour des Recherches sur les Ruminants,* 8: 153–156.

Arricau-Bouvery, N., Souriau, A., Lechopier, P. and Rodolakis, A. (2003). Experimental *Coxiella burnetti* infection in pregnant goats: excretion routes. *Veterinary Research,* 34: 423–433.

Astobiza, I., Barandika, J. F., Hurtado, A., Juste, R. A. and Garcia-Perez, A. L. (2010). Kinetics of *Coxiella burnetti* excretion in a commercial dairy sheep flock after treatment with oxytetracycline. *Veterinary Journal,* 184*:* 172–175.

Babudieri, B. (1959). Q fever: a zoonosis. *Advances in Veterinary Science,* 5: 82–154.

Baca, O. G. and Paretsky, D. (1983). Q fever and *Coxiella burnetti*: a model for host-parasite interactions. *Microbiological Reviews,* 47(2): 127–149.

Baca, O. G., Klassen, D. A. and Aragon, A. S. (1993). Entry of *Coxiella burnetti* into host cells. *Acta Virology,* 37: 143–155.

Baca, O. G., Scott, T. O., Akporiaye, E. T., DeBlassie, R. and Crissman, H. A. (1985). Cell cycle distribution patterns and generation times of L929 fibroblast cells persistently infected with *Coxiella burnetti. Infection and Immunity,* 47(2): 366–369.

Behymer, D. E., Biberstein, E. L., Riemann, H. P., Franti. C. E., Sawyer, M., Ruppanner, R. and Crenshaw, G. L. (1976). Q fever *(Coxiella burnetti)* investigations in dairy cattle: challenge of immunity after vaccination. *American Journal of Veterinary Research,* 37:631–634.

Bell, J. F., Lackman, D. B., Meis, A. and Hadlow, W. J. (1964). Recurrent reaction at site of Q fever vaccination in a sensitized person. *Mile Medicine* 7:591–595.

Benenson, S. and Tigertt, W.D. (1956). Studies on Q fever in man. *Transactions of the Association of American Physicians,* 69: 98–104.

Berri, M., Rousset,E. and Champion, J.L. (2005). Spread of *Coxiella burnetti* infection in a flock of sheep after an episode of Q fever. *Veterinary Record,* 157: 737–740.

Bildfell, R. J., Thomson, G. W., Haines, D. M., McEwen, B. J. and Smart, N. (2000). *Coxiella burnetti* infection is associated with placentitis in cases of bovine abortion. *Journal of Veterinary Diagnostic Investigation,* 12(5): 419–425.

Blondeau, J. M., Yates, L., Martin, R., Marrie T., Ukoli, P.and Thomas, A. (1990). Q fever in Sokoto, Nigeria. *Annals of the New York Academy of Sciences,* 590: 281-282.

Blondeau, J.M., Williams, J.C., and Marrie, T.J. (1990). The immune response to Phase I and Phase II *Coxiella burnetti* antigens as measured by western immunoblotting. *Annals of the New York Academy of Sciences,* 590:187-202.

Brooks, D. L., Ermel, R. W., Franti, C. E., Ruppanner, R., Behymer, D. E., Williams, J. C. and Stephenson J. C. (1986). Q fever vaccination of sheep: challenge of immunity in ewes. *American Journal of Veterinary Research*, 47:1235-1238.

Bruneval, P., Choucair, J., Paraf, F., Casalta, J.P., Raoult, D., Scherchen, F. and Mainardi, J. (2001). Detection of fastidious bacteria in cardiac valves in cases of blood culture negative endocarditis. *Journal of Clinical Pathology*, 54: 238–240.

Buhariwalla, F., Cann, B. and Marrie, T. J. (1996). A dog related outbreak of Q fever. *Clinical Infectious Diseases,* 23:753–755.

Burnet, F. M. and Freeman, M. (1937). Experimental studies on the virus of Q fever. *Medical Journal of Australia,* 2:299–302.

Burnet, F. M. and Freeman, M. (1983). Experimental studies on the virus of "Q" fever (reprinted). *Reviews of Infectious Disease,* 5: 800–808.

Cabassi, C.S., Taddei, S., Donofrio, G., Ghidini, F., Piancastelli, C., Flammini, C.F.and Cavirani, S. (2006). Association between *Coxiella burnetti* seropositivity and abortion in dairy cattle of Northern Italy. *New Microbiology*, 29: 211-214.

Cantas, H., Muwonge, A., Sareyupoglu, B., Yardimci, H. and Skjerve, E. (2011). Q fever abortions in ruminants and associated on-farm risk factors in Northen Cyprus. *BMC Veterinary Research*, 7:13.

Capuano, F., Landolfi, M.C. and Monetti, D.M. (2001). Influence of three types of farm management on the seroprevalance of Q fever as assessed by an indirect immunofluorescence assay. *Veterinary Record*, 149: 669-671.

