SEROEPIDEMIOLOGICAL SURVEY OF Q- FEVER IN LIVESTOCK AND HUMANS IN BURA, TANA RIVER COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To,

My husband Tequiero and my families, The Mwololos and Okumus

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LIST OF ABBREVIATIONS

EAAPP- East African Agricultural Productivity Project

DDDAC- Dynamic Drivers of disease in African Continents

ILRI- International Livestock Research Institute

TBDs- Tick-borne Diseases

PCR-RLB- Polymerase Chain Reaction- Reverse Line Blot

ELISA- Enzyme Linked Immunosorbent Assay

DNA- Deoxyribonucleic Acid

CFT- Complement Fixation Test

IF- Immunofluorescence

IFAT- Indirect Fluorescent Antibody Test

GIS- Geographical Information System

NIB- National Irrigation Board

AMREF-ESRC- African Medical and Research Foundation-Ethics and Scientific Review Committee

µL- Microlitres

OD- Optical density

IgG- immunoglobulin G

IgA- Immunoglobulin A

IgM- Immunoglobulin M

OR- Odds ratio

AIC- Akaike Information Criteria

CI- Confidence Interval

ABSTRACT

Q fever is a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii.* It was first described in 1935. Ruminants are considered to be the primary source of infection to humans who become infected through inhalation of aerosols from infected ruminants and also through exposure to animal products such as unpasteurized dairy products. Clinical disease in animals is characterized by abortion and still births in sheep and goats while in cattle there is infertility and mastitis. In humans the disease syndrome can be divided into acute and chronic forms with the acute form manifesting as a relatively mild self-limiting febrile illness while the chronic form is a more severe disease characterized by hepatitis, pneumonia and chronic fatigue. This disease is therefore important due to its public health implications in humans and reproductive losses in animals.

There are reports on the occurrence of this disease in Kenya, though very few studies have been published recently on the epidemiology of the disease and specifically in pastoral communities where livestock is the main source of livelihood. This study was therefore designed with the following objectives 1) To assess the knowledge, attitude and practices of the livestock owners in relation to Q fever and its control in Bura, Tana River County; 2) To estimate the seroprevalence of Q fever in livestock and humans in the study area; and 3) To determine the risk factors associated with the disease in livestock and humans. A seroepidemiological study was therefore carried out using an ELISA test to determine the occurrence of *Coxiella burnetii* antibodies in livestock (cattle sheep and goats) and humans in Bura, Tana River County. Questionnaires were also administered to household heads to determine the risk factors for Q fever.

The prevalence of *Coxiella burnetii* antibodies in all animals surveyed was 13% (95% CI, 6.41-19.59%). The seroprevalence was higher in goats at 16% (95% CI, 8.81-23.19%) compared to sheep at 12% (95% CI, 5.63-18.37%) and cattle at 5.2 %, (95% CI, 0.85-9.55%). There were no statistically significant differences in the seroprevalence of *Coxiella burnetii* antibodies between animals sampled within the irrigation scheme versus those sampled within the *manyattas* at 12% (95% CI, 5.63-18.37%) and 14% (95% CI, 7.20-20.80%), respectively. Factors associated ($P<0.05$) with animal seropositivity were species, age and sex of the animal. Compared with adults, weaners were less likely to be seropositive (OR, 0.102; $P= 0.002$). With regard to species, goats were more likely to be seropositive compared to cattle (OR, 3.49; $P=0.01$).

Two-hundred-and seventy-two humans were screened for antibodies against *Coxiella burnetii*. The seroprevalence of *Coxiella burnetii* was 26.8% (95% CI, 18.12-35.48%). The difference in the seroprevalence of *Coxiella burnetii* in individuals sampled within the irrigation scheme at 30.2% (95% CI, 21.20-39.20%), and those sampled in the non-irrigated areas at 16.4% (95% CI, 9.14-23.66%), was statistically significance. The seroprevalence for *Coxiella burnetii* among adults, children and adolescents was 34.2% (95% CI, 24.90-43.50%), 26.8% (95% CI, 18.12-35.48%) and 23.2% (95% CI, 14.93-31.47%), respectively. With regard to sex, there were slight differences in the seroprevalence of *Coxiella burnetii* among males and females occurring at 28% (95% CI, 19.20-36.80%) and 26% (95% CI, 17.40-34.60%) respectively.

There were differences in the seroprevalence between different occupation groups with higher frequencies being reported amongst herdsmen and farmers at 42.9% (95% CI, 33.20-52.60%) and 30.2% (95% CI, 21.20-39.20%), respectively. This difference was not significant. Sixteen percent (95% CI, 8.81-23.19%) and 11.8% (95% CI, 5.48-18.12%) of the individuals

reported having experienced malaria and flu like symptoms within a period of two months before the study begun out of which 39.5% (95% CI, 29.92-49.08%) and 25% (95% CI, 16.51-33.49%) were seropositive for *Coxiella burnetii* antibodies, respectively. Risk factors associated ($P<0.05$) with individual and household seropositivity were occupation, irrigation status and animals housing within the family house ($P<0.05$).

In the questionnaire survey, the respondents were neither aware of Q fever nor its risk factors. The only risk factor associated with *Coxiella burnetii* seropositivity was housing of animals within the family house whose odds were two times higher compared to households who did not house their animals within their living house.

The present study confirmed the occurrence of *Coxiella burnetii* in the study area. The high prevalences in both the livestock and humans sampled may have been due to the low levels of awareness on this condition and the subsequent lack of control measures. Land use change to irrigation may also have increased the risk of infection especially in the human population. It is therefore necessary to employ appropriate strategies for diagnosis of Q- fever patients by equipping local and national laboratories and increasing the knowledge of physicians of potential clinical signs of the disease and how to make a diagnosis of the same. Community education and awareness creation would also be very helpful in preventing Q -fever infection. There is therefore need for the government to have control strategies for the disease such as introduction of vaccination which is currently not being carried out in the country. Further research to determine the effect of land use change on tick-borne diseases in Kenya should also be carried out.

CHAPTER ONE

1. INTRODUCTION

Q fever is a zoonotic disease and ruminants are considered to be the primary source of infection to humans (Van Den Brom *et al.,* 2013). This disease was first described in 1935 in Queensland, Australia during an outbreak of a febrile illness among abattoir workers (Derrick, 1973). Humans become infected through inhalation of aerosols from infected ruminants such as cattle, sheep and goats and also through exposure to animal products such as unpasteurized dairy products (Whitney *et al.,* 2009). However, person-to-person transmission is rare but has been reported to occur through sexual contact and aerosol transmission (Whitney *et al.,* 2009).

A study by Wu *et al*. (2013) has shown that Q fever is one of the most commonly observed human tick-borne diseases (TBDs) in China. *Coxiella burnetii* positive ticks might pose a risk for transmission to animals and humans through tick fecal excretions although the risk appears negligible based on current data (Sprong *et al*., 2012). Some of the important drivers for the emergence and spread of vector- borne parasites include habitat change, alteration in irrigation habits, atmospheric and climate change (Sprong *et al*., 2012; Jaleta *et al*., 2013). Infected animals shed the organism in urine, feces, milk, placental and birth fluids (Dorko *et al.,* 2008) and clinical disease of *Coxiella burnetii* infection in domestic ruminants consists of abortion and still births in sheep and goats while causing infertility and mastitis in cattle (Woldehiwet, 2004; Ruiz- Fons *et al.,* 2010). In humans the disease syndrome can be divided into acute and chronic forms with the acute form manifesting as a relatively mild self-limiting febrile illness while the chronic form is a more severe disease characterized by hepatitis, pneumonia and chronic fatigue (Woldehiwet, 2004; Dorko *et al.,* 2008).

Q fever is likely to impact negatively on humans because of its public health importance and on livestock due to losses incurred as a result of abortions. Unfortunately very few studies have been published recently on the epidemiology of the disease in Kenya and especially in pastoral communities where livestock is the main source of livelihood.

1.1 Objectives

1.1.1 Overall objective

To determine the epidemiology of Q fever in livestock and humans in Bura, Tana River County

1.1.2 Specific objectives

1. To assess the knowledge, attitude and practices of the livestock owners in relation to Q fever and its control in Bura, Tana River County.
2. To estimate the seroprevalence of Q fever in livestock and humans in the study area.
3. To determine the risk factors associated with the disease in livestock and humans.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Epidemiology of the disease

2.1.1 Aetiology

Q fever is caused by *Coxiella burnetii* which is an obligate intracellular bacterial pathogen with a worldwide distribution and can persist in the environment in a spore-like state for weeks; once airborne it can be transported for long distances by wind (Tissot-Dupont *et al*, 2004; Gilsdorf *et al*., 2005; Wallenstein *et al*., 2010). Initially it was named *Rickettsia burnetti* because morphologically it resembles Rickettsia species but in 1948 a scientist by the name Philip Becker proposed to create a new genus of *Coxiella* and classified the bacterium as a new species *Coxiella burnetii* in honor of the discoverers Cox and Burnet (Chmielewski and Wierzbanowska, 2012).

