

**RISK MAPPING AND ECO –ANTHROPOLOGICAL ASSESSMENT OF
ANTHRAX IN THE UPPER ZAMBEZI BASIN**

BY

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DECLARATION

The contents of the dissertation are the author's own works. The dissertation has not previously been submitted for the award of degree to any University.

.....
Harvey Kakoma Kamboyi
.....

Date

DEDICATION

This manuscript is dedicated to my dear wife, Sylvia, and our two beautiful daughters, Chloé and Channah who allowed me to be away from them especially during the festive season when they needed me most.

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CERTIFICATE OF APPROVAL

This dissertation submitted by **Harvey Kakoma Kamboyi** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology (OHAE) at the University of Zambia.

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ABSTRACT

Anthrax is an acute and invariably fatal zoonotic disease affecting multi-species and caused by a bacteria, *Bacillus anthracis*. In Zambia, anthrax has emerged as a serious ecosystem disease resulting in mortalities across humans, livestock and wildlife. The disease has had devastating effects on tourism resulting in socio-economic destabilisation of three pristine wildlife sanctuaries, namely the upper Zambezi basin, the Lower Zambezi and the Luangwa valley. Preliminary trend analysis revealed sporadic annual outbreaks in the upper Zambezi basin. Consequently, the thrust of this study was to establish the spatial distribution of anthrax and determine ecological drivers of its recurrence, maintenance and epidemiological linkage to human activities in the study area. Particular emphasis was given to establish an epidemiological linkage between socio-cultural and economic activities of the inhabitants and the incidence of anthrax at ecosystem level.

Environmental and biological samples were collected within the livestock production and conservation areas in upper Zambezi Basin ($n=80$). Questionnaires ($n=113$) and focused group interviews ($n=4$) were conducted at independently distinct villages in terms of space and time. Interviewees admitted consuming animals that died from anthrax and consequently suffered from the cutaneous form of anthrax. They were not aware of the fatal pulmonary and gastrointestinal forms of the disease. "Hunger for meat" was the main driver of consumption of anthrax carcasses. Dressing of anthrax where they lay contributed to environmental contamination with anthrax spores which was a major source of primary infection for livestock and wildlife. These anthropogenic activities together with lack of knowledge about anthrax strongly intimated their contribution to anthrax recurrence and maintenance in the upper Zambezi basin.

From the samples analysed, fifteen (15) pure isolates of anthrax were obtained which were spatially distributed across four districts. Twelve, biologically plausible variables were found to be highly significant on multivariable logistic regression analysis model for questionnaires which included poor access to veterinary services (OR = 10.87; CI = 4.8-15.9; $p \leq 0.004$), herd size (OR = 10.46; CI 8.8-16; $p \leq 0.005$), anthrax carcass dressing (OR = 6.9; CI = 3.4-9.8; $p \leq 0.001$ and management system (OR = 2.57; CI = 1.3-7.5; $p \leq 0.001$).

In summary, the majority (78.7%) of anthrax outbreaks were observed in areas with low veterinary services ($\chi^2 = 8.6162$, $p \leq 0.013$) within the newly created districts of Nalolo, Mwandi and Luampa. This study has revealed that access to good quality veterinary services is critical in preventing anthrax outbreaks. We therefore highly recommend strengthening of veterinary and livestock extension services delivery and establishing a government anthrax surveillance programme for early detection, vaccination and decontamination of anthrax graves within the upper Zambezi basin. In that effect, there is need to embark on public health awareness campaigns aimed at promoting active participation of the general public in the control of anthrax in the identified hotspots and their surrounding areas.

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LIST OF ABBREVIATIONS

BSL:	Biosafety Level
BA:	<i>Bacillus anthracis</i>
CAP:	Capsule
CFSPH:	Centre for Food Security and Public Health
dATP:	Deoxyadenosine triphosphate
dCTP:	Deoxycytidine triphosphate
dGTP:	Deoxyguanosine triphosphate
DNA:	Deoxyribonucleic acid
dNTPs:	Deoxynucleotide triphosphates
dTTP:	Deoxythiamidine triphosphate
EDTA:	Ethylenediaminetetraacetic acid
EF :	Edema Factor
FAO :	Food and Agriculture Organization
FGD:	Focus Group Discussion
GPS:	Global Positioning System
HS:	Heat stable
ID:	Identity
IUCN:	International Union for Conservation of Nature

KAZA TFCA:	Kavango-Zambezi Transfrontier Conservation Area
LT:	Lethal Toxin
NALEIC :	National Livestock epidemiology and Information Centre
O.B :	Out Break
OIE :	World Organisation for Animal Health
PA :	Protective Antigen
PI:	Principle Investigator
PCR :	Polymerase Chain Reaction
RAs :	Research Assistants
TAE :	Tris-acetate-EDTA
USAF :	United States Air Force
UV :	Ultra Violet
WHO :	World Health Organization
ZAWA :	Zambia Wildlife Authority

CHAPTER ONE

1.0 INTRODUCTION

Anthrax is an acute febrile disease of virtually all warm-blooded animals, including man, caused by a gram positive, non-motile, spore-forming bacterium called *Bacillus anthracis*. When this multi-host pathogen is discharged from an infected animal or when an opened carcass exposes the bacilli to free oxygen, they form spores that are resistant to extremes of temperature, chemical disinfectants, and desiccation (The Merck Veterinary Manual, 1986). The spores can remain infective in soil for many years and during this time, they are a potential source of infection for grazing livestock, but generally do not represent a direct infection risk for humans (Turnbull *et al.*, 1998). Grazing animals may become infected when they ingest sufficient quantities of these spores from the soil. Feed contaminated with bone or blood meal from infected animals can serve as a source of infection for livestock, as can hay that is heavily contaminated with infected soil (The Merck Veterinary Manual, 1986). Raw or poorly cooked contaminated meat is a source of infection for carnivores and omnivores. Anthrax resulting from contaminated meat consumption has been reported in pigs, dogs, cats, mink, wild carnivores, and humans (The Merck Veterinary Manual, 2014).

In Zambia, anthrax has emerged as a serious ecological disease in livestock and wildlife with a devastating effect on the ecotourism and a potential threat on the socio-economic viability of three pristine wildlife sanctuaries of the upper Zambezi basin, the Lower Zambezi and the Luangwa valley. Although outbreaks of the disease have occasionally been reported from different parts of the country (Tuchili *et al.*, 1993; Turnbull *et al.*, 1991; Turnbull *et al.*, 1999), the disease has increased to alarming levels in recent past (Siamudaala *et al.*, 2006). The upper Zambezi basin has reached an endemic status of anthrax which means the area has an ecological threshold ideal for the appropriate mix of suitable parameters that favor the

maintenance and transmission of anthrax pathogen to susceptible population existing within the ecological habitat. For the period 1999 – 2007, Munang'andu *et al.*, (2012), reported a total of 1,216 bovine cases and 1,790 human cases with a human case fatality rate of 4.63% (83/1,790) in the Zambezi flood plain. Human cases were highly correlated with cattle cases ($r=0.94$). The initiation of outbreaks of anthrax depends on interrelated factors which include specific properties of the bacterium, environmental factors, and factors affecting the dissemination of the organism, animal densities and certain human activities.

1.1. STUDY JUSTIFICATION

Anthrax is re-emerging as a serious ecosystem disease with mortalities being recorded in humans, livestock and wildlife (Blackburn *et al.*, 2007; Hampson *et al.*, 2011; Siamudaala *et al.*, 2006). It has proved to have a devastating effect on ecotourism and a potential threat on socio-economic viability of three pristine wildlife sanctuaries. Initial trend analysis revealed sporadic annual outbreaks in the upper Zambezi basin. Consequently, the thrust of this study was to determine ecological drivers of anthrax recurrence, its maintenance and epidemiological linkage to anthropological activities in the upper Zambezi basin. Absence of zoonotic disease investigations that address the whole ecosystem and prevailing local anthropogenic activities, have largely contributed to speculative nature of what deterministic and risk factors are at play in Zambia with regard to anthrax outbreaks. The exact spatial distribution of anthrax is also unknown, but it is generally described in terms of the ecosystems affected (Fig. 2.1). This makes it practically impossible for local authorities to adopt remedial activities in order to reduce the frequency of the outbreaks. The generalization of the distribution does not indicate if anthrax is restricted to a particular locality or it is

actually spreading to green zones. Therefore, it is also the thrust of this study to establish the present spatial distribution from which anthrax distribution trends will stem from.

1.2. OBJECTIVES

1.3. GENERAL OBJECTIVE

The overall objective of this study was to map and identify high risk areas and to carry out an eco-anthropogenic assessment for anthrax in the upper Zambezi basin, Zambia.

1.4. SPECIFIC OBJECTIVES

The specific objectives of this study were:

- a) To map, identify and assess anthrax risk areas in the upper Zambezi basin.
- b) To identify the ecological drivers of anthrax recurrence and maintenance and their epidemiological linkage to anthropogenic activities in the upper Zambezi basin.

1.5. STUDY HYPOTHESES

- a) There is an epidemiological linkage between anthropogenic activities and recurrence of anthrax outbreaks in the upper Zambezi basin
- b) Precise spatial distribution of anthrax in the upper Zambezi basin is unknown.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. AETIOLOGY

Anthrax is a per-acute to acute multi-specie zoonotic disease caused by the gram positive spore-forming rod shaped bacterium called *Bacillus anthracis* (Hampson *et al.* 2011). Anthrax is known by many names around the world including charbon, woolsorters disease, ragpickers disease, malignant carbuncle, malignant pustule and Siberian ulcer (OIE, 2012). According to the CFSPH, 2007), *B. anthracis* has been found to be genetically very homogeneous; however, researchers have identified several genetically distinct groups that appear to be derived from clones. Some of these clones are distributed worldwide, while others are found in limited geographic areas. *B. anthracis* is a member of the *Bacillus cereus* group, which also contains *B. cereus* and *B. thuringiensis* (Coffin *et al.*, 2015; Pilo and Frey, 2011). These three organisms are very closely related. Based on genetic analysis, some authors consider them to be a single species; however, this idea is controversial (CFSPH, 2007). The differences in pathogenicity among the three species are mainly encoded on plasmids. *B. anthracis* possesses two plasmids, pXO1 and pXO2, both essential for virulence, that carry genes for toxin synthesis and capsule synthesis (Volokhov *et al.*, 2004; Klee *et al.*, 2006). A plasmid is a small circular DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. Typically, they are most commonly found in the cytoplasm of bacteria (Wikipedia, 2015). Plasmid pXO1 encodes two toxins: lethal factor (LF) and oedema factor (EF). These toxins share the same adhesion subunit, known as protective antigen (PA), that binds to the cellular receptors (Bradley *et al.*, 2001; Pilo and Frey, 2011). After binding, PA is cleaved by furin and oligomerizes and binds LF or EF for transport into cells via membrane lipid rafts (Abrami *et al.*, 2003). Once inside the cell, LF

exerts its toxic activity causing cell lysis or induction of pro-inflammatory cytokines leading to vascular collapse, shock and death (Moayeri *et al.*, 2003). The plasmid pXO2 encodes the enzymes that synthesize the poly-D-glutamic acid capsule. This virulence factor is necessary for survival in macrophages, which is a crucial step in the course of disease (Pilo and Frey, 2011). This allows *B. anthracis* to virtually multiply in the host without being impeded by the immune system (Abrami *et al.*, 2005). The poly-D-glutamic acid capsule and the two anthrax toxins are the key phenotypes distinguishing *B. anthracis* from closely related species (Pilo and Frey, 2011).

2.2 . EPIDEMIOLOGY

Although *B. anthracis* can be found worldwide, anthrax cases usually occur only in limited geographic regions. The global distribution of anthrax is largely determined by alkaline, calcareous soil with a pH above 6.1, a warm environment, and periodic episodes of flooding. ‘Anthrax seasons’ are characterized by hot-dry weather which stresses animals and reduces their innate resistance to infection allowing low doses of spores to be infective (CFSPH, 2007; Hugh-Jones and Blackburn, 2009). Anthrax is particularly common in parts of Africa, Asia and the Middle East. In Sub-Saharan Africa, where the disease is endemic (Hampson *et al.*, 2011) , it has taken up a seasonal trend; its incidence in any one place is related to the temperature, rain or drought. However conditions which predispose to outbreaks vary widely from location to location. Climate probably acts directly or indirectly by influencing the way in which the animal comes into contact with spores (for example, grazing closer to the soil in dry periods when the grass is short and sparse or movement of herds into restricted sites when water becomes scarce), or affecting the general state of health of the hosts and thereby affecting their level of resistance to infection (Turnbull *et. al.*, 1998). Much of the

literature addresses the effects of rainfall, temperature, season, soil, vegetation, host condition and population density on the epidemiology of anthrax, but little agreement exists on the roles played by these factors in the incidence of the disease. Most of the theories are based on concepts of conditions under which dormant *Bacillus anthracis* spores may germinate and multiply in the environment, but hard scientific supportive data are not readily available (Blackburn *et al.*, 2007; Turnbull *et al.* 1998).

Anthrax in Zambia dates as far back as 1914 particularly in Luambe National Park. In wildlife no official report was made until in 1987 when the disease covered most of the Luangwa Valley and wiped out most of the animals particularly the *Hippopotamus amphibious* (hippo), *Loxodonta Africana* (elephant), *Equus quagga burchellii* (zebra) and *Aepyceros melampus* (impala) species (Siamudaala *et al.*, 2005). In 2011 it was reported in the Lower Zambezi National Park where it affected in hippo, elephant, *Syncerus caffer* (buffalo), *Tragelaphus strepsiceros* (greater kudu) and *Panthera leo* (lion) species (Zambia Wildlife Authority (ZAWA), 2011) and in Musalangu game management area of the North Luangwa National Park and Chama district where it did not only cause mortalities in hippo species but also human populations (Hang'ombe *et al.*, 2012). In November 2013, anthrax broke out in Luampa district of western province where humans were affected with dozens hospitalized due to exposure to diseased cattle tissues through consumption of contaminated meat. From literature, three ecological anthrax hotspots have been identified in Zambia by the Ministry of Livestock and Fisheries National Livestock and Epidemiology information Centre. These include the upper Zambezi basin, Lower Zambezi valley and the Luangwa valley as depicted in figure 2.1 (Mataa, 2015).