Casadevall, A. and Pirofski, L. A. (2006). A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. *Advanced Immunology,* 91: 1–44.

Cekani, M.M., Papa, A., Kota, M., Velo, E. and Berxholi, K. (2008). Report of a serological study of *Coxiella burnetii* in domestic animals in Albania. *Veterinary Journal*, 175:278.

Cetinkaya, B., Kalender, H., Ertas, H.B., Muz, A., Arslan, N., Ongor, H.and Gurcay, M. (2000). Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. *Veterinary Record*, 146: 131-136.

Coleman, S. A., Fischer, E. R., Howe, D., Mead, D. J. and Heinzen, R. A. (2004). Temporal analysis of *Coxiella burnetti* morphological differentiation. *Journal of Bacteriology*, 186: 7344–7352.

Coleman, T.J. (2000). The Public Health Laboratory Service (PHLS) and its role in the control of zoonotic disease. *Acta Tropica*, 21: 71-75.

Collard, P and Udeoze, I.O.K. (1959). Serological evidence of the existence of Q fever in Ibadan. *West African Medical Journal*, 8: 137-141.

Collard, P. (1962). A sample survey to estimate the prevalence of certain communicable diseases in Katsina Province, Nigeria. *West African Medical Journal*, 11: 3-31.

Cox, H.R. (1938). A filter passing infectious agent isolated from ticks. Description of organism and cultivation experiments. *Public Health Report*, 53: 2270-2276.

Cox, H.R. (1940*). Rickettsia diaporica* and American Q fever. *American Journal of Tropical Medicine and Hygiene*, 20: 463-465.

Cutler, S.J., Paiba, G.A., Howells, J. and Morgan, K.L. (2002). Q fever-a forgotten disease? *Lancet infectious Diseases*, 2: 717-718.

Cutler, S.J., Bouzid, M and Cutler, R.R. (2007). Q fever. *Journal of infectious diseases*, 54: 313-318.

Davis, G.E. (1939). *Rickettsia diaporica*: recovery of three strains from *Dermacentor andersoni* collected in Southeastern Wyoming: their identity with Montana strain. *Public Health Report*, 54: 2219-2225.

Dellacasagrande, J., Ghigo, E., Machergui, L. S., Hammami, Toman, R., Raoult, D., Capo, C and Mege, J.L. (2000). Alpha (v) beta (3) integrin and bacteriallipopolysaccharide are involved in *Coxiella burnetti*-stimulated production of tumor necrosis factor by human monocytes. *Infection and Immunology*, 68: 5673–5678.

Delsing, C. E. and Kullberg, B. J. (2008). Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak. *Netherlands Journal of Medicine*, 66(9): 365–367.

Derrick, E.H. (1937). "Q" fever, a new fever entity: Clinical features, diagnosis and laboratory investigation. *Medical Journal of Australia, 2*:281-299.

Deutch, D. L. and Peterson, E. T. (1950). Q fever: transmission from one human being to others. *Journal of the American Medical Association,* 143(4): 348–350.

Dilbeck, P. M. and McElwain, T. F. (1994). Immunohistochemical detection of *Coxiella burnetti* in formalin-fixed placenta. *Journal of Veterinary Diagnostic Investigation,* 6 (1): 125–127.

Doller, G., Doller, P.C. and Gerth, H.J. (1984). Early diagnosis of Q fever: Detection of immunoglobulin M by radioimmunoassay and enzyme immunoassay. *European Journal of Clinical Microbiology and Infectious Disease,* 3:550-553.

Dorko, E., Kalinová, Z. and Pilipčinec, E. (2008). Seroprevalence of *Coxiella burnetti* antibodies among students of the Faculty of Medicine in Košice (Slovakia). *Folia Microbiologica,* 53(6): 563–568.

European Food Safety Authority (EFSA) (2010a). Scientific opinion on Q fever, "EFSA panel on animal and welfare and EFSA panel on biological hazards," *EFSA Journal,* 8(5): 1595.

Eklund, C. M., Parker, R. R. and Lackman, D. B. (1947). A case of Q fever probably contracted by exposure to ticks in nature. *Public Health Responses,* 62:1413–1416.

Elliott, J. J., Ruble, D. L., Zaucha, G. M., Jaax, G. P. and Waag, D. M. (1998). Comparison of Q fever cellular and chloroform-methanol residue vaccines as skin test antigens in the sensitized guinea pig. *Acta Virology,* 42:147–155.

Enserink, M. (2010). Questions abound in Q-fever explosion in the Netherlands. *Science,* 327: 266–267.

Fenollar, F., Fournier, P. E. and Raoult, D. (2004). Molecular detection of *Coxiella burnetti* in the sera of patients with Q fever endocarditis or vascular infection. *Journal of Clinical Microbiology,* 42(11): 4919–4924.