2.1.2 Mode of transmission

Coxiella burnetii is found in high numbers in amniotic fluid, placenta and fetal membranes as well as in milk, urine and faeces of infected animals (Rodolakis, 2009). The primary mode of human infection involves the aerosol route, and the domesticated animals most often implicated in human disease are sheep, cattle and goats (Bosnjak *et al.,* 2010). Consumption of raw milk and contact with placentae of livestock has been shown to be high-risk human behaviors for the acquisition of Q fever (Schelling *et al*., 2003).

Currently ticks are considered to be a reservoir in the environment though their role in the epidemiological process is not clear (Psaroulaki *et al.,* 2006); in many cases it is thought to be limited to the passive spread of the pathogen in the environment (Chmielewski and

Wierzbanowska, 2012; Knobel *et al.*, 2013). In one study, a total of 1482 adult ticks collected from vegetation and animals in central Spain in 2003-2005. They were tested for the presence of *Coxiella burnetii* by polymerase chain reaction and subsequent reverse line blot hybridization (PCR-RLB). *Coxiella burnetii* was identified in 7.7% of questing ticks (80/1039) and 3.4% of ticks collected from animals (15/443) belonging to four species: *Hyalomma lusitanicum, Dermacentor marginatus, Rhiphicephalus sanguineus, and Rhiphicephalus pusillus*. These findings suggest a role of ticks in maintaining *Coxiella burnetii* in wild and peridomestic cycles in central Spain (Toledo *et al*., 2008).

2.1.3 Occurrence and distribution

Q fever has a worldwide distribution with the exception of New Zealand (Maurin and Raoult, 1999; Angelakis and Raoult, 2010; Roest *et al.,* 2011). Information on the prevalence of infection in Sub-Saharan Africa is scanty with a study done in Western Kenya showing the prevalence of antibodies to *Coxiella burnetii* detected in human sera to be 30.9%, 28.3% in cattle, 32.0% in goats and 18.2% in sheep (Knobel *et al.,* 2013).

A study done in Southern Taiwan demonstrated the overall seroprevalence of Q fever as 26.3% in humans engaging in veterinary and animal-related work and exposure to goats was significantly associated with seropositivity (Chang *et al.,* 2009). Lidwien *et al.* (2012) associated a high density of goats in a densely populated region with the occurrence of Q fever in humans in the south of the Netherlands.

Q fever is considered an occupational disease in farmers, abattoir workers, and veterinarians, although community outbreaks around farms with infected ruminants, especially during the kidding season have also been reported (Maurin and Raoult, 1999). Schimmer *et al*. (2010) and Dijkstra *et al*. (2011) have shown that farms with abortion waves confirmed as

Coxiella burnetii positive were mainly located in the same area where human cases occurred. Some of the risk factors associated with the introduction, spread and persistence of *Coxiella burnetii* within farms in the Netherlands include high cattle density, importation of straw from abroad, access to the stable by cats and dogs and the use of artificial insemination. This study also found that large farms with over 800 goats are at an increased risk for infection (Schimmer *et al.,* 2010).

A study on the spread of Q fever within herds by Courcoul *et al*. (2010) revealed that animals having antibodies shed the bacterium for a longer period of time compared to those without antibodies. Age, residence, occupation, use of manure in the garden, animal ownership (especially goats), presence of infested ticks or aborting animals have also been shown to be important risk factors associated with Q fever seropositivity in humans (Psaroulaki *et al.*, 2006).

2.2 Diagnosis

Infection with *Coxiella burnetii* can be diagnosed using culture, serological and genetic methods (Woldehiwet, 2004). Field *et al.* (1983) and Schelling *et al*. (2003) have used Indirect Enzyme Linked Immunosorbent Assay (ELISA) (CHEKIT®-Q–FEVER IDEXX Switzerland AG, Liebefld-Bern, switzerland) to assay for antibodies against *C burnetti* in sera of livestock and humans. Impression smears and histological sections can also be used to demonstrate antigens by direct or indirect immunofluorescence and immunoperoxidase techniques (Woldehiwet and Aitken, 1993). Guinea pigs, which are extremely susceptible to *Coxiella burnetii* develop fever 5–8 days after intraperitoneal innoculation and the infected spleens can then be used for further isolation in cell cultures or yolk sacs (Ormsbee, 1952). The polymerase chain reaction (PCR) has also been used to detect specific DNA in clinical samples and to confirm the identity of culture isolates (Stein and Raoult, 1992).

The same serological tests used to investigate human Q fever are also used for epidemiological examination in domestic and other animals. The most widely used methods for diagnosis are complement-fixation test (CFT) (Murphy and Field, 1970; Herr *et al*., 1985), microagglutination (Babudieri, 1959), radioimmunoassay (Doller *et al*., 1984), immunofluorescence (IF) (Field *et al*., 1983), indirect fluorescent antibody test (IFAT) (Nakoun´e *et al*., 2004) and ELISA.

2.3 Treatment

Doxycycline, Chloramphenicol, Chlortetracycline, Erythromycin and Fluoroquinolones have been reported to be effective in the treatment of human Q-fever (Perez-del-Molino *et al*., 1991). Erythromycin was shown to be effective against acute *Coxiella burnetii* pneumonia (Yeaman and Baca, 1990). Rifampin was also reported to be effective against acute Q fever. However relapses occured in eight patients with chronic Q fever after treatment for 2 years with Tetracycline and Cotrimoxazole (Marrie, 1990)

2.4 Control

Destruction of straw and beddings contaminated by birth fluids and other secretions from ewes, goats and cows, pasteurization of milk and disinfection of vehicles used for animal transport are measures that can be used to prevent Q fever and hence protect those people handling animals or living in areas near the animals (Woldehiwet, 2004). Vaccination of cattle, sheep and goats has been reported to be effective in endemic areas (Biberstein *et al*., 1977). Vaccination may also be considered in livestock handlers, processors of animal products, veterinarians and laboratory workers likely to handle infected specimens (Ruiz and Wolfe, 2014).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study area

This study was carried out in Bura Irrigation Scheme and the *manyattas* within a 20 km radius from the scheme (Figure 3.1). For the purpose of this study a *manyatta* was defined as a temporary settlement often established by a pastoral family or a clan outside the irrigation scheme. The reason for the choice of study area was because of high livestock population and close contact between people and livestock. Tana River County covers an area of 35,375.8 Km^2 and has a human population of 240,075 according to the 2009 national census. The county has 58,056 head of cattle, 141,698 sheep and 365,544 goats (KNBS, 2010). The major ethnic groups living in this area are the Pokomo, many of whom are farmers, and the Orma and Wardei, who are predominantly nomadic. The county is generally dry and prone to drought, rainfall is erratic, with rainy seasons in March–May and October–December. Flooding is also a common occurrence caused by heavy rainfall in upstream areas of the Tana River. The county is divided into seven administrative units, namely, Bangale, Bura, Galole (Hola), Garsen, Kipini, Madogo and Wenje.