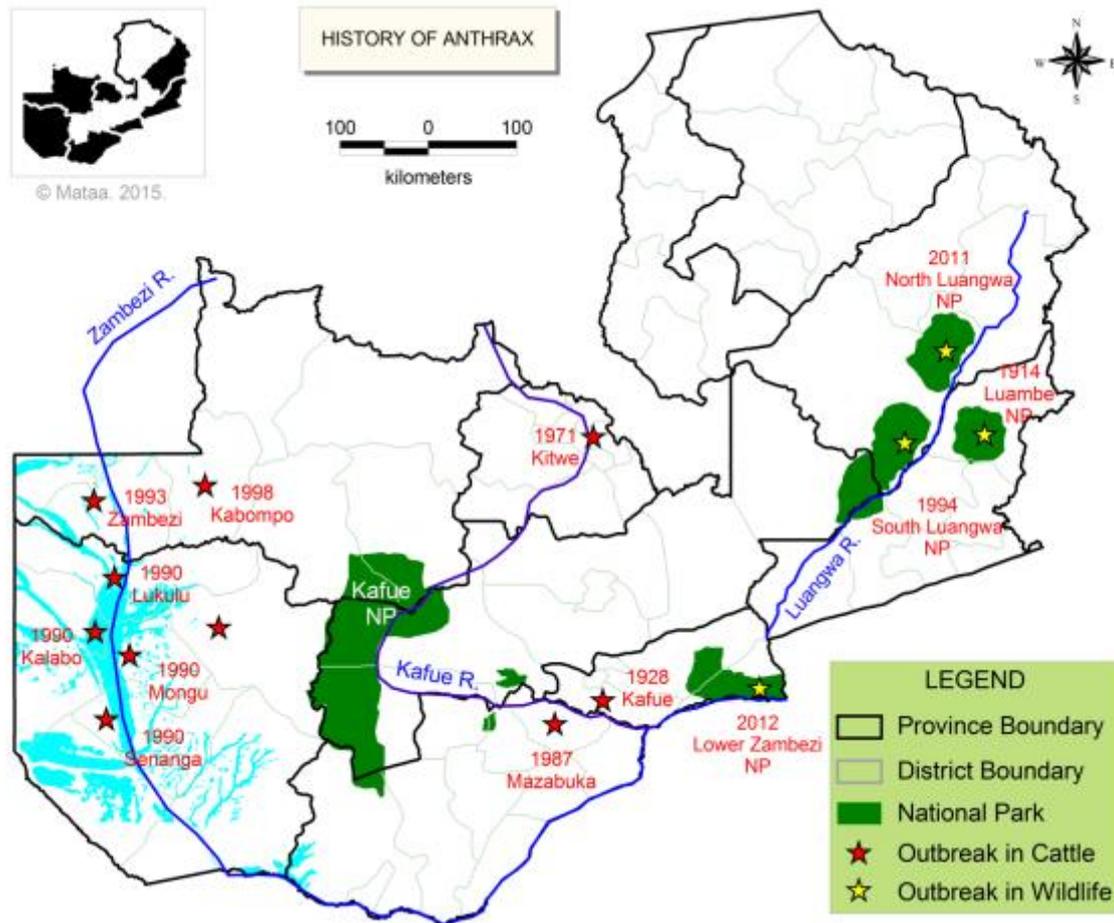


Figure 2.1: History of anthrax in three pristine ecological hotspots in Zambia (Mataa 2015)

Fluctuations in the ecological parameters such as increase in the susceptible livestock population, transhumance grazing system, seasonal anthropogenic pressure, poor public awareness and the general lack of systematic intervention program may have led to a cyclical trend in the recurrence of anthrax outbreaks on the upper Zambezi flood plains (Turnbull *et al.*, 1991). However, despite the varying views on the trigger mechanism of the disease mainly in the Luangwa valley and more recently in the Lower Zambezi valley, the reservoir for the bacteria that causes the disease is suggested to be environmental (soil borne) (Siamudala *et al.*, 2005).

2.3. HOST RANGE AND TRANSMISSION DYNAMICS

Anthrax is a highly contagious multi-host disease of domestic and wild animals to which man is susceptible. It is primarily a disease of herbivores, its occurrence in dogs scavenging anthrax carcasses and in carnivorous animals in zoological gardens and wildlife sanctuaries or parks are not entirely uncommon (Turnbull *et al.*, 1998). In livestock, ruminants such as cattle, sheep, and goats are the most susceptible and commonly affected, followed by horses, and then swine (Siamudala *et al.*, 2006). In wild animals, Lembo *et al.*, 2011, observed a high seroprevalence among carnivores suggested regular nonfatal exposure. Seropositive wildebeest and buffalo showed that infection was not invariably fatal among herbivores, whereas absence of seropositivity in zebras and frequent detection of fatal cases indicated high susceptibility.

In the host, the bacterium is often found in the vegetative form but on exposure to air from a carcass, it forms highly resistant spores that can remain viable for many years in some soils (de Vos, 1990) and therefore serves as a source of infection to grazing herbivores. In animals, transmission occurs by ingestion and possibly inhalation of spores, although entry through skin lesions has not been ruled out. Herbivores usually become infected when they ingest sufficient numbers of spores in soil or on plants in pastures (Turner *et al.*, 2014). Outbreaks are often associated with heavy rainfall, flooding or drought (Shiferaw *et al.*, 2002). The environmental factors at play for the precipitation of outbreaks remain unclear and but may vary from one location to another. Outbreaks associated with droughts (Prins and Weyerhaeuser, 1987; de Vos, 1990; Turnbull *et al.*, 1991; Lindeque and Turnbull, 1994; de Vos and Bryden, 1996; Bryden, 1999; Pollack, 1999; Smith *et al.*, 1999; Shiferaw *et al.*, 2002; Barrett, 2006; Dudley, 2006; Clegg *et al.*, 2007; Muoria *et al.*, 2007; Wafula *et al.*, 2008) have been suggested to result from animals congregating in areas where common pool resources (pasture and water) are diminishing leading to spore ingestion or inhalation (Dragon

and Rennie, 1995; Beyer and Turnbull, 2009) or contamination of limited water resources (Clegg *et al.*, 2007; Turner *et al.*, 2013). Alternatively, climate may indirectly affect animal health; hot and dry conditions may lower host immunity (Hugh-Jones and Blackburn, 2009), thereby increasing infection probabilities (de Vos, 1990; Turner *et al.*, 2014).

Contaminated bone meal and other feed can also spread this disease (CFSPH, 2007). The major sources of human anthrax infection are direct or indirect contact with infected animals, or occupational exposure to infected or contaminated animal products (Turnbull *et al.*, 1998). Carnivores get the disease from eating carcasses of animals that have died of anthrax. Vultures and flies may disseminate anthrax mechanically after feeding on carcasses (CFSPH, 2007). After the spores enter an animal, they germinate, changing from the spore resistant form into the growing and dividing vegetative form which multiplies rapidly and cause disease. Figure 2.2 below illustrates anthrax ecology and transmission cycle.

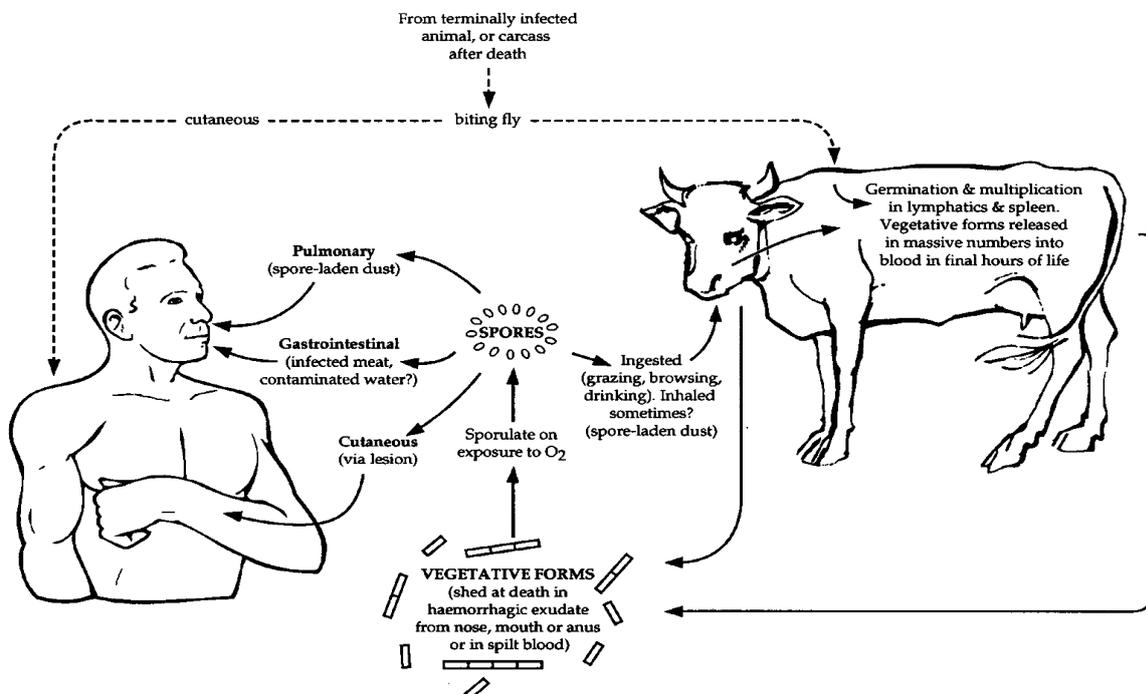


Figure 2.2: Cycle of infection of anthrax between man and livestock (Turnbull *et al.*, 1998)

The spore is central to the cycle, although infection can also be acquired through uptake of the vegetative forms when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease (Turnbull *et al.*, 1998).

Anthrax is a disease of high pathogenicity and high virulence mainly owed to two factors which are encoded on the plasmids, pXO1 and pXO2 (Okinaka *et al.*, 1999; Volokhov *et al.*, 2004; Klee *et al.*, 2006). The pXO2 encodes proteins required for the production of a poly- D- glutamyl capsule, which mediates the invasive stage of the infection in the host. The capsule inhibits macrophages from engulfing and destroying the vegetative cells, impeding the host's immune response (Fang *et al.*, 2005; Paccani, 2005; Fang *et al.*, 2006). Thus the capsule allows virulent anthrax bacilli to grow virtually unimpeded in the infected host. The virulent plasmid pXO1 encodes for production of the multi-component anthrax toxin which mediates the toxigenic stage (Turnbull *et al.*, 1998; Lixin *et al.*, 2007). The multi-component anthrax toxin consists of three polypeptides; the protective antigen (PA) which binds to cellular receptors, cleaved by cellular furin, oligomerizes, and transports the lethal factor (LF, a protease) and edema factor (EF, an adenylcyclase that inhibits the immune response, including phagocytosis by macrophages) into cells. The lethal toxin (LT, the combination of PA and LF) and edema toxin (ET, the combination of PA and EF) are sufficient to produce many of the symptoms of anthrax infection (Klein *et al.*, 1962; Moayeri *et al.*, 2003; Moayeri *et al.*, 2004; Lixin *et al.*, 2007). The endothelial cell linings of the capillary network may also be susceptible to lethal toxin and the resulting histologically visible necrosis of lymphatic elements and blood vessel walls is presumably responsible for systemic release of the bacilli and for the characteristic terminal hemorrhage from the nose, mouth and anus of the victim (Turnbull *et al.*, 1998).

2.4. CLINICAL SIGNS

Herbivores such as cattle, sheep, goats and most wild ruminants mainly manifest peracute and acute symptoms. The course of the peracute clinical disease is usually less than 2 hours and the acute form less than 72 hours (de Vos, 2002). Clinical manifestations to look for in herbivores include sudden death with incomplete rigor mortis and oozing of dark unclotted or partially clotted blood or blood-stained fluid from the natural body openings, subcutaneous hemorrhages (Dragon *et al.*, 1999; Hampson *et al.*, 2011; Coffin *et al.*, 2014) and severe bloating with extended limbs (de Vos, 2002) (Fig. 3A). If an anthrax carcass is opened, dark unclotted blood and an enlarged, haemorrhagic spleen are immediate indicators of anthrax (de Vos, 1994). However, an enlarged spleen (splenomegaly), cited as a characteristic feature of anthrax and regularly seen in cattle, is uncommon in sheep, pigs and horses (Animal Health Australia, 2005). Pigs, carnivores, primates in which the disease is subacute to chronic (de Vos, 2002), usually have local edemas and swelling of face and neck or of lymph nodes (Fig. 2.3B), particularly mandibular and pharyngeal and/or mesenteric (Turnbull *et al.*, 1998; de Vos, 2002).



Figure 3.3: Hippo anthrax carcass in Zambezi river and edematous swelling of the neck and face of a lioness in Lower Zambezi (Lower Zambezi anthrax outbreak in 2012).

Anthrax in humans occurs in three forms reflecting the route by which the disease was acquired. It is distinguished into **cutaneous anthrax** acquired through a skin lesion, **gastrointestinal tract anthrax** contracted from ingestion of anthrax contaminated food, primarily meat from an animal that died of the disease, or conceivably from ingestion of contaminated water and **pulmonary (inhalation) anthrax** from breathing in airborne anthrax spores (Turnbull *et al.*, 1998; Mock and Fouet, 2001; Siamudaala *et al.*, 2005; Animal Health Australia, 2005; Xu and Frucht, 2007; Munang'andu *et al.*, 2012; Turner *et al.*, 2013).

Cutaneous anthrax is said to account for 95% or more of human cases globally. All three forms, cutaneous, gastro.intestinal tract and pulmonary, are potentially fatal if untreated, but the cutaneous form is often self-limiting (Turnbull *et al.*, 1998; FAO, 2014). According to the United States Air Force (USAF) Public Health Information and Resources (2014), cutaneous anthrax ("malignant pustule") accounts for the majority of the cases in naturally acquired infections, it can be self-limiting but 20% of patients develop septicemia (Franz *et al.*, 1997; Tomaso *et al.*, 2006). An erythematous papule appears on an exposed area of broken skin which becomes vesicular, with a purple to black center. The center of the lesion finally forms a necrotic eschar and sloughs (Munang'andu *et al.*, 2012). Regional adenopathy, fever, malaise, headache, and nausea and vomiting may be present. After the eschar sloughs, hematogenous spread and sepsis may occur, resulting in shock, cyanosis, sweating, and collapse. Hemorrhagic meningitis may also occur with 20% fatality if not treated (Xu and Frucht, 2007).