Field, P.R., Hunt, J.G., and Murphy, A.M. (1983). Detection and persistence of specific IgM antibody to *Coxiella burnetti* by enzyme-linked immunosorbent assay: A comparison with immunofluorescence and complement fixation tests. *Journal of Infectious Diseases,* 148:477-487.

Fiset, P. (1957). Phase variation of *Rickettsia (Coxiella) burnetti,* study of the antibody response in guinea pigs and rabbits. *Canadian Journal of Microbiology,* 3: 435–*445.*

Fiset, P., Ormsbee, R.A., Silberman, R., Peacock, M. and Spielman, S.H. (1969). A microagglutination technique for detection and measurement of Rickettsial antibodies. *Acta Virology,* 13:60-66.

Fournier, P. E. and Raoult, D. (2003). Comparison of PCR and serology assays for early diagnosis of acute Q fever. *Journal of Clinical Microbiology,* 41(11): 5094–5098.

Fournier, P. E., Marrie, T. J. and Raoult, D. (1998). Diagnosis of Q fever. *Journal of Clinical Microbiology,* 36(7): 1823–1834.

Frangoulidis, D., Rodolakis, A., Heiser, V., Landt, O., Splettstoesser, V and Meyer, H. (2009). DNA microarray-chip based diagnosis of Q fever (*Coxiella burnetti*). *Clinical Microbiology and Infection,* 15(2): 165–166.

Gazyagci, S., Aktas, M.S., Kilic, S., Babur, C., Celebi, B.and Duru, S.Y. (2011). Seroprevalence of Q fever in dairy cattle in the Konya province, Turkey. *Revue de Elevage et de Medecine Veterinaire des Pays Tropicaux*, 162, 8-9, 387-390.

Giroud, P. and Jardin, J. (1954). Infectione latente et conservation de *Rickettsia burnetti* chez l'homme: le rôle du pou. *Bulletin Society Pathology Exotic*, 45:764–765.

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Jolyc, A. and Seegers, H. (2006). Shedding routes of *Coxiella burnetti* in dairy cows: implications for detection and control. *Veterinary Research,* 37(6): 827–833.

Harman, J. B. (1949). Q fever in Great Britain, clinical account of eight cases. *The Lancet,* 254(6588): 1028–1030.

Herr, S., Huchzermeyer, H. F., Te Brugge, L. A., Williamson, C. C., Roos, J. A. and Schiele, G. J. (1985). The use of a single complement fixation test technique in bovine brucellosis, Johne's disease, dourine, equine piroplasmosis and Q fever serology. *The Onderstepoort Journal of Veterinary Research*, 52(4): 279–282.

Hilbink, F., Penrose, M., Kovácová, E. and Kazár, J. (1993). Q fever is absent from New Zealand. *International Journal of Epidemiology*, 22: 945–*949.*

Hirai, K. and To, H. (1998). Advances in the understanding of *Coxiella burnetti* infection in Japan. *Journal of Veterinary Medical Science,* 60(7): 781–790.

Ho, T., Htwe, K. K., Yamasaki, N., Zhang, G. Q., Ogawa, M., Yamagushi, T., Fukushi, H. and Hirai, K. (1995). Isolation of *Coxiella burnetti* from dairy cattle and ticks, and some characteristics of the isolates in Japan. *Microbiology and Immunology*, 39:663–671.

Honstettre, A., Ghigo, E., Moynault, A., Capo, C., Toman, R., Akira, S., Takeuchi, O., Lepidi, H., Raoult, D. and Mege, J.L. (2004). Lipopolysaccharide from *Coxiella burnetti* is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. *Journal of Immunology*, 172: 3695–3703.

Howe, D. and Mallavia, L.P. (2000). *Coxiella burnetti* exhibits morphological change and delays phagolysosomal fusion after internalization by J774A.1 cells. *Infection and Immunology,* 68: 3815–382

Janssens, S. and Beyaert, R. (2003). Role of Toll-like receptors in pathogen recognition. *Clinical Microbiology Reviews,* 16: 637–646.

Johnson, J.E. and Kadull, P.J. (1966). Laboratory acquired Q fever. A report of fifty cases. *American Journal of Medicine*, 41: 391-403.

Kaduna State Government, (KDSG) (2008). Kaduna State Achievements in: Data on Estimated Annual Animal Population and Fish Production Investment Opportunities in Kaduna State. pp 16-18.

Kazar, J. (2005). *Coxiella burnetti* infection. *Annals of the New York Academy of Sciences*, 1063:105 - 114.

Kazar, J. and Rehacek, J. (1987). Q fever vaccines: present status and application in man. *Zentralblatt fur Bakteriologie Abt Originale Hygiene,* 267:74–78.