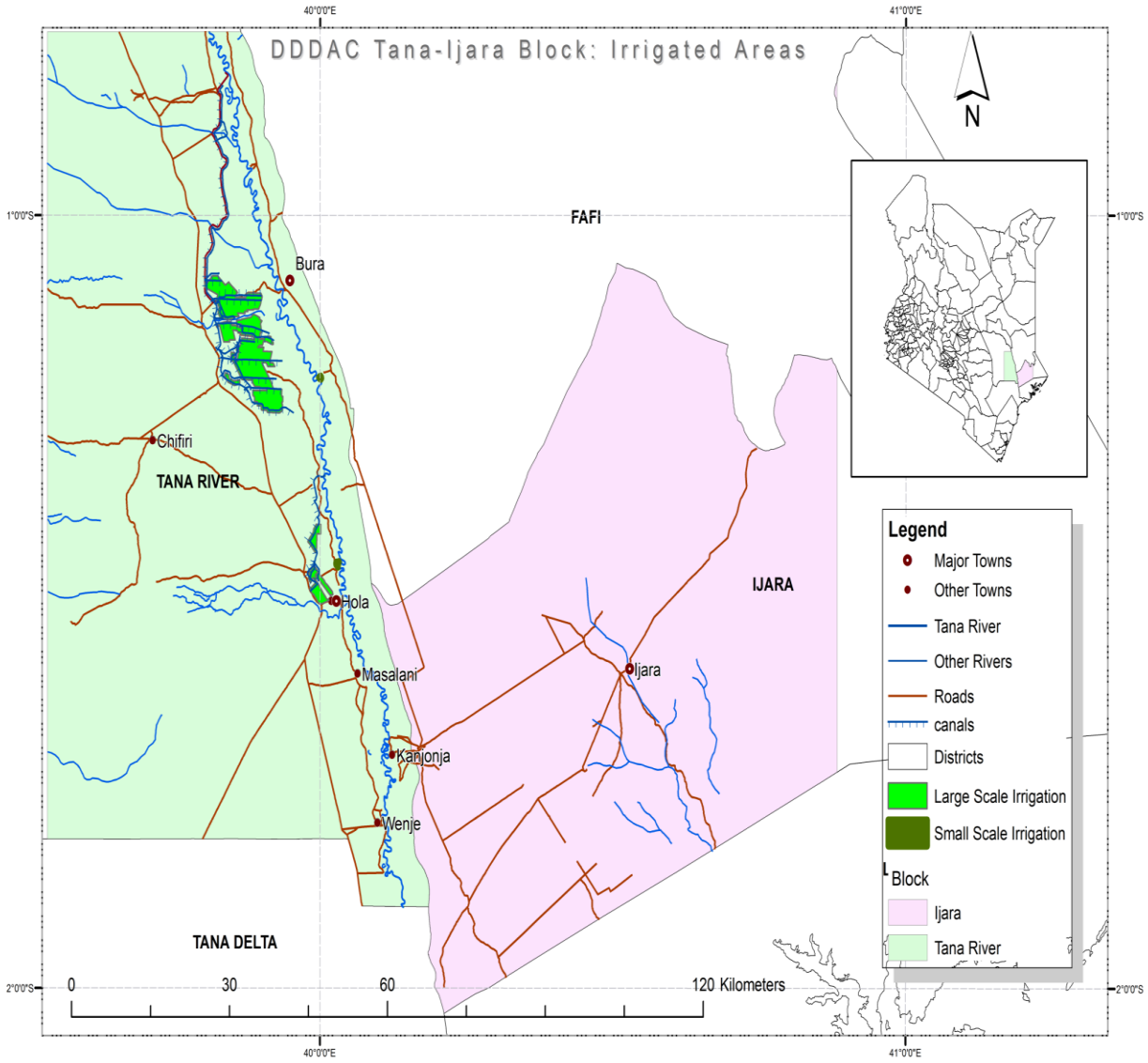

Figure 3.1: Map showing the study site (ILRI GIS Map, 2013)

3.2 Study design

A cross- sectional survey was conducted whereby households were randomly selected from a list of farmers who own livestock. The list of farmers was obtained from the National Irrigation Board (NIB) from which a sampling frame was generated only using farmers who own livestock.

3.2.1 Selection of study households

During the initial phase of the study, a census of households in the study sites was drawn up with the help of the local leaders including the village headmen and the NIB office. Bura Irrigation Scheme for instance had 10 defined villages within the scheme and therefore it was easy to enumerate households within each village. Lists generated were then used as a sampling frame for a random selection of households where livestock and human sampling was done. A herd was defined as a group of animals owned by a particular household while a household was defined as a group of people who live together and share common livelihood activities under a common household head.

Multi-stage sampling was carried out in livestock whereby secondary sampling units (animals) were randomly selected within the primary sampling unit which in this case was the household. Human sampling was done in the same households where livestock sampling was done and this involved random selection of 5 members above 5 years of age within the household. Where there were less than 5 members in the household all members present at the time of the survey were sampled. For the purpose of this study, herdsmen were defined as those individuals employed to look after livestock on other people's farms; students were the school-going either in primary school, high school or college, while farmers were those individuals

growing crops and keeping livestock and were sedentary at the irrigation scheme. Pastoralists were individuals who purely kept livestock as a source of livelihood and were mainly from the *manyattas* outside the irrigation scheme. They were nomadic, moving around looking for pasture.

3.2.2 Sample size determination

The sample size was determined using the following formula (Dohoo *et al*., 2009).

$$n = Z_{\alpha}^2 \, pq / L^2$$

Where; n = sample size, Z_{α} = value of the Z statistic that corresponds to a level of confidence of 95%, P = 28.3%, 32%, 18.2% and 30.9% in cattle, goats, sheep and humans respectively (Knobel *et al*., 2013), q=1-p, L = precision of the estimate set at 0.05.

3.3 Data Collection

3.3.1 Questionnaire administration

Semi- structured questionnaires (Appendix 1) were administered to the household heads. This collected information on participants' demographics, knowledge, attitudes and practices of the communities in relation to Q fever transmission and control as well as information on possible risk factors. Sampling forms (Appendix 2 and 3) were used to collect further information on livestock ownership, herd size, type of livestock owned, age, gender and occupation.

3.3.2 Ethical considerations

Ethical clearance was obtained from African Medical and Research Foundation-Ethics and Scientific Review Committee (AMREF-ESRC) reference number AMREF-ESRC P65/2013 dated 4th October 2013. Written consent/assent was sought from all subjects selected for the survey (Appendices 4-6).

3.4 Prevalence survey in livestock

3.4.1 Blood collection and serum separation

Five to ten Millilitres of blood were collected in plain tubes from the Jugular vein using vacutainer needle gauge 19 for cattle and 21 for small stock after obtaining informed consent from the farmers. The tubes with blood sample were then placed in a cool box with ice packs and transported to the local livestock office laboratory for separation to obtain serum.

Serum separation was done at the end of each day's sampling activity at the local livestock office. Serum was obtained by centrifuging the blood in the vacutainer tubes at 3000 rotations per minute for 5 minutes. Spontaneous clotting could be achieved between 30-60 minutes at room temperature by placing the vacutainer tube on a tube rack in a slightly tilted manner away from direct sunlight. Serum was then drawn using sterile Pasteur pipette into sterile bar-coded cryovials, placed in cryo boxes and stored in a refrigerator at -20 degrees Celsius or dry ice until transported to the ILRI laboratory in Nairobi for storage.

3.4.2 Sample analysis

A commercial ELISA kit (IDEXX Switzerland AG, Liebefld-Bern, switzerland) was used for the detection of antibodies in serum directed against *Coxiella burnetii.* The ELISA procedure

was performed as previously described by Berri *et al.* (2001). Components of the ELISA kit were; *Coxiella burnetii* antigen coated plate, positive control, negative control, conjugate, substrate, stop solution and wash concentrate. Antibodies directed against *Coxiella burnetii* binds to the antigen in the wells and forms an antigen/antibody complex on the plate well surface. Unbound material was then removed from the wells by washing. A peroxidase labelled anti-ruminant IgG conjugate was added which binds to the ruminant antibodies complexed with the *Coxiella burnetii* antigen. Unbound conjugate was removed by washing and the substrate (Para-nitrophenylphosphate in solvent free buffer) added to the wells. The degree of colour that develops is directly proportional to the amount of antibody specific for *Coxiella burnetii* present in the sample.

Sera were considered to be ELISA positive if they had an optical density (OD) value of ≥40%, suspect(borderline) if the OD value was between 30% and 40%, and negative if the OD value was <30%.

3.5 Prevalence survey in humans

3.5.1 Blood collection

3.5.1.1 Individual consent for sampling

After randomly selecting the participants in a household, the consenting process was done in a private area by the clinician. If the selected individual was a child 5-12 years of age, the parents' consent was sought (Appendix 4) and if the selected individual was between 13-17 years of age, the consent form (Appendix 5) was used in addition to parent/guardian consent

For adults (18 years or more), their individual consent was sought (Appendix 6). Once the consenting process was completed, the nurse proceeded to administer the individual sampling form questionnaire to each of the selected individuals that had consented.

3.5.1.2 Equipment and materials used

Materials used for blood collection in humans included; disposable latex gloves, 70% alcohol swabs, tourniquet (latex rubber or Velcro), vacutainer needles (21G (adult) and 23G (child)), vacutainer needle holders, plain vacutainer specimen tubes, cotton wool balls, elastoplast®, sharps disposal container, waste bin/ waste bags, 10% household bleach (Jik®), barcodes for labelling the samples, human blood sample collection record form, tube rack and a cool box with icepacks.

3.5.1.3 Human blood collection procedure

All the required equipment and materials were assembled before the blood collection procedure after obtaining informed consent and clearly explaining the blood collection procedure to the participant. The participant was then allowed to sit in a comfortable chair with the hand from which the blood sample was collected supported and tourniquet applied above the elbow to distend the veins. The blood collection site was then disinfected using alcohol swabs avoiding touching the disinfected site.

The appropriate vacutainer needle gauge (21G for adults and 23G for children) was selected and vacutainer needle holder attached, the needle tip was then inserted into the vein, a plain bar-coded vacutainer tube attached, 5-10ml of blood was collected and the tourniquet released.

The needle was then removed from the vein and carefully disposed in the sharps container without recapping and pressure applied to the puncture site for 30seconds using a cotton swab and the swab carefully disposed of and elastoplast® applied to the puncture site. The tubes with human blood sample were then placed in a cool box with ice packs at 4

degrees Celsius and transported to the local health facility laboratory for separation to obtain serum. Serum separation was done as described in section 3.4.1.