The pulmonary form ("wool sorter's disease") occurs following inhalation of spores from hides, bristles, or wool. It has an incubation period of 1 to 5 days. It is characterized by fever, cough, dyspnea, respiratory failure and death in 24 hours (Turnbull *et al.*, 1998; Siamudaala *et al.*, 2006).

The gastrointestinal form which may exert a substantial burden, it is both underreported and under-diagnosed. It is contracted from the consumption of of handling and consumption of inadequately cooked products from infected animals (Sirisanthana and Brown, 2002. In countries where hunger is a serious problem, ingestion of contaminated meat is more of a risk (Sirisanthana and Brown, 2002. The intestinal form has an incubation period of 12 hours to 5 days, and is characterized by anorexia, vomiting and diarrhea (may be hemorrhagic) (USAF Public Health Information and Resources, 2014). Gastrointestinal and inhalational anthrax commonly progress to fatal bacteremia and toxemia with a mortality rate greater than 80% (Tomaso, 2006).

2.5. TREATMENT, PREVENTION AND CONTROL

Anthrax control measures are aimed at breaking the cycle of infection and basically consist of disease surveillance, prophylactic procedures and disease regulatory actions which include quarantine, immunisation, treatment, proper disposal of carcasses and disinfection (de Vos, 2002). Prompt and timely antibiotic therapy usually results in dramatic recovery of the individual or animal infected with anthrax (Vogler *et al.*, 2002; Price *et al.*, 2003; Coffin *et al.*, 2015). Almost all isolates of *B. anthracis* can be expected to be highly sensitive to penicillin (Lindeque *et al.*, 1994; Slamti *et al.*, 2004; Klee *et al.*, 2006; Ross *et al.*, 2009; Habrun, 2011; Pilo *et al.*, 2011; Hang'ombe *et al.*, 2012) and, being cheap and readily available in most parts of the world, this remains the basis of treatment schedules in both animals and in humans in developing countries (Turnbull *et al.*, 1998). The organism is also sensitive to numerous other broad spectrum antibiotics. Should the use of penicillin be contraindicated, a wide range of alternative choices exist from among the aminoglycosides, macrolides, quinolones and tetracyclines (Athamna *et al.*, 2004; Habrun *et al.*, 2011. Chloramphenicol is also a satisfactory alternative (Turnbull *et al.*, 1998). Following the first incident of anthrax in a herd, the remaining animals should be moved immediately from the

field or area where the index case was found . Any animal showing anthrax signs should be separated from the herd and given immediate treatment (WHO, 2008). It should be remembered that vaccination and treatment should not be done simultaneously; treatment will prevent the live vaccine taking effect. Prohibit contact of humans with infected animals and their products; establish environmental and personal hygiene (ventilation and protective clothing) where a special risk exists (Animal Health Australia, 2005). In endemic areas, or if there is concern that the outbreak may spread, the herd should be vaccinated.

Decontamination of the site(s) where the index case or other case(s) died should be carried out using 10% formaldehyde or 4% glutaraldehyde (pH 8.0–8.5) with at least two hours exposure time (Turnbull *et al.*, 1998; Animal Health Australia, 2005). Anthrax carcasses must be destroyed by burning, removal for rendering or, as a last resort, deep burial after disinfection, preferably with 10% formalin. If carcass disposal has to be delayed, the carcass and surrounding ground should be disinfected with 10% formalin and the carcass covered with strong plastic to prevent access of scavengers (WHO, 2008). Disposal should be done at site by burial in quick lime where wood fuel is scarce or by complete burning of the carcass in a pit (Turnbull *et al.*, 1998).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY AREA

This study was carried out in the upper Zambezi basin also called the Barotse floodplain in Western Province which lies $14^{\circ}19'-16^{\circ}32'S$ and $23^{\circ}15'-23^{\circ}33'E$ and covering about $5,500\text{km}^2$ in extent (IUCN, 2003). The study area includes part of the Kavango-Zambezi Trans-Frontier Conservation Area (KAZA TFCA). The districts covered included Sioma Ngwezi, Nalolo, Senanga, Sesheke, Mwandu, Mulobezi and Luampa.

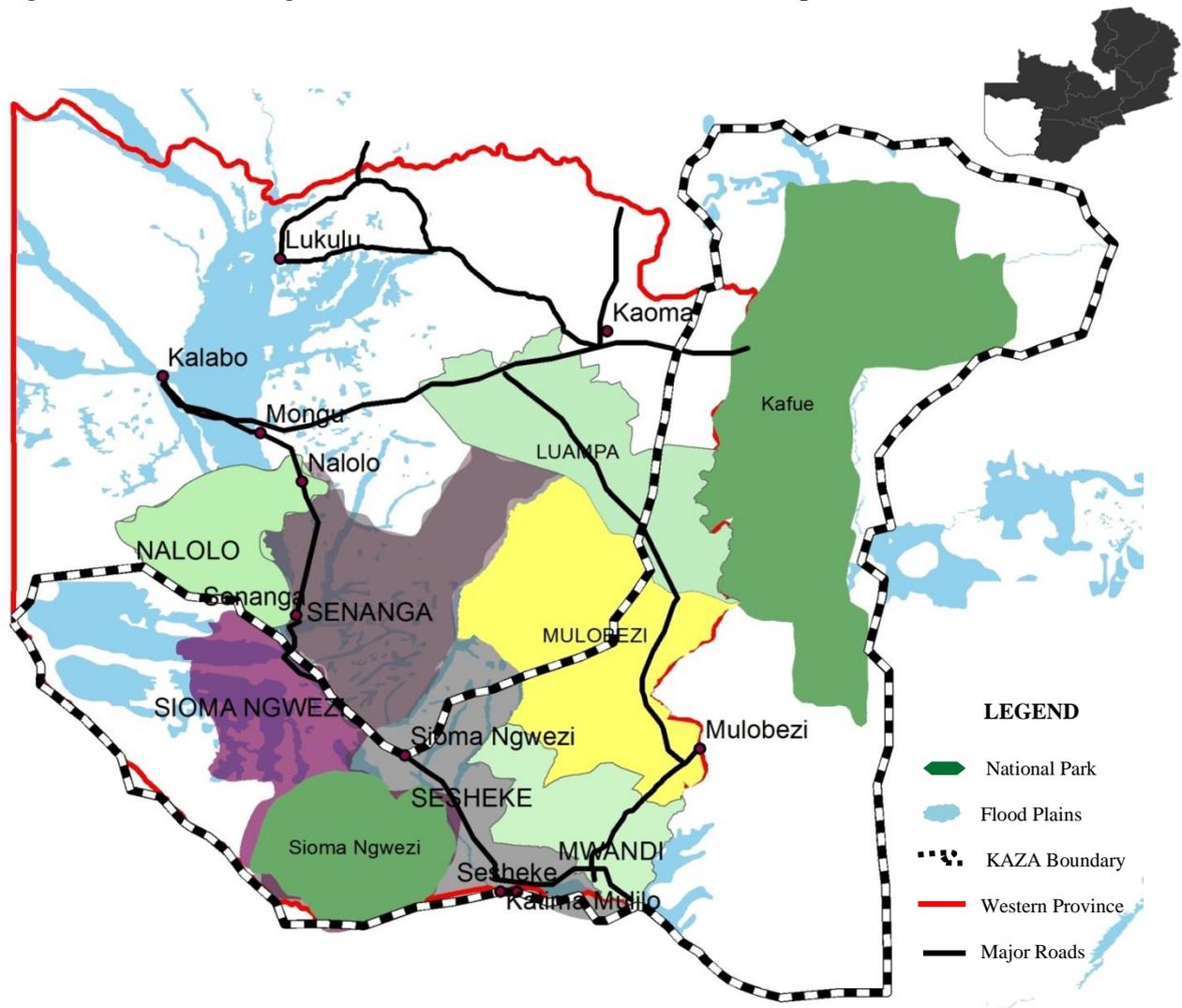


Figure 3.1: Map of Western Province of Zambia showing the study area with important physical features.

The major human activities in these districts are traditional cattle farming, fishing and rice farming and to a lesser extent, maize farming in the upper lands. The cattle population in the study area is estimated at 400,000 as of 2014 according to the National Livestock Epidemiology and Information Centre (NALEIC) a unit of the Department of Veterinary Services mandated to coordinate surveillance and information processing activities.

The maximum flooded area is estimated at 10,750km² (Welcomme, 1975) when floods of all tributaries of the Zambezi river are taken into account (Fig. 3.1). The floodplain stretches from the confluence of the Lungwebungu River with the Zambezi River in the north extending southwards for a distance of 250km until Ngonye falls (Fig. 3.1). On traversing the plain the river meanders so much that its length exceeds 590 km. Soils are composed of the Kalahari sands stretching several meters deep underlain by calcareous rocks. Elsewhere calcareous soils have been associated with prolonged survival of anthrax spores (Hugh-Jones and Hussain, 1975; New *et al.*, 2002).

3.2 STUDY DESIGN

This study utilized both cross-sectional and retrospective cohort studies. Under cross sectional study, opportunistic biological sampling of carcasses and/or their remains, active environmental sampling soil near carcasses and watering points and use of pre-tested structured questionnaires were done. The retrospective cohort component relied on available metadata based on records for the period 2004 up to 2014. Records of anthrax cases in livestock and wildlife were obtained from annual reports and/or case files provided by the Provincial Veterinary Office for Western Province and the Sioma Ngwezi National Park Area Warden (Zambia Wildlife Authority) respectively. Questionnaires were also utilized to obtain retrospective information on anthrax cases in livestock directly from individual farmers as baseline data indicated sparsely distributed veterinary/livestock officers and poor surveillance

system in the study areas, therefore, the officers may not have accurate data and most livestock cases go without being reported (Munang'andu *et al.*, 2012).

3.3 SAMPLE SIZE

Selecting the correct sample size from a large population was imperative in order to avoid research and/or inference bias. To have statistically amenable results, the required sample size for questionnaire surveys was based on the following calculations (Stockwell and Peterson, 2002):

Assumption: 50% of the sample has the specified attributes given the high cattle anthrax prevalence during the hot dry season in the study area and that the exact prevalence of the disease is unknown.

The formula that was used in calculating the sample size was as follows:

$$n = p\% \times q\% \times \left(\frac{Z}{e\%}\right)^2$$

Where;

n is the minimum sample size required

p% is the proportion belonging to the specified category

q% is the proportion not belonging to the specified category

Z is the value corresponding to the level of confidence required

e% is the margin of error required

Applying the formula for sample size:

$$n = 50 \times 50 \times \left(\frac{1.96}{5}\right)^2 = 2500 \times (0.392)^2 = 384.16 = \mathbf{384}$$

Then adjusting for the finite population:

$$n' = \frac{n}{1 + \left(\frac{n}{N}\right)}$$

Where;

n' is the adjusted minimum sample size, the total finite population to be sampled.

n is the minimum sample size (as calculated above).

N is the total cattle population of the anthrax cases, according to previous records for the period 2004 to 2007 within study area drained by the upper Zambezi Basin (Munang'andu *et al*, 2012). Cattle population was used in the study since Munang'andu *et al.*, 2012 concluded that anthrax outbreak in humans was highly correlated ($r = 0.94$) with that in cattle. Given that the gastrointestinal and probably the pulmonary forms in humans were misdiagnosed, therefore, the human data was inaccurate to be used in the calculation of sample size.

$$n' = \frac{n}{1 + \left(\frac{n}{N}\right)} = \frac{384}{1 + \left(\frac{384}{160}\right)} = \frac{384}{3.4} = 112.94118 = 113$$

Therefore, a sample size of 113 questionnaires was distributed in previously known anthrax outbreak areas which were randomly selected within the study area. The target population included all cattle farmers in the upper Zambezi basin and the study population comprised simple randomly selected villages with cattle farmers. The unit of interest was a village and the sampling unit was an individual cattle farmer in each of the selected villages.

The sample size for biological and environmental sample was not restricted because selected kraals where cattle died or where buried were sampled purposively. Anthrax being a highly

pathogenic and highly virulent infectious disease, a single case represents an outbreak; therefore, there was no need for restricting the environmental and biological samples.

3.4 BIOLOGICAL AND ENVIRONMENTAL SAMPLES

Biological samples comprised fresh carcass tissues and exudates as well as old bones and hides of animals presumed to have died of anthrax. The size of the sample ranged from the entire shaft of the long bone (femur/tibia), entire spleen to 2cm diameter of dried hide. Environmental samples included soil, dung and water specimens collected from wallows and open meadows and also from carcass disposal sites. Mostly environmental specimens were composed of a heterogeneous mixture, including soil, animal faces from carcass and vegetation near disposal sites. Both biological and environmental samples were purposively and conveniently collected in areas where cattle anthrax cases had been reported. During collection of specimens, the areas were considered contaminated and appropriate safety precautions were taken. The precautions included putting on gloves, face masks, limiting skin exposure and facing in the direction of wind (for soil/dung sampling).

Traditionally, anthrax soil sampling involves scooping of soil at the grave site. This method has low sensitivity to detect the spores because it does not take into consideration dispersal of spores from original death site by wind drifts, surface water run-off and ploughing. To overcome this shortcoming, this study utilized the transect system over and around the grave site whose radius depended on the micro-ecology of an individual sampling site. For sites that were located in open highlands, within flood plains and anthrax grave sites that had been turned into crop fields, a larger transect area of up to 400m² was covered (Fig. 3.2A). For sites in low lying areas with vegetation cover and around small water pools, a smaller transect area of up to 200m² was covered (Fig. 3.2B). Within the transect area, about 10 (small transect) to

15 (large transect) soil/dung samples were randomly collected from each grid point. This was achieved by making foot step counts.