Kelly, P.J., Mathhewman, L.A., Mason P.R. and Raoult, D. (1993). Q fever in Zimbabwe. A review and the results of a serosurvey of humans, cattle, goats and dogs. *South African Medical Journa*l, 83:21-25.

Kim, S.G., Kim, E.H., Lafferty, C.J. and Dubovi, E. (2005). *Coxiella burnetti* in bulk tank milk samples in the United States. *Emerging Infectious Diseases,* 11:619-21.

Kosatsky, T. (1984). Household outbreak of Qfever pneumonia related to a parturient cat. *Lancet* 11:1447–1449.

Kovacova, E. and Kazar, J. (2002). Q fever–still a query and underestimated infectious disease. *Acta Virology,* 46: 193–210.

Kovacova, E., Gallo, J. Schramek, S., Kazar, J. and Brezina, R. (1987). *Coxiella burnetti* antigens for detection of Q fever antibodies by ELISA in human sera. *Acta Virology,* 31:254-259.

Krt, B. (2003). The influence of *Coxiella burnetti* phase I and phase II antigens on the serological diagnosis of Q fever in cattle. *Slovenian Veterinary Research,* 40: 203–207.

Kruszewska, D., Lembowicz, K. and Tylewska-Wierzbanowska, S. (1996). Possible sexual transmission of Q fever among humans. *Clinical Infectious Diseases*, 22(6): 1087–1088.

Kruszewska, D., Tylewska-Wierbanowska, S. (1997). Isolation of *Coxiella burnetti* from bull semen. *Research Veterinary Science* 62, 299-300.

Lang,G.H.(1990). Coxiellosis (Q fever) in animals. In: "*Q fever: The disease".* (Edited by Marrie, T.J). CRC Press, Boca Raton, FL. Vol 1, pp 23-48.

Little, T.W.A. (1983). Q fever? An enigma. *British Veterinary Journal*, 139: 277-283.

Luoto, L. (1960). Report on the nationwide occurrence of Q fever infections in cattle. *Public Health Report*, 75: 135-140.

Lyytikainen, O., Ziese, T., Schwartlander, B., Matzdorff, P., Kuhnhen, C., Jager, C.and Petersen, L. (1998). An outbreak of sheep-associated Q fever in a rural community in Germany. *European Journal of Epidemiology*, 14: 193-199.

Mantovani, A. and Benazzi, P.(1953). The isolation of *Coxiella burnetti* from *Rhipicephalus sanguineus* on naturally infected dogs. *Journal of American Veterinary Medical Association*, 122:117–120.

Marrie, T. J. *(*1990). Epidemiology of Q fever. In: "*Q fever: The disease"*. (Edited by Marrie, T. J).CRC Press, Boca Raton, FL. Pp 49–70.

Marrie, T. J., Durant, H., Williams, J. C., Mintz, E. and Waag, D. M. (1988). Exposure to parturient cats is a risk factor for acquisition of Q fever in Maritime. *Canadian Journal of Infectious Disease*, 158:101–108.

Marrie, T. J., Schlech, W. F., Williams, J. C. and Yates, L. (1986). Q fever pneumonia associated with exposure to wild rabbits. *Lancet* 1: 427–429.

Masala, G., Porcu, R., Sanna, G., Chessa, G., Cillara, G., Chisu, V and Tola, S. (2004). Occurrence, distribution and role of abortion *of Coxiella burnettii* in sheep and goats in Sardinia, Italy. *Veterinary Microbiology*, 99:301-305.

Maurin, M. and Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews,* 12: 518–*553.*

McCaul, T. F. (1991). The developmental cycle of *Coxiella burnetti*. In: *"Q Fever: The Biology of Coxiella burnetti"* (Edited by Williams, J. C. and Thompson, H. A.) CRC Press, Boca Raton, FL. Pp 223–258.

McCaul, T. F. and Williams, J. C. (1981). Developmental cycle of *Coxiella burnetti*: structure and morphogenesis of vegetative and sporogenic differentiations. *Journal of Bacteriology*, 147: 1063–1076.

McDade, J.E. (1990). Historical aspects of Q fever. In: *"Q Fever: The Disease"* (.Edited by Marrie, T.J). CRC Press, Boca Raton, FL.Vol 1. Pp. 5-21.

McDade, J.E. and Joseph, E. (1990). Historical aspects of Q fever In: *Q fever: The Disease*, (Edited by Marrie and Thomas, J.C). CRC press, Boca Raton, FL. Pp 5-22.

McQuiston, J.H. and Childs, J.E. (2002). Q fever in humans and animals the in the United States. *Vector Borne and Zoonotic Diseases*, 2:179-191.

McQuiston, J.H., Childs J.E. and Thompson, H. A. (2002). Q fever. *Journal of American Veterinary Medical Association*, 221:790-799.