3.5.2 Sample analysis

A commercial ELISA antibody test kit (SERION ELISA classic *Coxiella burnetii* Phase 1 IgG) was used for the detection of human antibodies in serum directed against *Coxiella burnetii* Phase 1. The ELISA procedure was performed as previously described by Berri et al., (2001). The *Coxiella burnetii* Phase 1 IgG tests are used for the diagnosis of chronic Q-fever disease by detecting any antibodies present in the patients samples which if present binds to the antigen coated plate which constitutes the solid phase.

Components of the ELISA kit were: microtitre strips coated with inactivated antigen; cut-off (ready to use)-human serum in phosphate buffer with protein; negative control serum (ready to use) – human serum in phosphate buffer with protein in 0.1% sodium azide as the preservative, anti- human IgG-, IgA-, IgM-conjugate (ready to use; anti-human IgG from goat (polyclonal), conjugated to alkaline phosphatase and stabilized with protein stabilizing solution; washing solution concentrate (sufficient for 1 litre)-sodium chloride solution in <0.1% sodium azide as preservative; phosphate dilution buffer; substrate and stopping solution ($2NH_2SO_4$). Seropositivity was indicated by a yellow end point colour as shown in figure 3.1.

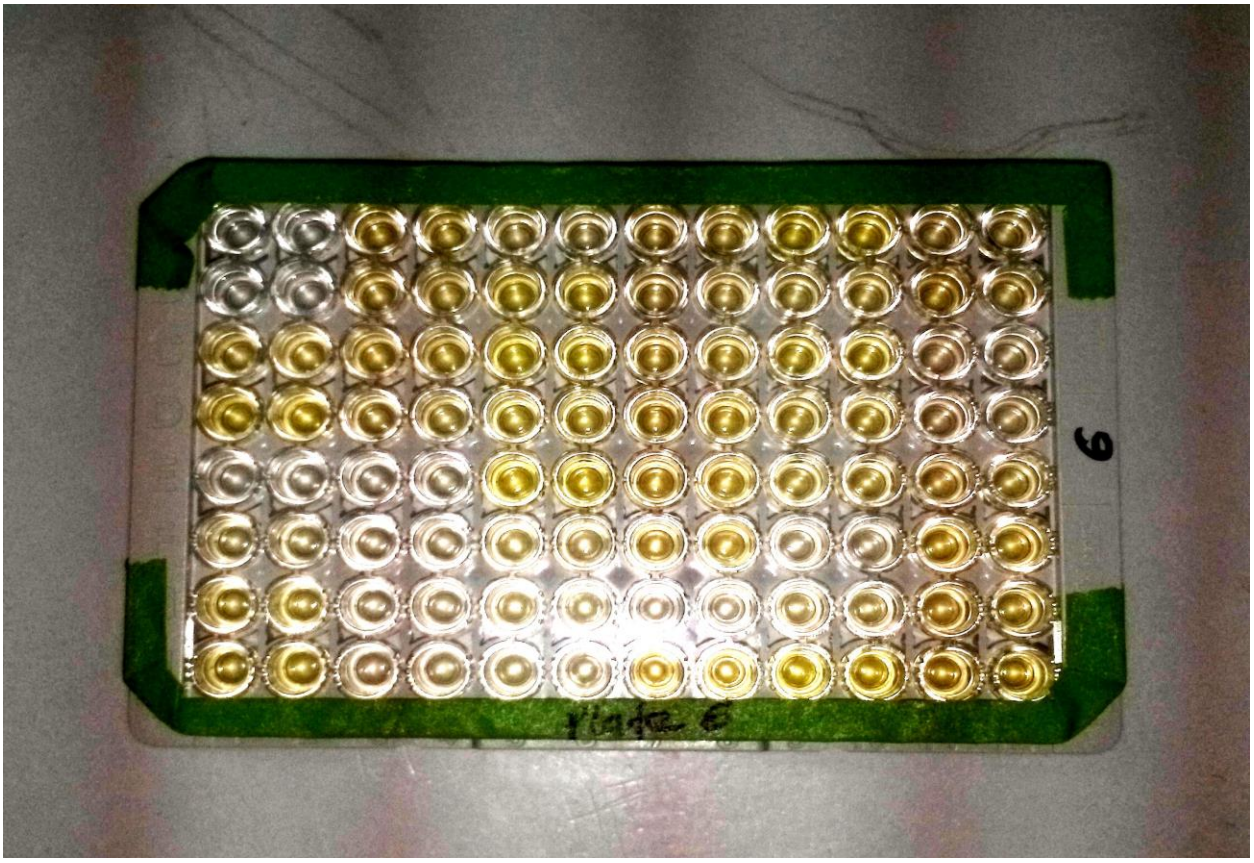

Plate 3.1: A 96-well ELISA plate for screening antibodies to Coxiella burnetii. Note the hue of yellow indicating seropositivity

All samples were tested in duplicates and the optical densities (OD) of the samples were averaged. The results were expressed as S/P (sample to positive control) values calculated using the following equation:

S/P =OD sample –OD negative control/OD positive control –OD negative control×100

Sera were considered to be ELISA positive if they had an optical density (OD) value of >10% above cut-off, suspect(borderline) if the OD value was +/- 10% of cut off, and negative if the OD value was >10% below cut off. The cut-off ranges were fixed by multiplying the mean value of the measured standard OD with the numerical data on the quality control certificate.

3.6 Data management

Data from the household questionnaire survey, sampling form and serological tests were entered and stored in Microsoft Office Excel 2010 (Microsoft Corporation, 2010). The data were then screened for any entry errors and imported into R version 3.1.1 software for analysis (R Development core team, 2014) and merged using the doBy® package in R.

3.6.1 Statistical data analysis

The apparent prevalence was calculated as the total number of animals that tested positive for *Coxiella burnetii* antibodies divided by the total number of animal samples analyzed multiplied by 100. Statistical differences prevalences were assessed by comparing the confidence intervals (CI). The differences were statistically significant where the CI of the prevalences overlapped. Descriptive statistics were performed using frequency tables in R software. Analysis was carried out to calculate odd's ratio (OR) with 95% confidence intervals. The OR was defined in this context as the odds of a given exposure among individuals/animals seropositive for *Coxiella burnetii* divided by the odds of the exposure among seronegative individuals (Dohoo *et al*., 2009).

Logistic regression was used to model for potential risk factors on univariable and multivariable risk factor analyses for human and livestock data separately. Factors that had p value < 0.2 on univariate analyses were then included in the multivariate risk factor analyses. Stepwise backward logistic regression was carried out starting with a model with all variables selected from univariate analyses and excluding stepwise each variable that had a p value >0.05. Variables that had a p value <0.05 were considered to be risk or protective factors for Q fever. Selection of the best fitting model was based on the value of Akaike's Information Criteria (AIC) (Akaike, 1981; Bozdogan, 2000). The model with the lowest AIC value was considered the best

fitting model. The decision to retain or eliminate a variable from the model was arrived at by performing the likelihood ratio test (lrtest) to compare two models and if the p value from the lrtest was <0.05 then the variable was retained in the model.

Poisson regression was used to model for potential risk factors at household/herd level after merging the questionnaire data and the *Coxiella burnetii* ELISA results/outcome.

CHAPTER FOUR

RESULTS

4.1 Livestock

4.1.1 Descriptive analysis

4.1.1.1 General characteristics of the sampled herds/animals

Of the 524 animals included in the study, 96(18.32%), 280(53.43%), and 148(28.24%) were cattle, goats and sheep, respectively. Herd size of all animals ranged from 2 to 350 with a mean of 112.7 and a median of 57 and most of the animals sampled 414 (79%), were females. The mean of the goat flock sizes was 36.05 (range of 0 to 120, median 25) while that of cattle herds and sheep flocks was 37 (range 0 to 200, median 2) and 38.9 (range 0 to 180, median 30), respectively.

Fifty three percent of the animals were sampled from herds in villages within the irrigation scheme while 47% were sampled within the *manyattas* outside the irrigation scheme. Most animals were adults (n=368; 70.2%), others being weaners (n=97; 18.5%), kids (n=34; 6.5%), calves (n=16; 3.1%), and lambs (n=9; 1.7%).

Twenty eight percent (147/524) of the animals sampled were the Black head Persian sheep which was a common breed amongst most farmers. The *Orma boran* cattle formed 18% (94/524) of the total animals sampled and this was also a popular breed of cattle kept by the farmers in the study area. The rest of the cattle were crosses.

4.1.1.2 Sero-surveillance in animals

A total of 68/524 tested samples were positive for *Coxiella burnetii* antibodies equivalent to an overall apparent seroprevalence of 13% (95% CI, 6.41-19.59%). The seroprevalence in

goats was 16% (n=45, 95% CI, 8.81-23.19%) and 12% (n=18, 95% CI, 5.63-18.36%) in sheep which were significantly (P<0.05) higher than that in cattle at 5.2 %,(n=5, 95% CI, 0.85-9.55%). There was no significant difference in the seroprevalence of *Coxiella burnetii* antibodies between animals sampled within the irrigation scheme verses those sampled within the *manyattas* at 12% (95% CI, , 5.63-18.37%) and 14% (95% CI,7.20-20.80%), respectively. Seroprevalence was also higher among females than males though the difference was not statistically (P<0.05) significant as shown in Table 4.1.