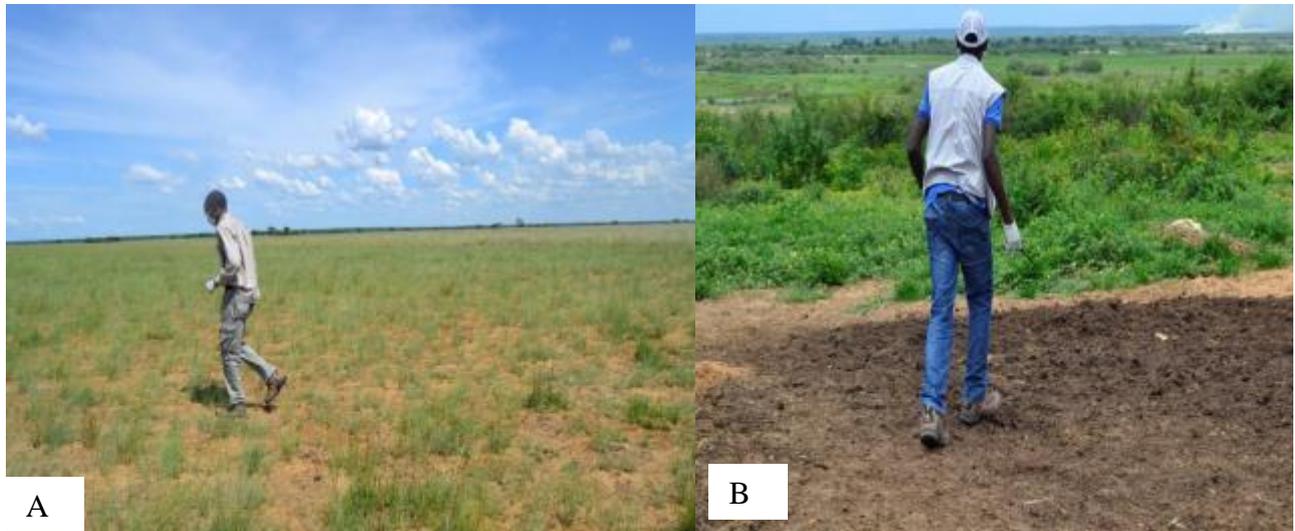


Figure 3.2: Using step counts to make transects around an identified grave site.

A minimum of 10 grams of soil/dung from each grid point was obtained from the top and 20 cm underneath the soil using a metal ladle. Each of these 10 to 15 individual heterogeneous samples from within the transect area were compounded to make one homogenous sample for that site weighing at least 200g. Global positioning system (GPS) coordinates were recorded for each of the transect area from which a compounded homogenous sample was be collected using a hand held etrex 10 Garmin® GPS. Both fresh and dry biological samples were put in sterile hermetically sealable zip-lock polystyrene bags of variable sizes. The environmental samples were put in sterile laboratory specimen tubes. Thereafter, zip-lock bags and specimen tubes containing the samples were disinfected externally using 10% formaldehyde. Several disinfected specimen tubes were packaged into sterile hermetically sealable zip-lock polystyrene bags. Sample containing zip-loc polystyrene bags were in turn packaged in the styrofoam cooler box. Fresh samples were stored at 2-8°C using a 12 volts mobile fridge. Table 3.1 below shows a summary of the number and range of the samples collected (more

details in appendix 1) while Fig 3.3 shows the samples collected and packaging. Before transportation, the styrofoam cooler box was also disinfected externally using 10% formaldehyde and then the samples were taken to the University of Zambia School of Veterinary Medicine in laboratory for isolation and further analysis.

Table 3.1: Samples collected ($n = 80$): environmental ($n = 56$) and biological ($n = 24$).

SAMPLE TYPE	Soil/dung	Water	Dry Bone	Dry Skin	Fresh Tissues
NUMBER OF SAMPLES	52	4	14	4	6



Figure 3.3: Field sampling. A and B: Grave sites in maize field. C: Water sampling in flood plains where cattle were grazing. D: Cutting a piece of cow hide. E: Disinfection of packaged sample in zip-loc polystyrene bag. F: Styrofoam cooler box used to transport samples from the field to the laboratory.

3.5 ISOLATION OF ANTHRAX

The protocol followed was as that described in the OIE terrestrial manual version adopted by the world assembly of delegates of the OIE in May 2012 with a few modifications. The following procedure was followed:

- a. Bone samples had to be crushed open so as to obtain the red marrow which was more likely to contain the spore since anthrax is a systemic disease.
- b. Then 3g of each of the 80 homogenous samples collected were put in sterile tube containing 10ml of sterile distilled water. The mixture was then placed in a water bath at 90°C for 10 minutes. According to WHO (2008) and OIE (2012) heat activation of spores can be conducted at a temperature range of 60–70°C with holding times not exceeding 15–30 minutes for best recovery. Heating of the samples also provided an opportunity for destruction of heat sensitive bacteria that may become contaminants.
- c. The heat activated samples were allowed to cool for 10 minutes before inoculation on freshly prepared blood agar media.
- d. Blood agar was prepared by suspending 40g of Blood Agar Base in 100ml of distilled water in a conical flask. The suspension was boiled to completely dissolve all the suspended particles. The solution was then sterilized at 151 bars and 121°C for 15 minutes and thereafter allowed to cool to 50°C. Then 5% v/v of sterile defibrinated sheep blood was aseptically added to the cooled Blood Agar Base solution and mixed carefully. The molten mixture was then poured onto sterile plates and allowed to solidify before inoculation.
- e. All inoculations and examinations of the colonies were done in the safety cabinet in the Biosafety Level 2 (BSL-2) in Para-clinicals Studies Department (Fig. 3.4) and Biosafety Level 3 (BSL-3) in Disease Control Department.

- f. A streak plate method was used to inoculate the samples on the blood agar plate and all plates were incubated at 37°C.
- g. Blood agar plate inoculations were examined for typical colonies for *B. anthracis* after 24 hours of incubation.
- h. Colonies that were suspected to be *B. anthracis* were sub-cultured on blood agar media for another 24 hours in order to obtain pure colonies without contaminants.
- i. Confirmation of the identity of suspect colonies as *B. anthracis* was done. Colonies that were suspected to be those of *B. anthracis* were subjected to grain stain for further validation.



Figure 3.4: Activated samples being inoculated under the biosafety cabinet.

3.6 GRAM STAINING OF SUSPECTED ANTHRAX COLONIES

The materials and reagents required for gram stain were clean glass slides, inoculating loop, Bunsen burner, distilled water, crystal violet (primary stain), gram's iodine (mordant), ethyl alcohol (decolourizer) and safranin (secondary Stain).

With a sterile cooled inoculation loop, a drop of sterile water was placed on a clean microscope slide. The inoculation loop was sterilized using the flame from the bunsen burner and cooled to pick up a very small sample of a bacterial colony and gently stirred into the drop of water on the slide to create an emulsion. The smear was allowed to air dry and then whilst holding the slide at one end, passed the entire slide through the flame of a Bunsen burner twice with the smear-side up. Heat fixing killed the bacteria on the smear, firmly adhered the smear to the slide, and allowed the sample to more readily take up stains. All of this was done under the BSL-2 biosafety cabinet for precaution. The following was the procedure for gram staining:

- i. Placed the slide with heat fixed smear on staining tray and then gently flooded smear with crystal violet which was allowed to stand for 1 minute. The crystal violet was poured off and then gently rinsed the slide with tap water.
- ii. Gently flooded the smear with gram's iodine (Fig. 3.5A) and let to stand for 1 minute. The gram's stain was poured off and then gently rinsed the slide with tap water.
- iii. Decolorized the purple appearing smear using 95% ethyl alcohol for five seconds when the alcohol ran almost clear. Immediately the alcohol was rinsed with tap water.
- iv. Gently flooded with safranin to counter-stain and let stand for 1 minute and then gently rinse with tap water.

The smear was viewed using a light-microscope initially at 10X magnification to get an idea of the location of a good area for observation and then switched to 100X magnification after

addition of immersion oil. Figure 3.5 below shows the part of the gram staining technique and viewing of the smears under the light microscope.

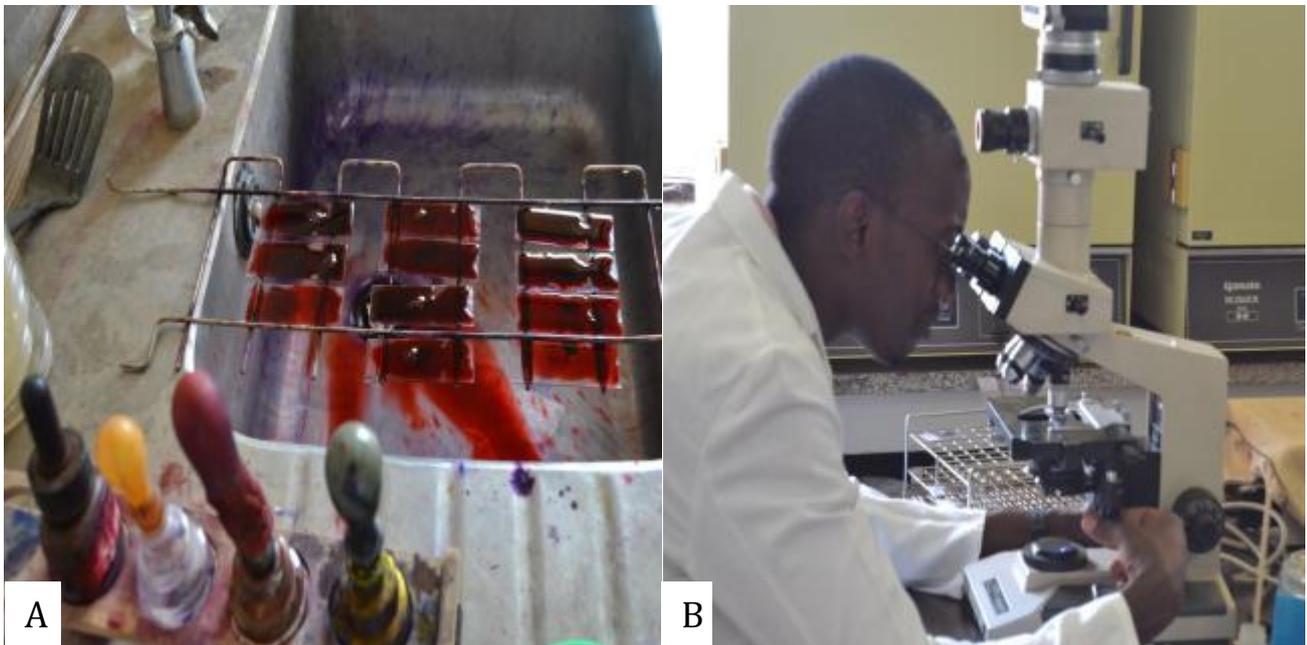


Figure 3.5A: Smears flooded with gram's iodine during gram staining. **B:** Viewing of smears using a light microscope.

3.7 MOLECULAR CONFIRMATION *B. ANTHRACIS*

3.7.1 Deoxyribonucleic Acid (DNA) Extraction

Samples suspected to be anthrax positive from colony characterization and gram stain were taken for DNA extraction for molecular confirmation. To extract DNA from colonies on blood agar, 200 μ l of milli-Q (deionized) water was put in the appendoff tube using a pipette. A flame sterilized inoculation loop was used to pick three to four suspected colonies and transferred to the appendoff tube with milli-Q water. The appendoff tubes with the samples were then vortexed to obtain a homogeneous mixture. The mixture was then heated at 95°C for 10 minutes using a dri-block bath and thereafter immediately put the tubes on ice for cooling. The cooled mixture was then centrifuged at 10,000 revolutions per minute for 2 minutes. The supernatant was taken using a micropipette and used as DNA template for polymerase chain reaction (PCR).

3.7.2 Polymerase Chain Reaction (PCR)

The PCR has not yet become a standard method for direct diagnosis of anthrax but it has become an important test for confirming the virulence of *B. anthracis* isolates (de Vos, 2002). PCR is exquisitely sensitive; it can detect extremely small amounts of genetic material. PCR was used to amplify DNA, and in the case of *B. anthracis* it was used to amplify four (4) specific genes namely:

- i. *B. anthracis* specific fragment (BA),
- ii. Toxin gene fragment - Protective antigen (PA)
- iii. Capsule gene fragment (CAP)
- iv. Bacteria specific fragment (16S)

Since multiple genes were being amplified at the same time, this type of PCR is called Multiplex-PCR. The PCR kit used was the TaKaRa Ex-taq Hot Start Version (code: RR006B: 1000U) and the thermal cycler was Applied Biosystems Veriti® 96-well.