Miceli, M. H., Veryser, A. K., Anderson, A. D., Hofinger, D., Lee, S. A. and Tancik, C. (2010). A case of person-to-person transmission of Q fever from an active duty serviceman to his spouse. *Vector Borne and Zoonotic Diseases,* 10(5): 539–541.

Milazzo, A., Hall, R., Storm, P.A., Harris, R.J., Wislow, W., and Marmion, B.P. (2001). Sexually transmitted Q fever. *Clinical Infectious Diseases*, 33: 399-402.

Nakoune, E.O., Debaere, F., Koumanda-Kotogne, B., Selekon, F., Samory and Talarmin, A. (2004). Serological Surveillance of Brucellosis and Q fever in cattle in the Central African Republic. *Acta Tropica*, 92:147-151.

Office International Des Epizootics (OIE) (2005). *Manuel terrestre de l'OIE*. *Fièvre Q*. Chapitre 2.2.10. Pp. 433–445.

Okimoto, N., Asaoka, N.and Osaki, K. (2004). Clinical features of Q fever pneumonia," *Respirology*, 9(2): 278–282.

Olson, J. G., Jones, F. R. and Blair, P. J. (2006). Rickettsia spp. In: *"Principles and Practice of Clinical Bacteriology"*. (Edited by Gillespie, S. H., Hawkey, P. M., Chichester, W and Waldhalm D. G) .Wiley, UK. Pp. 285–294.

Omsland, D., Cockrell, C. and Howe, D. (2009). Host cell-free growth of the Q fever bacterium *Coxiella burnetti*. *Proceedings of the National Academy of Sciences of the United States of America*, 106(11): 4430–4434.

Ormsbee, R. A. and Marmion, B. P. (1990). Prevention of *Coxiella burnetti* infection: vaccines and guidelines for those at risk. In: *"Q fever: The disease"*. (Edited by Marrie, T. J).CRC Press, Boca Raton, FL. Vol 1. Pp 225–248.

Ormsbee, R. A., Bell, E. J., Lackman, D. B. and Tallent, G. (1964). The influence of phase on the protective potency of Q fever vaccine. *Journal of Immunology*, 92:404–412.

Palmer, N. C., Kierstead, M., Key, D. W., Williams, J. C., Peacock, M. G. and Vellend, H. (1983). Placentitis and abortion in goats and sheep in Ontario caused by *Coxiella burnetti*. *Canadian Veterinary Journal*, 24: 60–61.

Palmer, S. R. and Young, S. E. J. (1982). Q fever endocarditis in England and Wales, 1975–81. *Lancet ii:* 1148–1149.

Parisi, A.R., Fraccalvieri, M., Cafiero, A., Miccolupo, C., Montagna, F., Capuano and Sottili, R. (2006). Diagnosis of *Coxiella burnetti* related abortion in Italian domestic ruminants using single-tube nested PCR. *Veterinary Microbiology*, 118:101-106.

Perrin, T. K. and Bengtson I. A. (1942). The histopathology of experimental Q fever in mice. *Public Health Response*, 57:790–794.

Philip, C.B. (1948). Comments on the name of the Q fever organism. *Public Health Response*, 63:58-59.

Paiba, G.A., Lloyld, G., Bewley, K., Webster, G.J., Green, L.E.and Morgan, K.L. (1999). An investigation of *Coxiella burnetti* infection at calving within a herd of dairy

cows in England using PCR. In: *"Rickettsiae and rickettsial diseases at the turn of the third millennium"* (Edited by Brouqui, P and D. Raoult). Amsterdam. *Elsevier*. Pp 387-392.

Plommet, M., Capponi, M., Gestin, J. and Renoux, G. (1973). Experimental Q fever in cattle. *Annales de Recherches Veterinaires,* 4(2): 325–346.

Pope, J.H., Scott, W. and Dweyer, R. (1960). *Coxiella burnetti* in kangaroos and kangaroo-ticks in Western Queensland. *Australian Journal of Experimental Biology,* 38: 17-19.

Raju, N.R., Collings, D.F. and Saville, P.H. (1988). Abortion in black belly Barbados sheep in Fiji caused by *Coxiella burnetti. Canadian Veterinary Journal*, 24:61-61.

Ransom, S. E. and Huebner, R. J. (1951). Studies on the resistance of *Coxiella burnetti* to physical and chemical agents. *American Journal of Epidemiology,* 53(1): 110–119.

Raoult, D. (1990). Host factors in the severity of Q fever. *Annals of the New York Academy of Sciences,* 590: 33–38.

Raoult, D. and Stein, A. (1994). Q fever during pregnancy:a risk for women, fetuses, and obstetricians. *The New England Journal of Medicine,* 330(5): 371.

Raoult, D., Fenollar, F. and Stein A. (2002). Q fever during pregnancy: diagnosis, treatment, and follow-up. *Archives of Internal Medicine,* 162(6) 701–704.