Table 4.1: Prevalence of *Coxiella burnetii* among livestock in Bura Sub-county of Tana river County, 2013

Species	**Sex**	**Number Sampled**	**Number Positive**	**Seroprevalence (%)**	**95% Confidence Interval**
Goats	Females	227	41	18	10.47-25.52
	Males	53	4	8	2.68-13.31
Sheep	Females	118	16	14	7.19-20.81
	Males	30	2	7	2-12
Cattle	Females	69	4	6	1.34-10.65
	Males	27	1	4	0.16-7.84

4.1.2 Risk factor analysis - livestock

4.1.2.1 Univariable analysis

Factors associated (P<0.05) with animal seropositivity based on univariable analysis were species, age and sex of the animal. Compared with adults, weaners were 9 times less likely to be seropositive (OR, 0.11; 95% CI, -4.10 -1.09; P= 0.002). With regard to species goats were more likely to be seropositive compared to cattle (OR, 3.49; 95% CI, -2.33 -0.38; P=0.01). In addition, males were 3 times less likely to be seropositive compared to females (OR, 0.01; 95% CI, -2.29 -0.33; P=0.29). The details are shown in Table 4.2.

Table 4.2: Univariable logistic regression analysis of associations between *Coxiella burnetii* antibody positivity and related risk factors on individual animal level in Bura sub county, Tana River County 2013

Variable	Levels	Seroprevalence (%)	Estimate	OR	95% CI (Estimate)	P Value
Species	Cattle (Ref)	18.32				
	Goat	53.43	1.25	3.49	0.38 2.33	0.01
	Sheep	28.24	-0.32	0.73	-0.035 2.06	0.28
Sex	Female (Ref)	79.01				
	Male	21	-0.93	0.39	-1.83 -0.18	0.02
Age	Adult (Ref)	70.23				
	Calf	3.05	-1.13	0.32	-4.03 0.49	0.28
	Kid	6.49	-1.19	0.30	-3.03 0.03	0.11
	Weaner	5.15	-2.28	0.11	-4.10 -1.09	0.002

4.1.2.2 Multivariable analysis

Of the variables that were found to be significant at the P<0.05 level in univariate analysis, only species and age were associated with *Coxiella burnetii* seropositivity in the multivariate analysis at a significant level of P<0.05 as displayed in Table 4.3. Weaners were 7 times less likely to be seropositive for Coxiella burnetii antibodies compared to adults. (OR 0.10, p=0.01). Goats were 0.3 times more likely (OR 2.94, P=0.05) to be seropositive compared to cattle.

Table 4.3: Multivariable logistic regression analysis of associations between *Coxiella burnetii* antibody positivity and related risk factors in Bura sub county, Tana River County 2013

Variable	Levels	Seroprevalence (%)	Estimate	OR	95% CI (Estimate)	P Value
Species	Cattle (Ref)	18.32				
	Goat	53.43	1.08	2.94	0.11 2.32	0.05
	Sheep	28.24	0.79	2.20	-0.26 2.07	0.18
Age	Adults (Ref)	70.23				
	Calf	3.05	-0.03	0.97	-3.05 2.01	0.98
	Kids	6.49	-1.34	0.26	-3.18 -0.09	0.01
	Weaner	5.2	-1.95	0.14	-3.78 -0.74	0.01

4.2 Humans

4.2.1 Descriptive analysis

4.2.1.1 General characteristics of the study participants

Samples from 272 individuals were analyzed for antibodies against *Coxiella burnetii*. One hundred and eighteen (43.38%) were males and 154 (56.62%) were females. With regard to their occupation, majority (38.97%) of the participants were farmers who kept livestock and grew crops. The rest were students (29.78%), pastoralists (11.76%), and herdsmen (2.57%) while 16.91% belonged to other occupations.

The age of the participants ranged from 5 to 82 years (median 23, mean 29); 27.9% were 5-12 years old, 15% were 13-17 years old and 57% were 18 years and above. Two hundred and five (75.4%) individuals were from the irrigation scheme while 67 (24.6%) were from the *manyattas* within a 20 km radius from the scheme.

4.2.1.2 Sero-surveillance

The seroprevalence of *Coxiella burnetii* in humans in this study was 26.8% (95% CI, 18.1-35.5%). The seroprevalence of *Coxiella burnetii* was higher in individuals sampled in the irrigation scheme at 30.2% (95% CI, 21.2-39.2%) relative to individuals sampled in the non-irrigated areas at 16.4% (95% CI, 9.14-23.7%).This difference was not significant. The seroprevalence for *Coxiella burnetii* among adults, children and adolescents was 34.2% (95% CI, 24.9-43.5%), 26.8% (95% CI, 18.1-43.5%) and 23.2% (95% CI, 14.9-31.5%), respectively. With regard to sex, there were no significant differences in the seroprevalence of *Coxiella burnetii* among males and females occurring at 28% (95% CI, 19.2-36.8%) and 26% (95% CI, 17.4-34.6%) respectively.

There were significant differences in the seroprevalence between different occupations with higher frequencies being reported amongst herdsmen and farmers at 42.9% (95% CI, 33.2-52.6%) and 30.2% (95% CI, 21.2-39.2%), respectively, while lower rates were reported amongst students and pastoralists at 24.7% (95% CI, 16.2-33.2%) and 6.3% (95% CI, 1.54-11.06%), respectively (Figure 4.1). Sixteen percent (95% CI, 8.8.1-23.19) and 11.8% (95% CI, 5.48-18.12%) individuals reported having experienced malaria and flu-like symptoms respectively, within a period of two months before the study begun out of which 39.5% (95% CI, 29.9-49.1%) and 25% (95% CI, 16.5-33.5%) were seropositive for *Coxiella burnetii* antibodies, respectively.

4.2.2 Risk factor analysis – humans

4.2.2.1 Univariable analysis

Risk factors associated ($P<0.05$) with individual and household seropositivity on univariate analysis were occupation and irrigation status (Table 4.4). Pastoralists were 6 times less likely to be seropositive for *Coxiella burnetii* antibodies (OR, 0.15; P=0.01) compared to farmers who grew crops and kept livestock. Individuals sampled within the *manyattas* (non-irrigated areas) were 2 times less likely to be seropositive for *Coxiella burnetii* (OR, 0.45; P=0.03. With regard to gender both males and female were equally exposed.

Table 4.4: Univariable logistic regression of associations between *Coxiella burnetii* antibody positivity and related factors on individual level in Bura sub county, Tana River County, 2013

Variable	Levels	Seroprevalence (%)	Estimate	OR	95% CI (Estimate)	P Value
Occupation	Farmers (Ref)	38.97				
	Herdsmen	72.57	0.55	1.72	-1.12 2.12	0.49
	Pastoralists	11.76	-1.87	0.15	-3.73 -0.59	0.01
	Students	29.78	-0.28	0.76	-0.94 0.37	0.41
	Other	16.91	0.21	1.23	-0.54 0.94	0.58
Irrigation Status	Irrigated (Ref)	75.37				
	Non-Irrigated	24.63	-0.79	0.45	-1.55 -0.11	0.03
Gender	Female (Ref)	56.62				
	Male	43.38	0.10	1.11	-0.44 0.64	0.71
Age	Adolescent (Ref)	15.07				
	Adult	56.99	0.71	2.03	-0.96 0.63	0.63
	Child	27.94	0.35	1.42	-0.47 1.21	0.41

4.2.2.2 Multivariable analysis

To control for potential confounding effects from various risk factors selected from the univariable analysis, multiple logistic regression models were built by starting with a model with all factors that had a P<0.20 and dropping variables that were not significant at P<0.05 based on the likelihood ratio test. Variables included in the full model were age, gender, occupation and

irrigation status. Occupation and irrigation status were significant in univariable analysis but only Occupation was significantly associated with *Coxiella burnetii* seropositivity in humans on multivariable analysis (Table 4.5). This could have been due to the confounding effect of irrigation status which has an association with both occupation and whether an individual was positive for *Coxiella burnetii* antibodies.