The oligonucleotide primers – forward (F) and reverse (R) reactions used were as indicated in table 3.2 below:

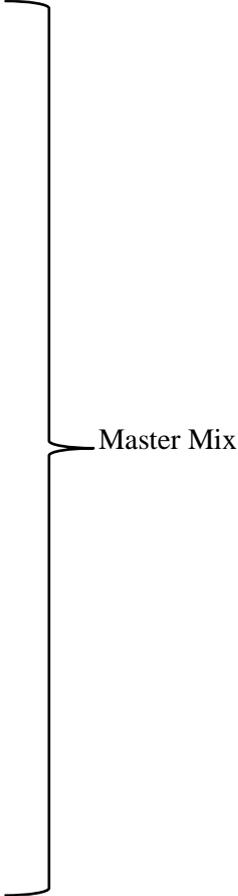
Table 3.2: Multiplex PCR primers

Name	Sequence (5' – 3')	Annealing (°C)	Expected Base Pairs
PA 5	TCC TAA CAC TAA CGA AGT CG	50	596
PA 8	GAG GTA GAA GGA TAT ACG GT		
BA813 R1	TTA ATT CAC TTG CAA CTG ATG GG	56	152
BA813 R2	AAC GAT AGC TCC TAC ATT TGG AG		
CAP 1	TAG GAG TTA CAC TGA GCC	56	341
CAP 2	TAA TGG TAA CCC TTG TCT TTG		
16S-63F	CAG GCC TAA CAC ATG CAA GTC	58.5	1324
16S-1387R	GGG GAC CAG CTC ATG GTG G	62.5	
16S-1387R2	GGG CGG AGT GTA CAA GGC	60.4	

The PCR reaction was carried out in 0.2µl microtubes with a final volume of 25µl. The multiplex PCR (Ex-taq HS) solution contained the components indicated in Table 3.3 below:

Table 3.3: Components of Multiplex PCR

SN	Component	Vol (μ l)	x36 samples (μ l)
1	10x Ex-Taq Buffer	2.5	90
2	dNTP Mix (containing 2.5mM of each)	2.0	72
3	BA 813 R1 (10 μ M) (final conc. 0.2 μ M)	0.5	18
4	BA 813 R2 (10 μ M) (final conc. 0.2 μ M)	0.5	18
5	CAP 1 (10 μ M) (final conc. 0.2 μ M)	0.5	18
6	CAP 2 (10 μ) (final conc. 0.2 μ M)	0.5	18
7	PA 5 (10 μ) (final conc. 0.2 μ M)	0.5	18
8	PA 8 (10 μ) (final conc. 0.2 μ M)	0.5	18
9	16S-63F (10 μ) (final conc. 0.2 μ M)	0.5	18
10	16S-1387 R1 (10 μ) (final conc. 0.2 μ M)	0.5	18
11	16S-1387 R2 (10 μ) (final conc. 0.2 μ M)	0.5	18
12	Distilled Water	14.875	535.5
13	Ex-Taq HS	0.125	4.5
14	DNA Template (sample)	1.0	
	Total Volume	25	864



- a) 10x Ex-taq Buffer: PCR buffer provides an optimal pH environment for the enzyme and DNA in the reaction
- b) Deoxynucleotide triphosphates (dNTP) mix: Consists of each of dATP, dCTP, dGTP and dTTP which supply the building blocks (nucleotides) for the enzyme DNA polymerase to utilize during the elongation stage of PCR.
- c) Primers: Already described in Table 3.2 above.

- d) Distilled water: Provides liquid environment in which the other components interact.
- e) Ex-taq HS: The enzyme taq (*Thermus aquaticus*) is heat stable DNA polymerase which adds dNTPs to primers for chain extension.

The master mix indicated in Table 3.3 above was prepared for the 36 reactions (34 suspected samples plus the negative and positive controls). Twenty-four microlitres (24µl) of the master mix was pipetted into each of the 36 PCR tubes (wells) and then 1µl of each of the 34 extracted DNA samples (template) was added to the master mix. In addition, 1µl of the *B. anthracis* DNA solution and distilled water were added to positive and negative control tubes respectively. The PCR tubes were then put in the 96-well thermal cycler, the PCR conditions were:

- a) 95°C for 2 minutes
- b) 95°C for 15 seconds
- c) 53°C for 30 seconds
- d) 72°C for 1 minute
- e) 72°C for 5 minutes
- f) 4°C for infinity

Steps b - d were set to ran for 35 cycles which represent the three (3) stages of the PCR namely:

1. Denaturation: 95°C for 15 seconds renders all DNA in the reaction single stranded.
2. Annealing: At 53°C for 30 seconds the primers (forward and reverse) bind to the complementary sequences of the target DNA.
3. Extension: 72°C for 1 minute allows the heat stable Ex-taq HS to use the dNTPs to synthesize DNA complementary to the template DNA.

Once the PCR was completed, the products were analyzed by electrophoresis on a 1% agarose gel stained with 0.5 μ g/ml ethidium bromide.

3.7.3 Agarose Gel Electrophoresis

The protocol for the agarose gel electrophoresis was as follows:

- a) 1.5 g of agarose powder was measure and put it into a 500 ml flask.
- b) Added 100 ml 1xTAE (Tris base-Acetate-EDTA) buffer to the flask to make the required 1.5% w/v gel.
- c) The preparation was put in the microwave for heating and repeated swirling until all the agarose powder dissolved; the solution became clear.
- d) Let the solution cool to about 50-55°C (warm to touch), and then added 1 μ l of ethidium bromide. Ethidium bromide stains the DNA so it can be see under UV light. Precautions for handling ethidium bromide were followed as it is a mutagen and therefore carcinogenic.
- e) Placed the combs in the gel casting tray and then slowly poured the molten agarose solution into the casting tray and let it to solidify on a flat surface.
- f) Carefully pulled out the combs from the hardened gel to create wells and placed the gel casting tray in the electrophoresis chamber so that the top of the gel (top = the end with the wells nearest to the edge) is at the negative (black) electrode-end of the tank.
- g) Poured enough 1xTAE buffer (use the same batch of buffer as used to create the gel) into the chamber such that the gel is completely submerged (Fig. 3.6).
- h) Mixed the 4 μ l of DNA sample (for all 36 PCR products) with the loading dye on a piece of parafilm and loaded the wells.
- i) 5 μ l of DNA ladder (Nippongene®) was also loaded onto the first well.

- j) Covered the chamber, then switched on the power supply (100 volts) and then run for 30 minutes. Thereafter, the gel was removed from the chamber and put in the ultra-violet viewing device to see the separated DNA bands.

After running the PCR for about three hours, the samples were put in solid agarose gel for separation of DNA fragments (genes) and then visualized under UV-illumination. In the solid agarose gel, the first lane had a gene ladder for estimation of gene base pairs (bp), the second lane had a negative control (-ve) which was distilled sterile water. A pure isolate of *B. anthracis* DNA was used as the positive control (+ve). The DNA samples (Sp) occupied the rest of the lanes on the gel. Positive DNA samples genes separated into four bands similar to the *B. anthracis* (positive control).

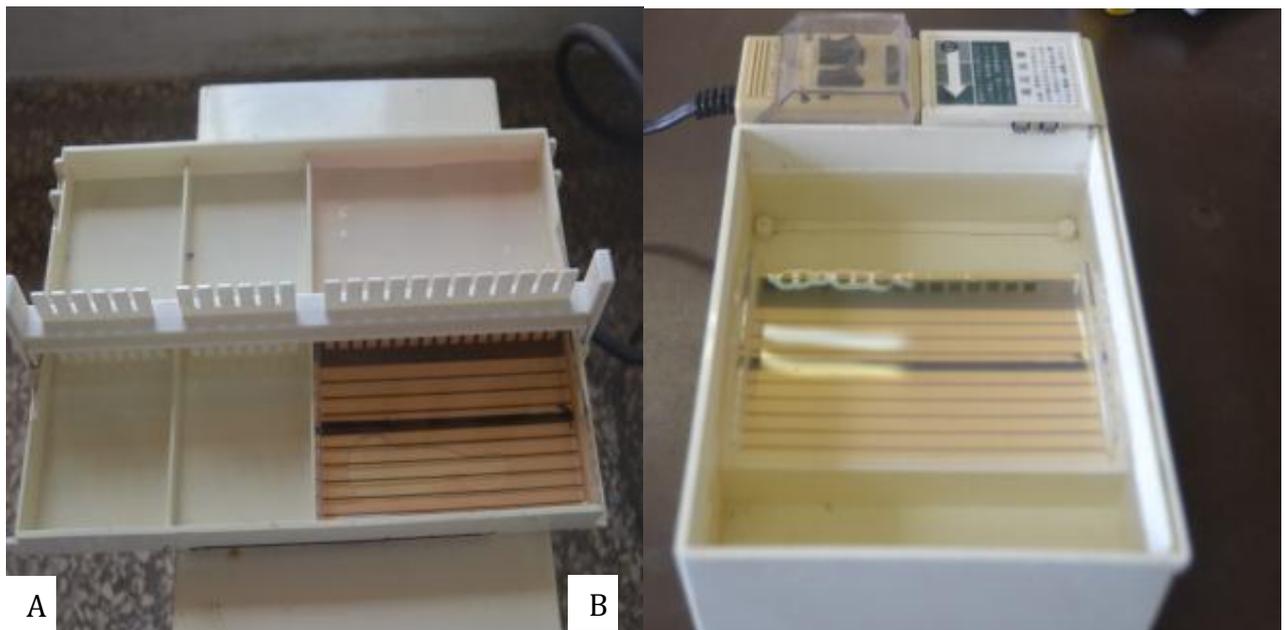




Figure 3.6A: Molten agarose gel in casting tray with combs in place. **B:** Hardened agarose gel in electrophoresis chamber submerged in 1xTAE buffer. **C:** Loading DNA samples into gel wells.

3.8 QUESTIONNAIRE SURVEY

A questionnaire survey was conducted during the data collection stage to determine the knowledge, attitudes, beliefs and opinions of the local people about anthrax that may help to explain the disease recurrence. The structured questionnaire had four (4) sections with a total of 49 closed and open-ended questions (see appendix 2). The questionnaire covered socio-demographic information, land use and livestock ownership, health problems of cattle and disease awareness and the impact. The data that was collected included among many other variables: the demographic data (i.e. age, sex, occupation, place of residence, family size), clinical information (any diagnosed anthrax, i.e. both in cattle or in humans/family members, date of diagnosis, treatment outcome), livestock owned (herd size, cattle management system) and the economic activities the people were involved in (agriculture, trade, fishing, hunting).

The questionnaire was pre-tested on a smaller scale within the study area to ensure that:

- Questions were understood by respondents,
- Questions were placed in the best order,
- All questions were necessary and sufficient and
- Instructions to interviewers were adequate.

The questionnaires were administered by the principle investigator (PI) and/ the trained research assistants (RAs) as the pre-testing exercised revealed that most of the target population could not read and understand English in which the questionnaires were printed. The role of the PI and RAs was to translate the questions into the native language, Lozi, the commonly spoken language in the study area. The RAs were fluent in English and Lozi while the PI was not as fluent in lozi.

The questionnaire sample size was determined as explained under section 4.1. A total of 113 questionnaires were distributed in villages had a history of anthrax and those without any history within the study area. This ensured that there was an unbiased evaluation of the levels of awareness about anthrax. The target population included all cattle farmers in the upper Zambezi basin and the study population comprised simple randomly selected villages with cattle farmers. The sampling unit of interest was a village and the secondary sampling unit was an individual cattle farmer in each of the selected villages. At village level, only one household was randomly selected to represent the entire village. On average, each village had about 9 households who lived as an extended family and had put together their livestock as one herd in one kraal. GPS data were also entered for each completed questionnaire; the information therein was used for the mapping of past anthrax cases. The questionnaire used in the study is attached as appendix 2.

3.9 FOCUSED GROUP DISCUSSIONS

The study conducted four focused group discussions (FDGs) within the study area: two were in Sioma Ngwezi district (Mbende and Nakapungu villages) and the other two in Mwandia districts (Njobwe and Katolo villages). The sampling unit of interest was the village which was randomly selected. The participants, drawn from the same village comprised adults and the elderly; both men and women made the cohort. The size of the group ranged from 7 to 12 participants who sat in a circle in an open environment under a tree (Fig. 3.7). The research team for the FDG comprised three members; the recorder (principle investigator), moderator (translator) and time keeper who were both trained research assistants. The major aims of discussion were to assess the levels of awareness of anthrax both in livestock and humans as well as the methods of disposal of suspected or confirmed anthrax livestock carcasses. This topic aimed at establishing and/or assessing the bio-sociocultural linkages between anthropogenic activities, past and present and occurrence as well as maintenance of anthrax in the upper Zambezi basin. The views, opinions and beliefs of the participants were recorded in the field note book which were later summarized in descriptive form after discussions were concluded. Prior to the discussions, consent was obtained from the informants to be part of the proceedings and to ensure confidentiality, they were not allowed to disclose their identities.



Figure 3.7: A focused group discussion in session at Katolo village in Mwandia district.

3.10 DATA ANALYSIS

1. Mapping

The location and elevation of all areas whose samples (biological or environmental) tested positive for anthrax on PCR and for questionnaire responses that were in the affirmative for history of anthrax in livestock. The X;Y data was first entered in Microsoft® excel as an extension package for ArcGIS 10 software (ESRI, Redlands, CA, USA). Thereafter, the X;Y data was imported from Microsoft® excel into ArcGIS version 10® to generate density maps showing the distribution of confirmed anthrax outbreaks at village level using GIS techniques. The data were geo-coded and matched to the area of occurrence using the software ArcGIS 10®. The coordinates were recorded in the format of longitude and latitude (X;Y data respectively) in decimal degrees with positional accuracy of within 4 meters.

2. Questionnaire Statistical Analysis

Raw data generated from questionnaires was initially coded and then it was entered in Microsoft® excel 2010 for storage and cleaning before statistical analysis. The excel data was then transferred to STATA™ version 13 software (StataCorp College Station, Texas 77845 USA) for quantitative analysis. The outcome of interest was occurrence of an outbreak at village level to obtain frequencies, proportions, risk factor association using chi square test and multiple logistic regression reporting odd ratios to test for causal association.

3. Focus Group Discussions (FGDs)

The contents of the interviews and observations made during FGDs were analysed to assess the levels anthrax awareness in livestock and the means of disposal of anthrax carcasses practiced at village level. At the end of the session, key statements that were recorded in lozi were translated into English with the help of the trained research assistants who understood the local language. The mode of analysis was fidelity to words spoken by the participants.

CHAPTER FOUR

4.0. RESULTS

4.1. BIOLOGICAL AND ENVIRONMENTAL SAMPLES

4.1.1 Culture

Out of the 80 samples that were collected and cultured on blood agar, 36 were suspected to be *B. anthracis* based on colony characteristics and morphology. The colonies were grey-white to white in colour and measuring 0.3–0.5 cm in diameter. They were non-haemolytic, with a ground-glass surface, and very tacky when teased with an inoculating loop. Some colonies had prominent tufts of growth trailing back towards the parent colony, all in the same direction; a characteristic feature described as “medusa head” or “curled hair” or “judge’s wig” appearance (Fig. 4.1).



Figure 4.1: Greyish white non-haemolytic colonies of *Bacillus anthracis* on blood agar.

4.1.2. Gram Stain

The samples suspected to be *B. anthracis* colonies on blood agar ($n=36$), were subjected to gram stain technique. Typical *B. anthracis* colonies were gram positive rods which sometimes

occurred in chains. The edges were sharp, a few with visible terminal endospore. Thirty-three (34) of the colonies tested positive on gram stain (Figure 4.2).