Raoult, D., Laurent, J.C. and Mutillod, M. (1994). Monoclonal antibodies to *Coxiella burnetti* for antigenic detection in cell cultures and in paraffin-embedded tissues. *American Journal of Clinical Pathology*, 101(3): 318–320.

Rauch, A. M., Tanner, M., Pacer, R. E., Barrett, M. J., Brokopp, C. D. and Schonberger, L. B. (1987). Sheep-associated outbreak of Q fever in Idaho. *Archives of Internal Medicine,* 147:341–344.

Rehacek, J. and Tarasevich, I.V. (1988). Acari-borne Rickettsiae and Rickettsioses in Eurasia. *Slovak Academy of Sciences*, pp 343.

Reinthaler, F.F., Mascher,F., Sixl W. and Arbesser,C.H. (1988). Incidence of Q fever among cattle, sheep, and goats in the Upper Nile province in Southern Sudan. *Veterinary Record*, 122:137-137.

Rey, D., Obadia,V., Tissot-Dupont,H. and Raoult. (2000). Seroprevalence of antibodies to *Coxiella burnetti* among pregnant women in South Eastern France. *European Journal of Obstetrics and Gyneacology and Reproduction Biology*, 93:151-156.

Rodolakis, A. (2005). Q fever, state of art: Epidemiology, diagnosis and prophylaxis. *Small Ruminant Research*, 62:121-124.

Rodolakis, A., Berri, M.and Héchard, C. (2007). Comparison of *Coxiella burnetti* shedding in milk of dairy bovine, caprine, and ovine herds. *Journal of Dairy Science,* 90(12): 5352–5360.

Rousset, E., Durand, B.and Berri, M. (2007). Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. *Veterinary Microbiology*, 124(3-4): 286–297.

Ruiz-Fons, F., Astobiza, I., Barandika, J.F., Hurtado, A., Alexandrio, R., Juste, R.A., and Garcia-Perez, A.L. (2010). Sero-epidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. *BMC Veterinary Research*, 6:3.

Russell-Lodrigue, K.E.,Zhang,G.Q., McMurray, D. N. and Samuel, J. E. (2006). Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. *Infection and Immunity,* 74(11): 6085–6091.

Schelling, E., Diguimbaye, C., Daoud, S.,Nicolet, J., Boerlin, P., Tanner, M and Zinsstag, (2003). Brucellosis and Q fever Seroprevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine,* 61:279-293.

Schmeer, N., Muller, H.P., Baumgartner, W., Wieda, J. and Krauss, H. (1988). Enzyme-linked immunosorbent fluorescence assay and high-pressure liquid chromatography for analysis of humoral immune responses to *Coxiella burnetti* proteins. *Journal of Clinical Microbiology,* 26:2520-2525.

Schmeer, N., Muller, P., Langel, J., Krauss, H., Frost, J. W. and Wieda J. (1987). Q fever vaccines for animals. *Zentralblatt Fur Bakteriologie Abt Originale Hygiene*, 267:79–88.

Schneeberger, P. M., Hermans, M. H. A.,Van Hannen, E., Schellekens, J. J. A., Leenders, A.C.A.P. and Wever, P.C. (2010). Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clinical and Vaccine Immunology,* 17(2): 286–290.

Schramek, S. and Mayer, H. (1982). Different sugar compositions of lipopolysaccharides isolated from phase I and pure phase II cells of *Coxiella burnetti*. *Infection and Immunity*, 38: 53–57.

Seshadri, R. and Samuel, J.E. (2001). Characterization of a stress-induced alternate Sigma factor, RpoS, of *Coxiella burnetti* and its expression during the development cycle. *Infection and Immunology*, 69: 4874–4883.

Shannon, J. G. and Heinzen, R. A. (2009). Adaptive immunity to the obligate intracellular pathogen *Coxiella burnetti. Immunological Research,* 43: 138–148.

Shannon, J. G., Cockrell, D. C., Takahashi, K., Stahl, G. L. and Heinzen, R. A. **(**2009**).** Antibody-mediated immunity to the obligate intracellular bacterial pathogen *Coxiella burnetti* is Fc receptor- and complement-independent. *BMC Immunology, 10- 26.*

Smith, D.J.W. and Derrick, E.H. (1940). Studies on the epidemiology of Q fever in the isolation of six strains of *Rickettsia burnetti* from the tick *Haemaphysalis humerosa*. *Australian Journal of Experimental Biology and Medical Science*, 18:1-5.

Smith, D. J. W. (1940). Studies on the epidemiology of Q fever.III. The transmission of Q fever by the tick *Haemaphysalis humerosa*. *Australian Journal of Experimental Biology and Medical Science*, 18: 103–106.