Table 4.5: Multivariate logistic regression analysis of associations between *Coxiella burnetii* antibody positivity and related risk factors in Bura sub county, Tana River County 2013

Variable	Levels	Seroprevalence (%)	Estimate	OR	95% CI (Estimate)	P Value
Occupation	Farmers	38.97	Ref			
	Herdsmen	72.57	-0.61	0.54	-2.97 1.36	0.57
	Pastoralists	11.76	-1.87	0.15	-3.73 -0.59	0.01
	Students	29.78	-2.04	0.13	-4.06 -0.48	0.02
	Other	16.91	-1.19	0.30	-3.11 0.19	0.14
Age	Adolescent	15.07	Ref			
	Adult	56.99	-1.45	4.26	-3.44 0.09	0.09
	child	27.94	0.49	1.63	-0.38 1.40	0.28

4.3 Household level analysis

Of the 85 semi structured questionnaires administered, 61% of the respondents were females while 39% were males. The respondents' age ranged from 20 to 78 years with a mean and median of 49 and 50 years respectively. Eighty percent of the total households sampled were within the irrigation scheme. The questionnaire was used to collect information on possible risk

factors such as drinking raw milk, assisting in birthing, disposal of aborted fetuses, animals housed within the family house, milking animals, and animals mixing at grazing and watering point among others. All respondents from the sampled households did not know what Q fever was and whether it affects humans and animals. Fourty percent of the households reported having been involved in drinking of raw milk and 78% were involved in disposal of aborted fetuses whose household seroprevalence was 11.7% (95% CI, 5.4-18.0%) and 17.9% (95% CI, 10.4-25.4%), respectively. Ninety four percent of the households housed animals within the family house and the household seroprevalence for *Coxiella burnetii* antibodies was 20.4% (95% CI, 12.5-28.3%). Respondents reported mixing of livestock from different households and this was especially during the dry season.

4.3.1 Univariate risk factor analysis on household/herd level

Poisson regression analysis was used to model for possible risk factors with the outcome being a weighted count of positive Q fever individuals in a household. The only risk factor associated with *Coxiella burnetii* seropositivity was housing of animals within the family house whose odds were two times higher compared to households who did not house their animals within their living house (Table 4.6).

Table 4.6: Univariable Poisson regression analysis of associations between *Coxiella burnetii* antibody positivity and related risk factors at household/herd level in Bura sub county, Tana River County 2013

Variable	Levels	Seroprevalence (%)	Estimate	OR	95% CI (Estimate)	P Value
Animals mixing during grazing	yes	19.3	-0.13	0.89	-0.71 0.51	0.68
	no	4	Ref			
Animals mixing at watering points	Yes	21.6	0.05	1.05	-0.59 0.79	0.88
	no	1.55	Ref			
Disposal of aborted foetuses	yes	17.9	0.10	1.11	-0.47 0.75	0.739
	no	5.5	Ref			
Milking animals	Yes	23	0.15	1.16	-1.01 1.96	0.84
	no	0.4	Ref			
Drinking raw milk	Yes	11.7	0.34	1.40	-0.13 0.82	0.16
	no	11.7	Ref			
Animals housed in the family house	yes	20.4	0.79	2.20	1.02 1.45	0.02
	no	2.9	Ref			
Slaughter of animals	yes	10.6	0.07	1.07	-0.42 0.59	0.78
	no	12.8	Ref			

CHAPTER FIVE

DISCUSSION

The results from this study demonstrated that *Coxiella burnetii* has been circulating in livestock populations in this region. The seroprevalence of *Coxiella burnetii* in sheep and goats (12% and 16%, respectively) found in this study did not differ significantly with a similar study done in Western Kenya by Knobel *et al*., (2013) (18% and 32%); however, the seroprevalence was significantly lower ($P<0.05$) among cattle (5%) compared to the same study which reported a seroprevalence of 28%. A recent literature review on the prevalence of *Coxiella burnetii* infection in domestic ruminants in different countries worldwide, revealed a wide variation in reported prevalence (Guatteo *et al*., 2011). The overall mean prevalence in animals were 15% and 27% for sheep and goats, respectively (Guatteo *et al*., 2011). Out of the 69 publications reviewed, four studies on small ruminants were performed in African countries, of which one study in Chad (Central Africa) found the seroprevalence in sheep and goats to be 11.0% and 13.0%, respectively (Schelling *et al*., 2003). In recent studies done in northwestern Iran the seroprevalence among sheep was 29.4% (Sakhaee and Khalili, 2010) and the seropositivity among goats and cattle was 65.8% and 10.7%, respectively (Khalili and Sakhaee 2009).

The seroprevalence of *Coxiella burnetii* was higher in females of all species relative to males. This was similar to reports by Esmaeili *et al* (2014) though the predominant female sex hormone estradiol has been shown to have a protective effect on intracellular bacterial infections (Leone *et al.,* 2004). This may be an indication the estradiol has no protective effect on this bacterium. There were slight differences in the seroprevalence among animals sampled within the irrigation scheme and those sampled within the *manyattas* at 12% and 14%, respectively. Furthermore, the lower seroprevalence observed in the present study in the pastoral areas could

be explained by the semi extensive management conditions in which animals move during part of the year over large land surfaces thus reducing the contact between animals.

The species of the animal was a risk factor for seropositivity in this study and the odds of being seropositive for goats was 1.2 (higher risk) as compared to cattle. The reason for this observation could be the fact that cattle are moved during various times of the year over large stretches of land and this reduces contact between sick animals as opposed to goats which were always grazed around the homesteads thus increasing the chances of contracting the disease.

The age of the animals in the present study was a significant risk factor for seropositivity. The older the animals were, the higher the risk of being seropositive. This difference was especially notable between weaners and adults. Weaners were less likely to be seropositive for *Coxiella burnetii* antibodies compared to adults. Age-related *Coxiella burnetii* serological patterns have also been reported in other studies (Psaroulaki *et al*., 2006; García-Pérez *et al*., 2009). Pathogen contact tends to increase with age as a result of higher probability of contact during lifespan.

In humans, the seroprevalence of *Coxiella burnetii* at 26.8% was similar to other studies (Chang *et al*., 2009; Knobel *et al*., 2013). Studies in different sub Saharan countries on apparently healthy people have shown the seroprevalence to range between 1% and 37% (Kelly *et al*., 1993; Kobbe *et al.*, 2008) and a recent study in Tanzania found a seroprevalence of 13.5% (Prabhu *et al*., 2011). The seropravalence was higher in adults (34%) compared to adolescents and children (23% and 26% respectively) in the current study. This could be due to their cumulative risk of exposure throughout their lives. The seropravalence in males and females were 28% and 26%, respectively, an indication that there were probably equal chances of exposure to the bacterium.

There was high seroprevalence observed in this study among herdsmen (42.9%) and farmers (30.2%) compared to that observed among students (24.7%) and pastoralists (6.3%). This could have been due to the low number of herdsmen sampled which therefore tended to overestimate their prevalence in the study population. Exposure to animals and animal products has been reported to be a risk factor to *Coxiella burnetii* infection (Shelling *et al.*, 2003). Farmers had a higher chance of infection since they housed their animals within the family house thus increasing the risk of *Coxiella burnetii* infection. The lower prevalence observed among pastoralists, may have been due to the fact that the animals were housed outside of their *manyattas*. In addition, their nomadic lifestyle may have led to less chances of infection due to reduced environmental contamination.

Individuals who reported having experienced malaria and flu like symptoms within a period of two months before the study begun were also found to be seropositive for *Coxiella burnetii* antibodies in this study. This may be an indication that these patients may have been misdiagnosed and treated for the wrong ailment, or that *Coxiella burnetii* co-occurred with malaria in these subjects. This finding was similar to a study by Kunda *et al.*, (2007) who reported that during the acute stages, Q- fever can easily be misdiagnosed for malaria especially in the developing world setting which can result in the administration of the wrong treatment and hence resulting in chronic disease.

The risk factors associated with seropositivity at individual and household level were occupation, irrigation status and housing animals within the family house. Pastoralists were less likely to be seropositive for *coxiella burnetii* compared to farmers (OR, 0.15; P=0.014); while individuals who housed animals within the family house were more likely to be seropositive for *Coxiella burnetii* antibodies (OR, 1.44; P=0.02). Irrigation status was however not significant on

multivariable analysis and this could have been due to its confounding effect of whether an individual lived within the irrigation scheme or not and hence the fact that the real exposure was the occupation of the individual and not the irrigation status. A study by Maurin and Raoult, (1999) considered Q fever to be an occupational disease in farmers, abattoir workers and veterinarians. They also noted that community outbreaks around farms with infected ruminants are common especially during kidding and calving season. Psaroulaki *et al*., (2006) in their epidemiological study of Q fever in humans, ruminant animals and ticks also identified occupation as a risk factor for seropositivity to *Coxiella burnetii*. Housing animals within the family house poses a risk of inhaling infected aerosols which is a major mode of transmission (Bosnjak *et al*., 2010; Rodolakis, 2009) of Q fever from animals to humans. Both males and females were equally exposed to *Coxiella burnetii* and this study suggests that sex has no influence on the serostatus as reported by others (Steinmann *et al*., 2005).

CHAPTER SIX

CONCLUSSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Q fever was not known in the study area. Out of the 85 respondents interviewed none of them knew about the disease however they were engaged in practices that could expose them to Q fever like drinking raw milk and assisting in birthing of animals.
2. The seroprevalence of *Coxiella burnetii* in human and livestock (26.8 and 13 %, respectively) populations was high in Bura sub county, Tana River County.
3. The risk factors associated with seropositivity in humans were occupation housing animals within the family house.
4. The risk factors associated with seropositivity in livestock in this study were age, species and sex of the animal.