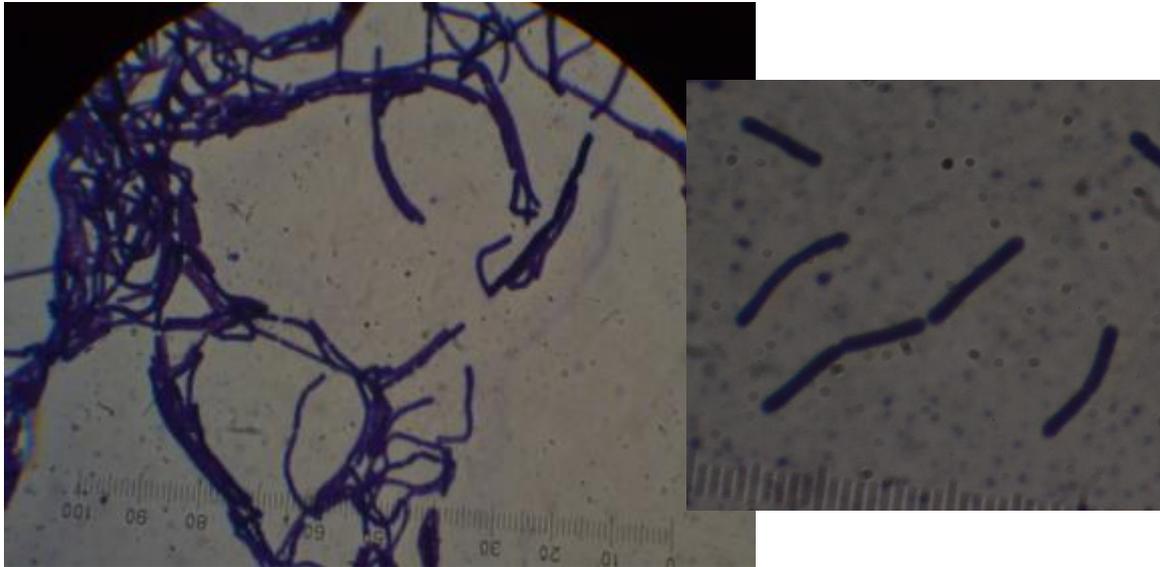


Figure 4.2: Gram positive *B. anthracis* rods at X100

4.1.3. Multiplex Polymerase Chain Reaction (MPCR)

Suspected samples that tested positive on both culture and gram stain ($n = 34$) were subjected to MPCR for confirmation. Of these, 15 were confirmed positive on MPCR (Fig. 4.3). Table 4.1 shows a summary of the distribution of samples that test positive on the three tests. Appendix 1 shows all the 80 samples and their individual test result.

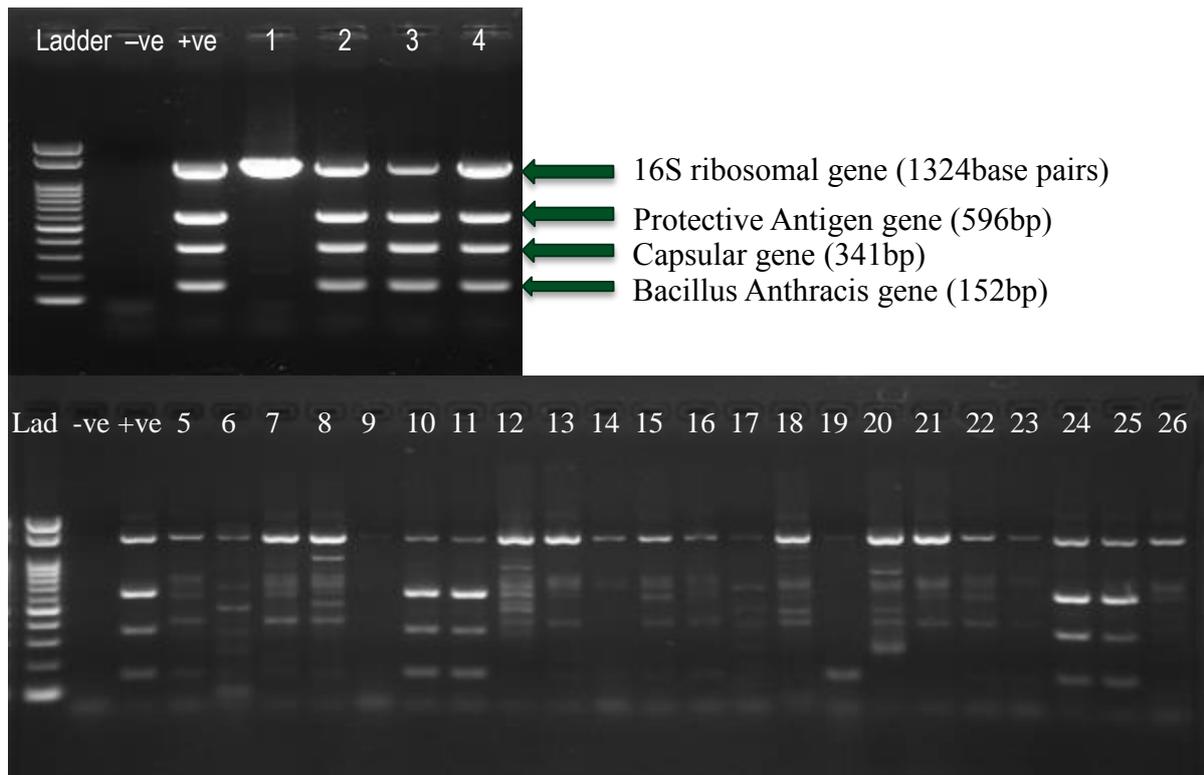


Figure 4.3: From left to right: 100 base pair ladder (Lad), negative control (-ve), positive control (+ve) and the rest are individual samples (numbers). Positive samples showing characteristic separation into four clear gene bands at the same level as the positive control. Negative samples had less or more than four unclear gene bands at different levels.

4.1.4. Mapping of Anthrax Positive Samples

The raw GIS data obtained during biological and environmental sampling (Appendix 1) and imported into ArcGIS for geoprocessing generated spatial distribution map in figure 4.4.

Table 4.1: Summary of the biological and environmental sample sources, GPS coordinates and test results. (P = Positive and N = Negative result)

SN	ID	DISTRICT	LATITUDE	LONGITUDE	SAMPLE	CULTURE	GRAM	PCR
1	Mbende	Sioma Ngwezi	-16.4523	23.40017	Soil	P	P	P
2	Mbende	Sioma Ngwezi	-16.4523	23.40017	Horn	P	P	P
3	Katolo	Mwandi	-17.1593	24.52506	Soil	P	P	P
4	Katolo	Mwandi	-17.1596	24.52514	Soil	P	P	P
5	Nambwele	Sioma Ngwezi	-16.20358	23.19485	Soil	P	P	P
6	Nangweshi Field 2	Sioma Ngwezi	-16.34428	23.34861	Soil	P	P	P
7	Nakapungu Kalongola 3	Sioma Ngwezi	-16.19932	23.17062	Ear	P	P	P
8	Nakapungu Kalongola 4	Sioma Ngwezi	-16.19932	23.17062	Spleen	P	P	P
9	Libunguta	Senanga	-15.83903	23.26246	Soil	P	P	P
10	Libunguta	Senanga	-15.83807	23.26484	Water	P	P	P
11	Ziba Zako Grave	Nalolo	-16.10482	23.10377	Soil	P	P	P
12	Maombe	Nalolo	-16.23252	23.07052	Soil	P	P	P
13	Luampa	Kaoma	-15.039	24.434	Soil	P	P	P
14	Namando	Kaoma	-15.239	24.565	Ear	P	P	P
15	Nalwashi	Sioma Ngwezi	-16.374	23.039	Soil	P	P	P

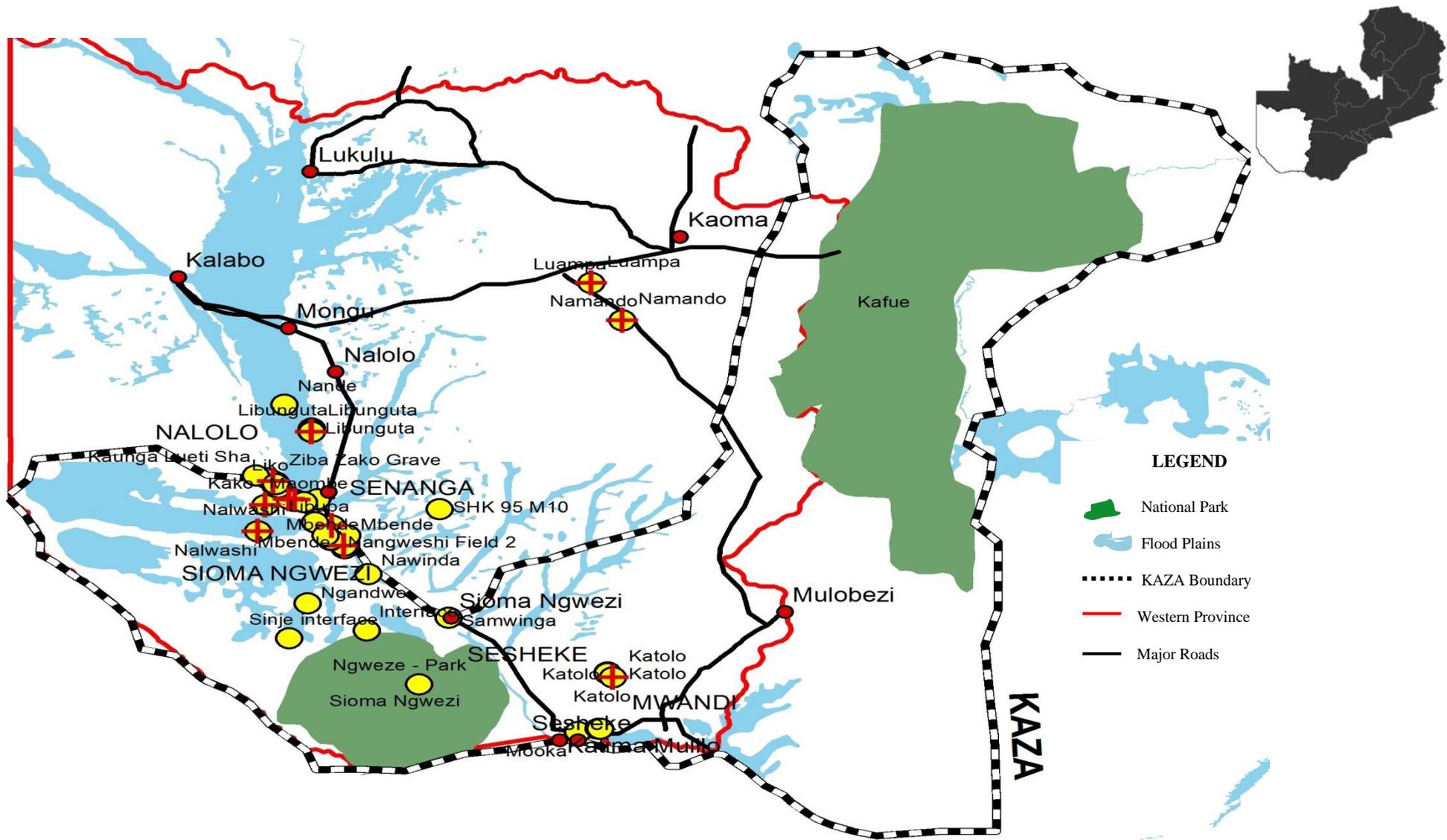


Figure 4.4: **Spatial distribution of anthrax at village level.** The yellow dots indicate the coverage of the sampling exercise (both within and outside the KAZA-TFCA) while the red crosses represent samples that tested positive on PCR (refer to Table 4.1)

4.2. QUESTIONNAIRE SURVEYS AND IDENTIFIED RISK FACTORS

ASSOCIATED WITH ANTHRAX

A total of 113 households were interviewed and these formed the core for which identified risk factors associated with anthrax were identified. All the 113 (100%) interviewed households were male headed and all owned livestock with 84.5% (60/70), [95% CI 75.8-93.1%] being married respondents. Biological plausible risk factors were considered and table 4.2 shows the significant factors on univariate analysis.

Table 4.2: Risk factors for anthrax in traditional cattle in upper Zambezi basin (questionnaire survey, November 2014 to August 2015, $n=113$)

Risk Factor	Levels	n	Percentage	No. of Anthrax Cases	Percentage of the Anthrax Cases
Herd Size	0 : ≤ 30	33	29.58	14	18.67
	1 : 31 - 60	41	50.70	41	54.67
	2 : 61 - 90	16	16.90	16	21.33
	3 : ≥ 91	4	2.82	4	5.33
Management System	0 : Transhumance	75	66.37	63	85.11
	1 : Free Range	38	33.63	12	14.89
Grazing Distance	0 : ≤ 5 Km	40	35.21	11	14.89
	1 : > 5 Km	73	64.79	64	85.11
Grazing Duration	0 : < 1 month	38	33.8	11	14.89
	1 : 1 - 2 months	6	5.63	3	4.26
	2 : > 2 months	68	60.56	61	80.85
Grazing Months	0 : April - Nov	62	54.93	53	70.21
	1 : Aug - Dec	8	7.04	5	6.38
	2 : Non-specific	43	38.03	18	23.41

Year of Observation	0 : 2010 – 2014	88	77.46	64	85.11
	1 : 2004 – 2009	25	22.54	11	14.89
No. in Kraal	0 : Yes	87	76.67	72	95.45
	1 : No	26	23.33	3	4.25
Kraal Mortality #	0 : ≤ 10	75	66.67	49	65.96
	1 : 11-50	35	31.25	24	31.91
	2 : ≥ 50	3	2.08	2	2.13
Village Mortality #	0 : ≤ 10	39	43.66	41	55.32
	1 : 11-50	27	23.94	24	31.91
	2 : ≥ 50	37	32.39	10	12.77
Disease Management	0 : Treatment	74	65.38	49	65.96
	1 : Do nothing	26	34.62	26	34.04
Carcass Disposal	0 : Consumed	104	92	69	91.49
	1 : Disposed	9	8	6	8.51
Vet-Services	0 : None	44	39.44	37	48.94
	1 : Low-Med	32	28.17	22	29.79
	2 : High	37	32.39	16	21.77

Variables considered to be significant on univariate analysis were assessed via a multivariable logistic regression model. All the main variables with their interaction-terms were included as predictors in the model, as each variable or its interaction could affect the odds of an animal being positive for anthrax. All the variables and their interactions were previously analyzed using contingency tables (Table 4.2), and those that were statistically significant ($p < 0.05$) on univariate analyses were included in the full model (Table 4.3).