Smith, D. J. W. (1941). Studies on the epidemiology of Q fever. VIII. The transmission of Q fever by the tick *Rhipicephalus sanguineus. Australian Journal of Experimental Biology and Medical Science*, 19: 119–122.

Sonenshine, D.E. (1993). "*Biology of Ticks*." Volume 2. Oxford University Press, New York.

Stein, A. and Raoult, D. (1999). Pigeon pneumonia in Provence: a bird-borne Q fever outbreak. *Clinical Infectious Diseases,* 29: 617*–620.*

Thomas, D.R., Treweek, L. Salmon,R.L., Kench S.M. and Coleman, T.J. (1995). The risk of acquiring Q fever on farms. A Seroepidemiological study. *Occupational and Environment Medicine*, 52:644-645.

Tigertt, W. D., Benenson, A. S. and Gochenour, W. S. (1961). Airborne Q fever. *Bacteriological Reviews,* 25: 285–293.

To, H., Htwe, K. K., Kako, N., Kim, H. J., Yamaguchi, T., Fukushi, H. and Hirai, K. (1998). Prevalence of *Coxiella burnetti* infection in dairy cattle with reproductive disorders. *Journal of Veterinary Medical Science*, 60: 859–861.

To, H., Kako, N., Zhang, G.Q. (1996). Q fever pneumonia in children in Japan. *Journal of Clinical Microbiology*, 34(3): 647–651.

Tokarevich, N. K., Schramek, S. and Daiter, A.B. (1990). Indirect Haemolysis Test in Q fever. *Acta Virology*, 34:358-360.

Ughetto, E., Gouriet, F. Raoult, D. and Rolain, J. M. (2009). Three years experience of real-time PCR for the diagnosis of Q fever. *Clinical Microbiology and Infection*, 15(2): 200–201.

Van der Hoek, W., Dijkstra, F., Wijers, N., Rietveld, A., Wijkmans, C.J. and Van Steenbergen, J.E. (2010). Q fever in the Netherlands: an update on the epidemiology and control measures. *European surveillance*, 15(12) 19526

Vasselon, T. and Detmers, P.A. (2002). Toll receptors: a central element in innate immune responses. *Infection and Immunology*, 70: 1033–1041.

.

Waldhalm, D. G., Stoenner, H. G., Simmons, R. E. and Thomas, L. A. (1978). Abortion associated with *Coxiella burnetti* infection in dairy goats. *Journal of American Veterinary Medical Association*, 137:1580–1581.

Watanabe, A. and Takahashi, H. (2008). Diagnosis and treatment of Q fever: attempts to clarify current problems in Japan. *Journal of Infection and Chemotherapy*, 14(1): 1–7.

Welsh, H.H., Lennette, E. H., Abinanti, F. R. and Winn, J. F. (1958). Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. *Annals of the New York Academy of Sciences*, 70(3): 528–540.

.

World Health Organisation (WHO) (2004). Health Aspects of Chemical and Biological Weapons, 2nd edition. Geneva.

Williams, J. C. and Cantrell, J. L. (1982). Biological and immunological properties of *Coxiella burnetti* vaccines in C57BL/10ScN endotoxin-non responder mice. *Infection and Immunity, 35:1091–1102.*

Williams, J. C., Peacock, M. G., Waag, D. M., Kent, G., England, M. J., Nelson, G. and Stephenson, E. H. (1993). Vaccines against Coxiellosis and Q fever. Development of a chloroform-methanol residue subunit of phase I *Coxiella burnetti* for the immunization of animals. *Annals of the New York Academy of Science*, 653:88–111.

Woldehiwet, Z. (2004). Q fever (Coxiellosis): Epidemiology and pathogenesis. *Research in Veterinary Science,* 77:93-100.

Zamboni, D.S., McGrath, S., Rabinovitch, M. and Roy, C.R. (2003). *Coxiella burnetti* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophila* Dot/Icm system. *Molecular Microbiology*, 49: 965–976.

Zeman, D.H., Kirkbride,C.A., Leslie-Steen, P. and Duimtra, J.R. (1989). Ovine abortion due to *Coxiella burnetti* infection. *Veterinary Diagnostic Investigation*, 1:178-180.

Zhang, G., Russell-Lodrigue, K. E., Andoh, M., Zhang, Y., Hendrix, L. R. and Samuel, J. E. (2007). Mechanisms of vaccine-induced protective immunity against *Coxiella burnetti* infection in BALB/c mice. *Journal of Immunology*, 179*:* 8372–*8380.*

Zusman, T., Yerushalmi, G. and Segal, G. (2003). Functional similarities between the 1cm/dot pathogenesis systems of *Coxiella burnetti* and *Legionella pneumophila*. *Infection and Immunology*, 71: 3714–3723.