6.2 Recommendations

1. It would be necessary to employ appropriate strategies for diagnosis of Q- fever patients by equipping local and national laboratories and increasing the knowledge of physicians of potential clinical signs of the disease and how to make a diagnosis of the same.
2. Community education and awareness creation especially on the risk factors for Q -fever would be very helpful in preventing infection.
3. There is need for the government to have control strategies for the disease like introduction of vaccination which is currently not being carried out in the country.
4. Further research to determine the effect of land use change on tick-borne diseases in Kenya is recommended.

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APPENDICES

APPENDIX I: Questionnaire
UNIVERSITY OF NAIROBI

Q FEVER HOUSEHOLD QUESTIONNAIRE

Date: ……

Name of interviewer: ……...

INTRODUCTION AND CONSENT

NOTE TO INTERVIEWERS

For all pre-coded answers, circle the choice that applies. Where the questions is open-ended then enter the respondent's answer in the space provided.

IDENTIFICATION

Division …….. Location …….

Sub-location ……..

Village/number ……..

Name of household head……

Household head phone number……..

GPS reading ……

1. Do you know Q fever Disease? **(if No, Go to 11.)**

1.	Yes
2.	No

2. What causes Q fever in animals?

1	Floods
2	Mosquito bites
3	Other biting insects (biting midges, ticks)
4	Mixing with neighboring herds
5	Don't know
6	Others (specify)……

3. What are the clinical signs and symptoms of Q fever in animals?

1	Bloody nasal discharge
2	Abortion
3	Fever
4	High mortality
5	Diarrhoea
6	Excessive salivation
7	Don't know
8	Other (specify)..…….

4. Are there animals that have suffered from Q fever in your household? **(If No, go to 12)**

1.	Yes
2.	No

5. If yes, which animals, how many, how old and when?

Animals	**Number of Males**	**Number of Females**	**Age**	**When**
Cattle				
Sheep				
Goat				

6. When you recognized the symptoms in the animal(s), what intervention was done?

1.	Treatment
2.	Vaccination
3.	Traditional quarantine
4.	Government quarantine
5.	Slaughter
6.	Report to veterinary office
7.	Sell them before anyone notices
8	Nothing

7. **(Don't ask if any intervention is given).** If no interventions, what were the pertinent reasons?

1.	
2.	
3.	
4.	
5.	
6.	
7.	

8. What can be done to effectively prevent cattle from contracting Q fever?

1.	Spray animals with acaricides
2.	Avoid communal grazing and watering
3.	Vaccinate
4.	Avoid purchasing cattle from infected origin
5.	Burning contaminated beddings
6.	Regular removal of manure
7.	Don’t know
8.	Other (specify)

9. Which month(s) of the year are the animals at greater risk of Q fever, and why?

	Month(s)	**Reason**

1.		
2.		
3.		
4.		

10. Do your animals mix with other neighbours' animals at watering point?

1.	Yes
2.	No

11. **Do your animals mix with other neighbours' animals during grazing?**

3.	Yes
4.	No

12. **When do they mainly mix?**

1.	Dry season
2.	Wet season
3	Other (specify)

(Ask 13, 14 and 15 only if respondent knows Q fever)

13. **Does Q fever affect humans?** 1=Yes 2=No 3=don't know

14. **What causes Q fever in humans?**

(*Can be more than one*)

1= Taking raw milk

2=Tick bites

3=Contact with sick animals

4=Handling blood/tissues of infected animals

5=Handling aborted fetuses

6=Aerosol transmission

7=Taking undercooked meat

8=Don't know

Other (specify)……

15. **What are the signs and symptoms of Q fever in humans?**

(*Can be more than one*)

1=Headache

2=Pneumonia

3=Body weakness

4=Fever

5=Lack of appetite

6=Joint/muscle pain

7=Don't know

Other……..

16. **How can Q fever be prevented in humans?**

(*Can be more than one*)

1=Wear personal protective equipment when assisting in delivery of animals

2=Washing hands after touching animals

3=Boiling milk

4=Taking properly cooked meat

5=Avoiding direct contact with animals

6=Vaccinating animals

7=Don't know

Other (specify)……

17. **Is any member of your household often involved in assisting animals when giving birth?**

1. Yes
2. No

18. **Do you dispose aborted fetuses?**

1. Yes
2. No

19. **Does any member of your household sleep next to animals?**

1. Yes
2. No

20. **Does any member of your household take raw milk?**

1. Yes
2. No

APPENDIX 2: Animal sampling form

Serial Number----------Household ID----------

Date----------Sampling Team----------Team Leader-----

Name of Owner----------Mobile phone no. ----------

County----------District----------Sub-location----------Village----------

GPS Waypoint----------Latitude----------Longitude----------Altitude---

Animal ID	Species	Age	Breed	Sex	Body condition score	Vaccination history	History of any illness	Herd size	Other comments

APPENDIX 3: Human sampling form

Household ID	**Village**	**District**	**Name of the household head (HH)**	**Sex of the HH**	**Age of the HH**	**Occupation of the HH**	**No. in the household**		**Name of the subject**	**Sex of the subject**	**Age of the subject**	**Occupation of the subject**	**Disease in the last 2 months**
							Male	Female					

APPENDIX 4: Assent form for 5-12 years

Ministry of Health

Assent for (Barcode for BSR tube): _____

.... child between 5 and 12 years who is participating in a survey to determine the prevalence of Rift Valley fever and related flu-like syndromes (dengue, yellow fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever) under the project: Dynamic Drivers of Disease in Africa: Ecosystems, livestock/wildlife, health and wellbeing (DDDAC)

Name of the lead scientist: **Dr. Ian Njeru**

Name of Organization: **DDSR, Ministry of Health**

Name of Sponsor: **Ecosystem Services for Poverty Alleviation**

Project **DDDAC**

PART A: Project Information

I am _____ and I work with _____ as a researcher. We are doing research on Rift Valley fever, which occurs periodically in this district following prolonged periods of heavy rainfall. This study will screen for yellow fever, dengue fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever because we want to know the types of diseases that cause fever in children in this area. HIV/AIDS will **not** be included. The results obtained will be used to build capacity of the medical personnel from this area on diagnosis of diseases that cause fever. You have been selected randomly to participate in the study and your participation is voluntary. This means that you are free to decline participation without any penalty. We have spoken to your parent(s)/guardian and they know we are also asking you for agreement. If you are going to participate, your parent(s)/guardian also have to agree. You do not have to decide now whether or not you will participate; you can also talk to them (your parents) first before making a final decision. Irrespective of whether you choose to participate or not, you will continue receiving medical services from any clinic in this area whenever you need them; you will not be denied access nor get preferential treatment. .

I will take time to explain more about the research, please stop me whenever you need clarifications or to clarify meanings of words that you don't understand.

This work involves asking a few questions about you and your household and collecting a blood sample. We will draw about 5 ml of blood (one tea spoonful) from your arm.

The blood will then be taken to KEMRI Nairobi for laboratory analysis. Some of samples will be shipped to specialised laboratories outside the country for more analyses. However, samples will be shipped only after obtaining approval from the relevant authorities and AMREF's ethical committee. Information that will be obtained from your sample (e.g. infection status) will be kept confidential. This information will be put under lock and key and no-one will be able to see it except you, a clinician from the local hospital that would provide treatment if needed, Ian Njeru, Delia Grace and Bernard Bett. All your medical records will be identified using number codes instead of your name so that other people do not identify you with the sample results.

General information generated from this research will be shared with the community in local community meetings before it is made widely available to the public. Confidential information will not be shared. This proposal has been reviewed by the AMREF Ethical Review Board. If you wish to know more about the work, please contact **Ephy Khaemba** on phone number **020 4223375**.

Benefits from the survey

The information we collect will help us learn more about diseases with unknown fevers in this area. This will help us know what we need to do to reduce the transmission and control the diseases.

Risks of being in the survey

Drawing blood will cause some little pain as the needle goes through your skin but the pain will subside once I remove the needle. But, should you be injured as a direct result of participating in this survey, we will notify your parent(s)/guardian and you will be provided with medical care at a local government health facility for free. You will not receive any money, only medical care.

If you agree to be in the study but change your mind later, that is still OK; you can stop any time.