Table 4.3: Final multivariate logistic regression analysis of risk factors for anthrax outbreaks in cattle ($n=113$)

Risk Factor	Odds Ratio <i>p-value</i>		95% CI		
Herd Size >30	10.46	0.005	8.8	-	16
Transhumance System	2.57	0.001	1.3	-	7.5
Grazing Distance >5km	14.99	0.0001	7.3	-	24.4
Grazing Duration > 2 months	14.84	0.0001	5.4	-	17.58
Grazing Months (April-Nov)	4.08	0.001	2.8	-	7.3
Year of Observation (2010-14)	3.1	0.003	1.5	-	4.9
No. in Kraal	14.57	0.0001	11.02	-	23.7
Kraal Mortality O.B History	17.96	0.001	15.4	-	27.9
Village O.B History	5.69	0.0001	3.6	-	7.2
No Disease Management	4.32	0.02	2.2	-	6.2
Carcass Consumption	6.9	0.001	3.4	-	9.8
Vet-Services (low to none)	10.87	0.004	4.8	-	15.9

Not significant ($p>0.05$), Likelihood ratio = 45.91

Number of observations in the model = 113

Hosmer – Lemeshow χ^2 (8) = 6.71, Prob > χ^2 = 0.938

Twelve biologically plausible variables were found to significantly increase the risk of anthrax outbreaks on multivariable logistic regression analysis model (Table 4.3). The model was tested for the goodness of fit using the Hosmer-Lemeshow statistic. The same test was used to test the model's sensitivity and specificity at different probability cut points.

4.3. FOCUS GROUP DISCUSSIONS (FGDs)

Pertinent data obtained from the FGDs were as follows:

1. The majority of the participants on average, especially the males were aware of anthrax in cattle which they said was characterized by bleeding from natural

openings and an enlarged spleen. In Lozi, they called cattle anthrax as **‘butuku bwa lubete’** which literally means **‘disease of the spleen’**. One of the participants, an elderly man at Katolo village said, “It is called the disease of the spleen because all anthrax carcasses have an enlarged spleen sometimes as big as his spleen”. However the participants did not know the cause of anthrax in cattle while others speculated that ticks transmitted the disease. “We just found animals are dead”, said the young cattle herder in Katolo village.

2. Both men and women on average were able to recognise human anthrax cases from the cutaneous non-painful sores with local swelling which they called **‘bitombotombo’** in Lozi. They were aware that these sores were usually as a result of coming into contact and consuming beef from anthrax carcasses and were a common occurrence once cattle mortalities had been experienced. Their knowledge on anthrax both in livestock and humans was obtained orally from their senior generations. When asked if they knew any other symptoms of human anthrax, the general response was not in the affirmative. However the participants were not aware of the other forms of anthrax; the pulmonary and gastrointestinal forms.
3. People in the study area were extremely reluctant to dispose of both suspected and confirmed anthrax carcasses through burial or burning, because this to them presented an opportunity to consume beef. When asked why they did not bury anthrax carcasses, most participants laughed at the question and showed no remorse towards this behaviour. They said that cattle were kept as a Lozi tradition and that one’s livelihood and social standing depended on having cattle. Therefore, slaughter for consumption was never encouraged. They dressed the carcasses wherever they lay and then distributed the beef to the villages for consumption. Participants were not aware that the process dressing and distribution of anthrax carcasses provided

means, through exuding blood and fluids, of contaminating the environment with anthrax spores which later provided a source of primary foci of infection for the rest of their livestock.

4. People in the study area were not fearful to dress, distribute and consume anthrax carcasses because they said “*bitombotombo*” were easily recognised and they would immediately seek medical services and recovered in no time. To them the opportunity cost of not consuming anthrax beef was higher compared to development of the easily treatable disease. During the study period, two isolated individuals (figures not included) of the same family, although not part of the FGDs were found to have cutaneous form of anthrax at Maombe village of Nalolo district ($S 16^{\circ}13.951, E 23^{\circ}04.231$). Figure 4.5 shows processed anthrax beef and typical anthrax symptoms in a dog at the same village.



Figure 4.5-A: Partially smoked beef from an anthrax carcass being sun-dried on the rack. **B:** A dog that had consumed cut-outs during processing of beef (A) developed oedematous facial and neck swelling and stupor in Maombe village, November 2014.

CHAPTER FIVE

5.0. DISCUSSION

The observed trend with regards to the re-emergence of anthrax cases as an annual disease event occurring simultaneously at particular times of the year in both the Luangwa and Zambezi basins of Zambia intimates a display of unique yet unrelated eco-anthropogenic factors of the disease and its distribution in these independent areas. Further, given the paucity of data as well as risk factors at play coupled with a general lack of organized epidemiological studies in these areas, arose as the main thrust behind the formulation of this study. As a first step, this study aimed at mapping and identifying possible high risk areas and assessing ecological as well as anthropological factors known to be associated with anthrax occurrence. The upper Zambezi basin was chosen given the long history of the disease and frequent annual outbreaks equaled only to the Luangwa basin (Turnbull *et al.*, 1998; Siamudaala *et al.*, 2006). Further, the relative ease of transmission of the disease to humans who readily consume the contaminated meat as opposed to disposal made the Zambezi basin an ideal area for this study.

The findings of this study have brought out critical factors known and some which were not known on the possible drivers for the spread and maintenance of anthrax in certain areas given its public health importance and implication. Some of these factors include herd size, cattle management system, access to veterinary services and dressing of anthrax carcasses. Further, the increasing and becoming acceptable assertion of the annual emergence of anthrax as a common trend in these areas was observed and studied. One important point worth noting and strongly linked to eco-anthropogenic factors is the spatial distribution and disease burden of anthrax in relation to common pool resource utilization. General human activities (transhumans cattle management system and dressing of anthrax carcass) as well

as the geographical factors (seasonal water distribution and vegetation cover) at play which at a distance glance appeared proxy, but were found to be risk factors in this study.

Before we look at detailed aspects from this present study, a few general aspects which were of interest need to be highlighted. In the most affected areas, a perception has seemingly developed among the inhabitants that the yearly outbreaks are not uncommon. This was very clear from the answers that were provided during the focused group discussions (FGDs). An elderly man at Mbende village of Sioma Ngwezi district said “ we have lived with cutaneous anthrax (*bitombotombo* in Lozi) for generations and we recover if you compare with malaria which claims lives”. They assume anthrax is not a fatal disease unlike malaria, as the only effect of anthrax is “*bitombotombo*”, a Lozi word literally meaning sores or wounds and nothing else. Given this background, the inhabitants readily open up anthrax carcasses, which results in the sporulation of the bacteria and environmental contamination (Siamudaala *et al.*, 2005), although they are not aware of this epidemiological consequence of their action. Turner *et al.* (2014) found that where wildlife anthrax carcasses died, the remains provided nutrition for vegetation growth in the long term and in turn, the vegetation attracted susceptible animals. Dressing of anthrax carcasses where they lay provided this fatal attraction to ingest the spores around the site. At Nakapungu village (-16.19932°S and 23.17062°E) in Sioma Ngwezi district, the District Veterinary Officer (DVO) conducted a post-mortem on a cow and strongly suspected anthrax. The DVO recommended and supervised the burial of the carcass where it had died in the plains. When we went for sample collection at the same site, we found that the carcass had been exhumed and the beef distributed for consumption. This shows that anthrax is not dreaded as it should be.

This scenario was worsened by the lack of veterinary intervention, as a direct association was found between lack of veterinary services and frequency of anthrax outbreaks in the study area. Areas that received veterinary intervention and good extension services were less

likely to experience frequent anthrax outbreaks. Additionally, in areas where such services were not available, no systemic disease surveillance nor monitoring programs are in place. Other major factors that were brought out by the study participants was that of the slow response time or lack of response by veterinary extension officers when suspected anthrax mortalities are encountered. They anticipated that veterinary extension officers should conduct a post mortem inspection and advise on the means of disposal as well as treatment or free vaccination of other in contact animals. In one of the remote villages (Njobwe), the participants alluded to the lack of response due to cumbersome government procedures. They said they were informed that in government, before a worker travels out of station, he or she needed to complete government procedures, which included among many other issues, obtaining authority to travel out of station from superiors, sometimes organizing transport and necessary funds for field work. By the time they get to the field, carcasses would have already been “disposed off” in the best way known by the villagers, which is consumption. This implies that the dead animals were opened within the premises of the village boundary thus perpetuating and sustaining the spores and increasing the risk of another outbreak in the same area. This was in agreement with the findings of Munang’andu *et al.*, 2012 who observed that the current passive surveillance system routinely requires veterinary assistants to visit cattle owners and identify animals that died of anthrax or other notifiable disease. This does not provide an adequate early warning system given the poor transportation and communication systems between the veterinary camps and district offices. It is probable that more animals will have died and the meat distributed before public health and district veterinary officers can travel to the site of the outbreaks (Munang’andu *et al.*, 2012).

Even during severe outbreaks, the course of the disease was dependent on “self-limiting factors” such as reduced cattle density during outbreaks (de Vos, 1990) whilst the people did

not dispose of the carcasses. The lack of veterinary services further resulted in data on outbreaks being patchy and most of the time outbreaks from such remote areas went unreported. Therefore, the information which reaches both the veterinary officials is mostly incomplete. The problem of not having updated (accurate) data by veterinary professionals can partly be blamed on their poor response or lack of response to outbreaks. The existing poor infrastructure, starting from the road networks, lack of funding to most rural veterinary and health outposts mean authorities in these areas have no planned activities to cover such issues like anthrax despite their serious nature (Munang'andu *et al.*, 2012). Information from veterinarians in the study area indicated that most of the resources and activities were directed towards the prevention and control of foot and mouth disease (FMD) and contagious bovine pleural pneumonia (CBPP). Anthrax control and prevention is left to the cattle farmers even when it is not only a notifiable disease but also a zoonotic one. The CBPP and FMD surveillance system could only switch to focus on anthrax when tens or hundreds of cattle would have died, the meat consumed by humans and domestic carnivores and the environment contaminated with anthrax spores. Further, despite the annual outbreaks, the disease is considered as a sporadic episode and nothing more, a view which had resulted in authorities considering the disease in isolation and not linked to the overall ecological perspectives and anthropogenic activities taking cognizance of the land use practices and traditional cultural practices of the people.

The first key finding from this study is that we have been able to map and identify high risk areas with regards to frequency of anthrax outbreaks in the upper Zambezi basin. Additionally, this study has elucidated the ecological and anthropogenic factors for anthrax occurrence and sustained outbreaks in the study area. This study also married biological data of environmental samples collected mainly from kraals with frequent outbreaks to the frequency and proportion of outbreaks. Biological samples were useful to positively link that

what was collected was indeed anthrax, and from our multiplex PCR analysis we got positive results which we linked to risk factors. Further, all points or areas which had positive biological samples were identified on the map and using GIS technology displayed according to the exact sampling site given the coordinates that were generated. The spatial distribution of most of confirmed anthrax samples emanated from low lying areas where transhumance grazing system was practiced and also those that had poor veterinary services. From the final multivariate multiple logistic regression model, anthropogenic factors that were strongly associated with anthrax outbreaks included herd size and cattle management systems (especially transhumance animals that were grazed in the flood plains most times of the year). Other anthropological factors included modes of carcass disposal (carcass dressing for consumption), veterinary services availability among many others. Ecological factors that were significant and seen on the mapping data included elevation/altitude, with almost all the outbreaks occurring in the low lying areas (flood plains) and seldom in the higher ecotone areas. This link in ecological determinants is well elucidated when outbreaks are overlaid according to topography as reports of anthrax in both the epizootic and sporadic form in the upper Zambezi were all confined in the lower flood plains with non in the higher plateau. This finding was also observed by Munang'andu *et al.*, 2012 who attributed this observation partly to the alluvial soils of the plains. Another significant anthropological risk factor of anthrax outbreak was history of having had an animal that had previously died in the same kraal. Those kraals which reported animals having died before in the same kraals were almost 18 times more likely to experience another outbreak than those that did not have a previous occurrence (odds ratio 17.96, CI 15.4-27.9, 95 % $P < 0.001$).

Geographical distribution of the positive anthrax isolates based on molecular typing of environmentally collected samples has helped us to elucidate essential characteristics linked to this disease in Western Province of Zambia. Frequent outbreaks in the same areas and

some anthropogenic activities were found to be directly associated with anthrax outbreaks. These observations are congruent with those of Siamudaala *et al.*, (2006), who found that dressing of anthrax carcasses by human were more at play in the continuous outbreaks of anthrax in the Zambezi basin of Western Province than in the Luangwa basin. Similarly, Munang'andu *et al.*, 2012 attributed this observation to consumption of dead carcasses as a sequel of an anthrax outbreak. They found that coincidentally, the ratio of carcasses that could be consumed by humans and therefore be sources of infection were also high (37:1), meaning that for every anthrax carcass consumed, 37 humans were at risk of being infected with anthrax (Munang'andu *et al.*, 2012). However, in South Luangwa, old anthrax graves of wild animals were the most probable sources of soil contamination (Turnbull *et al.*, 1991; Turnbull *et al.*, 1998). What was common among our present study, Turnbull *et al.*, (1998) and Munang'andu *et al.*, (2012), is the location of the hotspots in low lying areas.