## APPENDIX I

## QUESTIONNAIRE ON RISK FACTORS ASSOCIATED WITH COXIELLOSIS (Q FEVER)

Dear Sir/ Madam,

This questionnaire is a part of an M.Sc Research programme. We intend to find out the risk factors associated with Q fever infection in our domestic cattle. Any findings will be treated as confidential. We therefore seek your truthful and candid response to questions contained in the questionnaire.

Your cooperation will be highly appreciated.

Thank you.

**Dr. Habiba Bello Tukur**

Kindly fill and tick where appropriate

Demographic Information

1. Serial No _____
2. Name of farm _____
3. Location of farm _____
4. Name of Respondent _____
5. Respondent: Owner [ ] Manager [ ] Herdsman [ ]
6. Occupation: Farmer [ ] Civil servant [ ] Businessman [ ]
7. Others specify _____
8. Sex: Male [ ] Female [ ]
9. Education qualification: None [ ] Primary [ ] Secondary [ ] Tertiary [ ] Others

   Specify _____

**FLOCK/HERD DATA**

1. Type of livestock kept: Sheep [ ] Cattle [ ] Goats [ ] Others specify _____

2. Sources of livestock: Market [ ] Inheritance [ ] Gift [ ] Purchase from other farms [ ] combination [ ]

3. Sex and Age distribution of livestock

a. Sex Distribution

| **Animal species** | **Number** | | **Total** |
|---|---|---|---|
| | **M** | **F** | |
| Cattle | | | |

b. Age Distribution

| Animal species | | | | |
|---|---|---|---|---|
| Cattle | Less than 1 year | 1-3 years | Greater than 3 -6 years | Over 6 years |

4. Breed of Livestock

Cattle

a. White Fulani [ ]

b. Sokoto Gudali [ ]

c. Exotic [ ]

d. Crosses (exotic x local) [ ]

e. Others specify _____

5. Why do you keep the animals?

a. sources of meat [ ]

b. sources of milk [ ]

c. source of family income [ ]

d. festivities [ ]

e. cultural/religious [ ]

f. others specify _____

**MANAGEMENT**

1. Management system

   Intensive [ ] Semi intensive [ ] Extensive [ ]

2. Type of supplementary feed

   Commercial feed [ ] locally compounded [ ] others specify _____

3. Type of housing

   Complete housing [ ] fenced area [ ] animals left to continuously roam and graze in the field [ ]

4. How do you handle sick animals?

   Consultation with a veterinarian [ ] Local remedies [ ] combinations [ ] does not bother [ ] not aware of veterinary services [ ]

5. Do you practice: Ectoparasite control [ ] Routine dewarming [ ] vaccinations [ ] does not bother [ ]

6. Form of tick control

   Dippling/spraying [ ] Tick baths [ ] spot ons [ ] injectables [ ]

7. Type of breeding

   Artificial insemination [ ] Natural mating [ ]

8. Type of milking

   Hand milking [ ] Milking machines [ ]

9. How do you dispose of animal waste/manure

   Use on the farm [ ] sell to farmers [ ] burn [ ] others specify _____

10. Do you use protective clothing when handling animal manure? Yes [ ] No [ ]

11. Biosecurity measures on the farm

    a. Provision of foot bath [ ] Yes [ ] No [ ]

    b. Restriction of entry into farm [ ] Yes [ ] No [ ]

    c. Quarantine of new arrivals [ ] Yes [ ] No [ ]

    d. Cleaning and disinfection of equipment after use [ ] Yes [ ] No [ ]

    e. Rodent control [ ] Yes [ ] No [ ]

    f. No biosecurity measures [ ]

12. How often do you clean animals barns/titter

    Monthly [ ] every 2-3 months [ ] every 6 months [ ] yearly [ ]

13. Have you had any cases of abortions of stillbirths in the: last month [ ] past 2-6

    months [ ] over 6 months – 1 year [ ] none ever [ ]

14. If yes to the question above, how many?

    Still birth No _____

    Abortion No _____

15. Have you heard of Q fever? Yes [ ] No [ ]

16. How do you dispose of after birth materials?

    Burn [ ] Bury [ ] dispose in refuse bin [ ] Others specify _____

17. If animals abort, do you give any form of treatment? Yes [ ] No [ ]

18. Other animals on the farm

    Cats [ ] dogs [ ] poultry [ ] sheep [ ] goats [ ]

19. Do you consume fresh milk from your cattle?

    yes [ ] no [ ] don't drink milk [ ]

20. If yes, how do you consume it?

    Raw [ ] fermented [ ] Heating before drinking [ ]

**APPENDIX II**
**INDIVIDUAL ANIMAL RECORD FORM**

Farm/Head No: _____

Date of collection: _____

Serial number: _____

Age: _____

Breed: _____

Sex: Male [ ] Female [ ]

Physiological status if female: Milking [ ] not milking [ ]

History of abortion: Yes [ ] No [ ]

Presence of ticks on animals: Yes [ ] No [ ]