Will you be a part of this study? □ Yes □ No

PART II: Certificate of Assent

Parent/Guardian

I have witnessed the accurate reading of the assent form to my child, and the child has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name/ID of the parent/Guardian _____ID No. _____

Signature/thumb print of the parent/guardian _____Date _____

Witness:

I have witnessed the accurate reading of the assent form to the child, and the child has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name/ID of a witness _____ID No. _____

Signature/thumb print of the witness _____Date _____

Researcher

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Name/ID of the researcher _____ID No. _____

Signature/thumb print of the researcher _____Date _____

APPENDIX 5: Assent form for 13-17 years

Ministry of Health

Assent for (Barcode for BSR tube): _____

…. child between 13 and 17 years who is participating in a survey to determine the prevalence of Rift Valley fever and related flu-like syndromes (dengue, yellow fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever) under the project: Dynamic Drivers of Disease in Africa: Ecosystems, livestock/wildlife, health and wellbeing (DDDAC)

Name of lead scientist: **Dr. Ian Njeru**

Name of Organization: **DDSR, Ministry of Health**

Name of Sponsor: **Ecosystem Services for Poverty Alleviation**

Project **DDDAC**

PART A: Project Information

I am _____ and I work with _____ as a researcher. We are doing research on Rift Valley fever, which occurs periodically in this district following prolonged periods of heavy rainfall. This study will screen for yellow fever, dengue fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever because we want to know the types of diseases that cause fever in young people in this area. HIV/AIDS will **not** be included. The results obtained will be used to build capacity of the medical personnel from this area on diagnosis of diseases that cause fever. You have been selected randomly to participate in the study and your participation is voluntary. This means that you are free to decline participation without any penalty. We have spoken to your parent(s)/guardian and they know we are also asking you for agreement. If you are going to participate, your parent(s)/guardian also have to agree. You do not have to decide now whether or not you will participate; you can first talk to them (your parents) or any other person before making a final decision. Irrespective of whether you choose to participate or not, you will continue receiving medical services from any clinic in this area whenever you need them; you will not be denied access nor get preferential treatment. .

I will take time to explain more about the research, please stop me whenever you need clarifications or to clarify meanings of words that you don't understand.

This work involves asking a few questions about you and your household and collecting a blood sample. We will draw about 5 to 10 ml of blood (one to two tea spoonful) from your arm. The blood will then be taken to KEMRI Nairobi for laboratory analysis.

Some of samples will be shipped to specialised laboratories outside the country for more analyses. However, samples will be shipped only after obtaining approval from the relevant authorities and AMREF's ethical committee.

Information that will be obtained from your sample (e.g. infection status) will be kept confidential. This information will be put under lock and key and no-one will be able to see it except you, a clinician from the local hospital who would provide treatment if needed, Ian Njeru, Delia Grace and Bernard Bett. All your medical records will be identified using number codes instead of your name so that other people do not identify you with the sample results.

General information generated from this research will be shared with the community in local community meetings before it is made widely available to the public. Confidential information will not be shared. This proposal has been reviewed by the AMREF Ethical Committee. If you wish to know more about the work, please contact **Ephy Khaemba** on phone number **020 4223375**.

Benefits from the survey

The information we collect will help us learn more about diseases with unknown fevers that affect people in this area. This will help us know what we need to do to reduce the transmission and control the diseases.

Risks of being in the survey

Drawing blood will cause some little pain as the needle goes through your skin but the pain will subside once I remove the needle. But, should you be injured as a direct result of participating in this survey, we will notify your parent(s)/guardian and you will be provided with medical care at a local government health facility for free. You will not receive any money, only medical care.

If you agree to be in the study but change your mind later, that is still OK; you can stop any time.

Will you be a part of this study? □ Yes □ No

PART II: Certificate of Assent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research**.**

Name/ID of the participant _____

Signature/thumb print of participant _____Date _____

Witness:

I have witnessed the accurate reading of the assent form to the child, and the child has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name/ID of a witness _____ID No._____

Signature/thumb print of the witness _____Date _____

Parent/Gurdian

I have witnessed the accurate reading of the assent form to my child, and the child has had the opportunity to ask questions. I confirm that the child has given consent freely.

Name/ID of a witness _____ID No. _____

Signature/thumb print of the parent/guardian _____Date _____

Researcher

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Name/ID of the researcher _____ID No. _____

Signature/thumb print of the researcher _____Date _____

APPENDIX 6: Assent form for 18years and above

Ministry of Health

Assent for (Barcode for BSR tube): _____

…. who is participating in a survey to determine the prevalence of Rift Valley fever and related flu-like syndromes (dengue, yellow fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever) under the project: Dynamic Drivers of Disease in Africa: Ecosystems, livestock/wildlife, health and wellbeing (DDDAC)

Lead scientists: **Dr. Ian Njeru**

Name of Organization: **DDSR, Ministry of Health**

Name of Sponsor: **Ecosystem Services for Poverty Alleviation**

PART A: Project Information

I am _____ and I work with _____ as a researcher. We are doing research on Rift Valley fever, which occurs periodically in this district following prolonged periods of heavy rainfall. This study will screen for yellow fever, dengue fever, brucellosis, Q fever, malaria and Crimean-Congo haemorrhagic fever because we want to know the types of diseases that are present in this area. HIV/AIDS will **not** be included in the study. The results obtained will be used to build capacity of the medical personnel from this area on diagnosis of diseases that cause fever.

You have been selected randomly to participate in the study and your participation is voluntary. This means that you are free to decline participation without any penalty. You do not have to decide now whether or not you will participate; you can consult anyone you feel comfortable with before making the final decision. Irrespective of whether you choose to participate or not, you will continue receiving medical services from any clinic in this area whenever you need them. That means you will not be denied access nor get preferential treatment in these facilities. You can contact **Ephy Khaemba** on phone number **020 4223375** to verify the purpose of the study.

I will now take time to explain more about the research, please stop me whenever you need clarifications or to clarify meanings of words that you don't understand.

This work involves asking a few questions about you and your household and collecting a blood sample. We will draw about 10 ml of blood (two tea spoonful) from your arm. The blood will then be taken to KEMRI Nairobi for laboratory analysis. Some of samples will be shipped to specialised laboratories outside the country for more analyses. However, samples will be shipped only after obtaining approval from the relevant authorities and AMREF's ethical committee.

Information that will be obtained from your sample (e.g. infection status) will be kept confidential. This information will be put under lock and key and no-one will be able to see it except a clinician from the local hospital that would provide treatment if needed, the lead scientist: Ian Njeru, Grace Delia and Bernard Bett. All your medical records will be identified using number codes instead of your name so that other people do not identify you with the sample results. However, general information generated from this research will be shared with the community in community meetings before it is made widely available to the public. This proposal has been reviewed by the AMREF Ethical Review Board.

Benefits from the survey

The information we collect will help us learn more about diseases with unknown fevers in this area. This will help us know what we need to do to reduce the transmission and control the diseases in this area.

Risks of being in the survey

Drawing blood will cause some little pain as the needle goes through your skin but the pain will subside once I remove the needle. But, should you be injured as a direct result of participating in this survey, you will be provided with medical care at a local government health facility for free. You will not receive any money, only medical care.

If you agree to be in the study but change your mind later, that is still OK; you can stop any time.

Will you be a part of this study? □ Yes □ No

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research**.**

Name/ID of the participant _____

Signature/thumb print of participant _____Date _____

Witness:

I have witnessed the accurate reading of the consent form to the subject and the subject has had the opportunity to ask questions. I confirm that the subject has given consent freely.

Name/ID of a witness _____ID No._____

Signature/thumb print of the witness _____Date _____

Researcher

I have accurately read or witnessed the accurate reading of the consent form to the potential subject, and the subject has had the opportunity to ask questions. I confirm that the subject has given assent freely.

Name/ID of the researcher _____ID No._____

Signature/thumb print of researcher _____Date _____

APPENDIX 7: R Codes

QH.data<-read.csv(file.choose(),header=T) _import data

head(QHS.data)_Gives the first five rows of your data

summary(QH.data) _summarizes your variables

class(QH.data$Occupation) _check variable class

class(QH.data$Age)_check variable class

Occupmodel<-glm(formula=result~Occupation,family=binomial,data=QH.data)_code for running a glm

summary(Occupmodel)_Gives a summary of the model

confint(Occupmodel)_code for calculating confidence intervals

exp(estimate)_gives ORs of the coefficients

merged.data<-merge(QH.data,AnimalQFever.data,by="Household.ID")_code used to merge different data sets in R

write.table(merged.data,"D:/merged.data.txt",sep="\t")_ to view the data as an excel file. 'D' is the drive in the computer for saving the excel file

collapse<-summaryBy(nsamp+pos~Household.ID,data=merged.data2,FUN=c(sum))_command for collapsing data

ass.birthingmodel1<-glmer(pos.sum) ~ ass.birthing+offset(log(nsamp.sum))+(1|village)),family=poisson(link=log),data=Questionnaire.data,nAGQ=1)_command for poisson mixed models with outcome being a weighted count(rate)

Computation of confidence intervals in R

If in percentage (%) then 'n' becomes the denominator and 'Pos' the numerator for example 67%

n<-100

Pos<-67

prop<-pos/n

q<-1-prop

error<-qnorm(0.975)*sqrt((prop*q)/n)

lower<-prop-error_lower limit

upper<-prop+error_upper limit

lrtest(QAmodel4,QAmodel5)_ code for comparing two models to determine whether a variable can be dropped from the model

QHmodel3<-glm(formula=result~Occupation*Age.cat+Irrigation_Status,family=binomial,data=QH.data)_ code for checking for interactions