From the multiple logistic regression analysis, transhumance grazing system was one of the significant risk factors of anthrax occurrence in the upper Zambezi basin. Animals that were under the distant transhumance grazing system were approximately 15 times more likely to be affected by anthrax than those under local grazing. Cattle owners with a herd size greater than 30, were 9 times more likely to practice transhumance grazing. This explains why herd size is also a risk factor for anthrax outbreak. The transhumance grazing system along with fishing and farming on the wetlands increased human activities on the floodplain during the dry season where humans and animals share common pool resources (Simwinji, 1997; Namafe, 2004). Herdsmen who practiced transhumance grazing system that brought cattle into the wetlands during the dry season, 55% (n =75) took their cattle in the flood plains between the months of April to November. Cattle that grazed in the flood plains during this period were 4 times ($p < 0.001$) more likely to develop anthrax than those grazing in any other period. During this period it was observed that cattle herds concentrated around the

lagoons and oxbow lakes within the flood plains (Fig. 3.3-C). As the grass become overgrazed, cattle ingest the spores from the soil and grass (Hugh and de Vos, 2002; Munang'andu *et al.*, 2012). They also ingest spores by drinking water from the lagoons where spores have been concentrated by the incubator conditions (Turnbull *et al.*, 1991). Outbreaks start in the dry season extending until the onset of the rain season. By the peak of the rain season most animals were moved to the upper woodlands and no outbreaks were until the next dry season, this was in agreement with the findings of Lindique and Turnbull (1994), Hugh-Jones and Blackburn (2009) and Munang'andu *et al.* (2012). Suffice to mention that this transhumance grazing system extends up to Luampa district where herdsmen move their cattle from parts of the district to the floodplains of Mongu and Lukuludistricts for grazing during the dry season (figure 4.4).

The most critical public health implication of the scenario of continuous anthrax outbreaks on an annual basis in Western Province will be seen in the long-term. For a zoonosis like anthrax, increasing the susceptible host population has a multiple effects. Increasing the number of animals infected from each outbreak increases the number of spores being released back in the environment, thereby expanding the transmission capacity to humans as more carcasses are available for consumption from each outbreak.

From our field observations and what is known of anthrax, we can say that in the upper Zambezi basin, the epidemiology of anthrax is highly associated with anthropogenic and ecological parameters (hydrology, seasonal rainfall pattern, evaporation potential and temperature of the floodplain) (Moazeni, 2004; Animal Health Australia, 2005; Hugh-Jones and Blackburn, 2009). These parameters are likely to influence the survival and re-distribution of anthrax spores in the soil (Hugh-Jones and Hussaini, 1975; de Vos, 2002). This also agrees with the study of McGinley (2008), who looked at the ecology and biodiversity of the upper Zambezi basin and found that the survival and redistribution of

anthrax was largely depended to the soils apart from the activities of the people. Complementary to ecological parameters, increase in the susceptible livestock population, transhumance grazing system, seasonal anthropogenic activities and pressure, poor public awareness and the general lack of a systematic intervention program have led to a cyclical trend in the recurrence of anthrax outbreaks on the upper Zambezi basin.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

The upper Zambezi basin has reached an anthrax endemic situation resulting from a mix of ecological and anthropogenic parameters such as the cyclical rainfall pattern, flooding, evaporation potential, temperature, the geology of the floodplains, with complimentary epidemiological factors like the increase in cattle and human populations, transhumance grazing system, cultural norms, beliefs and anthropogenic pressure. Anthropogenic pressure resulted from partial awareness of anthrax in humans. The ecology and seasonal anthropogenic pressure impacted on the floodplain from transhumance grazing system and fishing, showed evidence that human livelihood is dependent on the floodplain which is endemic with spores. In the absence of systematic disease intervention programs, the recurrence of anthrax has become a cyclical trend that is highly associated with human activities on the floodplains. The spatial distribution of anthrax was observed to be restricted to the flood plains and more so in areas that had poor veterinary extension services within and on the periphery of KAZA. Anthrax epidemics occur in the dry season when the anthropogenic pressure is high on the floodplain with no reports occurring at the peak of the flooding when the anthropogenic pressure is at its lowest. Therefore, the only foreseeable solution lies in the following recommendations:

- Urgent improvement in government veterinary and livestock extension services delivery and carry out free or subsidized annual vaccination of livestock. Preventing the disease in livestock will ultimately prevent the disease in humans; a one health approach.
- Establishment of anthrax surveillance programme for early detection, vaccination and decontamination of anthrax graves within the upper Zambezi basin.

- Embark on public health awareness campaigns aimed at promoting active participation by the general public in the control of the disease in the identified hotspots and surrounding areas.
- Further longterm study the establish temporal-spatial distribution of anthrax will elucidate the pattern of disease spread over a period of years. This study did not prove if anthrax outbreaks are localized or spreading to new areas (emerging or re-emerging). The study would also establish strain characteristics, transmission dynamics at the wildlife/livestock interface and sensitivity to antimicrobials used in human anthrax cases.

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APPENDICES

APPENDIX 1: Distribution of environmental and biological samples and their respective test results

SN	ID/VILLAGE	DISTRICT	LATITUDE	LONGITUDE	SAMPLE TYPE	CULTURE TEST	GRAM STAIN	PCR
1	Mbende	Sioma Ngwezi	-16.4653	23.40098	Dung	N		
2	Mbende	Sioma Ngwezi	-16.4468	23.40266	Soil	P	P	N
3	Mbende	Sioma Ngwezi	-16.4523	23.40017	Soil	P	P	P
4	Mbende	Sioma Ngwezi	-16.4523	23.40017	Horn	P	P	P
5	Njobwe	Sesheke	-17.1335	24.50379	Soil	P	P	
6	Njobwe	Sesheke	-17.1335	24.50379	Tibia	N		
7	Njobwe	Sesheke	-17.1396	24.50194	Soil	P	P	N
8	Njobwe	Sesheke	-17.1376	24.50337	Humerus	N		
9	Katolo	Mwandi	-17.1593	24.52506	Soil	P	P	P
10	Katolo	Mwandi	-17.1593	24.52506	Jaw	N		
11	Katolo	Mwandi	-17.1594	24.52548	Soil	N		
12	Katolo	Mwandi	-17.1594	24.52548	Tibia	N		
13	Katolo	Mwandi	-17.1596	24.52514	Soil	P	P	P
14	Slowana Likondwama	Sioma Ngwezi	-16.19243	23.16576	Dung	N		
15	Slowana Likondwama	Sioma Ngwezi	-16.19243	23.16576	Soil	N		
16	Naluwe Village	Sioma Ngwezi	-16.3252	23.27817	Dung	N		
17	Slowana Plains 3	Sioma Ngwezi	-16.20166	23.28611	Dung	P	N	
18	Nambwele	Sioma Ngwezi	-16.20358	23.19485	Soil	P	P	P
19	Nambwele	Sioma Ngwezi	-16.20368	23.19059	Soil	P	P	N
20	Nangweshi Field 2	Sioma Ngwezi	-16.34428	23.34861	Soil	P	P	P
21	Nambwele 2	Sioma Ngwezi	-16.20357	23.19193	Water	P	P	N
22	Nambwele 1	Sioma Ngwezi	-16.20357	23.19193	Water	N		
23	Nangweshi Field 1	Sioma Ngwezi	-16.41966	23.34323	Dung	N		
24	Nangweshi Kraal 1	Sioma Ngwezi	-16.41966	23.34328	Soil	N		
25	Nangweshi Kraal 2	Sioma Ngwezi	-16.41961	23.34338	Dung	P	P	N

SN	ID/VILLAGE	DISTRICT	LATITUDE	LONGITUDE	SAMPLE TYPE	CULTURE TEST	GRAM STAIN	PCR
26	Naluwe	Sioma Ngwezi	-16.3252	23.27814	Dung	P	P	N
27	Luneta BP Kraal 1	Sioma Ngwezi	-16.39618	23.32287	Dung	P	P	N
28	Luneta BP Kraal 2	Sioma Ngwezi	-16.39618	23.32287	Soil	P	P	N
29	Nakapungu Induna	Sioma Ngwezi	-16.19977	23.17216	Skin	N		
30	Nakapungu subject	Sioma Ngwezi	-17.199	23.17018	Skin	N		
31	Nakapungu Kalongola 1	Sioma Ngwezi	-16.19932	23.17062	Heart	N		
32	Nakapungu Kalongola 2	Sioma Ngwezi	-16.19932	23.17062	Muscle	N		
33	Nakapungu Kalongola 3	Sioma Ngwezi	-16.19932	23.17062	Ear	P	P	P
34	Nakapungu Kalongola 4	Sioma Ngwezi	-16.19932	23.17062	Spleen	P	P	P
35	Nangweshi Field 3	Sioma Ngwezi	-16.41966	23.34323	Rib	N		
36	Namatoya 1	Senanga	-15.82947	23.26309	Soil	P	P	N
37	Namatoya 2	Senanga	-15.82073	23.26414	Soil	P	P	N
38	Namatoya 1	Senanga	-15.82947	23.26309	Bone	N		
39	Libunguta	Senanga	-15.83903	23.26246	Soil	P	P	P
40	Libunguta	Senanga	-15.83807	23.26484	Water	P	P	P
41	Powerline Bridge	Senanga	-16.396	23.41207	Water	P	P	N
42	Powerline Bridge	Senanga	-16.396	23.41207	Soil	N		
43	SHK 95 M10	Senanga	-16.25537	23.80027	Soil	P	P	N
44	Libuba	Nalolo	-16.13869	23.12512	Soil	P	P	N
45	Libuba	Nalolo	-16.13869	23.12512	Skin	N		
46	Libuba	Nalolo	-16.13869	23.12512	Jaw	P	P	N
47	Libuba	Nalolo	-16.13869	23.12512	Jaw	N		
48	Ziba Zako Grave	Nalolo	-16.10482	23.10377	Soil	P	P	P
49	Ziba Zako Field	Nalolo	-16.10482	23.10377	Soil	N		
50	Ziba Zako Kraal	Nalolo	-16.10482	23.10377	Dung	N		
51	Mbunda Makungu	Nalolo	-16.11382	23.11337	Soil	N		
52	Shanjamba	Nalolo	-16.10875	23.10927	Tibia	P	P	N
53	Shanjamba	Nalolo	-16.10875	23.10927	Jaw	N		

SN	ID/VILLAGE	DISTRICT	LATITUDE	LONGITUDE	SAMPLE TYPE	CULTURE TEST	GRAM STAIN	PCR
54	Shanjamba	Nalolo	-16.10875	23.10927	Skin	N	P	N
55	Shanjamba	Nalolo	-16.10875	23.10927	Skull	P	P	N
56	Kako	Nalolo	-16.08814	23.07927	Soil	P	N	
57	Maombe	Nalolo	-16.23252	23.07052	Soil	P	P	P
58	Kaunga Lueti Sha	Nalolo	-16.07315	23.03045	Soil	N		
59	Mooka	Sesheke	-17.45334	24.37827	Femur	N		
60	Mooka	Sesheke	-17.45333	24.37827	Soil	P	P	N
61	Katongo 368	Sesheke	-17.43591	24.46571	Soil	N		
62	Katongo 368	Sesheke	-17.43591	24.46571	Dung	P	P	N
63	Kazauli	Sioma Ngwezi	16.41902	23.34369	Soil	N		
64	Kazauli	Sioma Ngwezi	-16.41902	23.34369	Dung	N		
65	Kazauli	Sioma Ngwezi	-16.41902	23.34369	Bone	N		
66	Liko	Nalolo	-16.12086	23.114	Soil	P	P	N
67	Katundu	Sesheke	-17.43513	24.47181	Soil	N		
68	Nawinda	Sioma Ngwezi	-16.60383	23.50046	Soil	N		
69	Namita	Sioma Ngwezi	-16.21727	23.23068	Soil	N		
70	Namita	Sioma Ngwezi	-16.21727	23.23068	Dung	N		
71	Mushika	Sioma Ngwezi	-16.39578	23.32261	Dung	N		
72	Luampa	Kaoma	-15.039	24.434	Spleen	P	P	P
73	Namando	Kaoma	-15.239	24.565	Spleen	P	P	P
74	Nande	Nalolo	-15.694	23.15	Soil	P	N	
75	Nalwashi	Sioma Ngwezi	-16.374	23.039	Soil	N		
76	Samwinga	Sioma Ngwezi	-16.838	23.838	Soil	N		
77	Ngandwe	Sioma Ngwezi	-16.762	23.248	Soil	N		
78	Ngweze - Park	Sioma Ngwezi	-17.196	23.713	Soil/bone	N		
79	Interface	Sioma Ngwezi	-16.907	23.495	Soil/bone	N		
80	Sinje interface	Sioma Ngwezi	-16.949	23.17	Soil			

APPENDIX 2: QUESTIONNAIRE

THE UNIVERSITY OF ZAMBIA

School of Veterinary Medicine

Disease Control Department

QUESTIONNAIRE

**Mapping of High Risk Areas and Ecological Assessment for
Anthrax in the Upper Zambezi Basin with Sioma-Ngwezi as
Case Study**

Masters of Science One Health Analytical Epidemiology Research

STUDENT:

Dr. Harvey Kakoma Kamboyi

SUPERVISOR:

Dr. Musso Munyeme

CONSENT

Dear Participant,

My name is **Harvey Kakoma Kamboyi**, herein referred to as Principal Investigator (PI). I am a Master of Science student in One Health Analytical Epidemiology at the University of Zambia (UNZA), Great East Road Campus in Lusaka.

Your area has been identified as a very important focal point for the data our research is looking for. Therefore, you are being asked to complete this questionnaire survey as part of a research project being conducted by the PI. The research project is designed to determine the ecological drivers of anthrax recurrence and socio-cultural impact of anthrax in cattle in the upper Zambezi basin. You are required to complete this questionnaire before or by the 31st October, 2014.

Your responses are entirely voluntary, and you may refuse to complete any part or all of this questionnaire survey. This survey is designed to be anonymous, meaning that there will be no way to connect your responses to you. Towards that end, please do not sign your name to the survey or include any information in your responses that makes it easy to identify you. By completing and submitting this questionnaire, you affirm that you are at least 18 years old and that you give consent for the PI to use your answers in his research. If you have any questions about this research before or after you complete the questionnaire, please call or email the PI on +260 977 465 111 and docharveykk@yahoo.com respectively. If you have any concerns or questions about your rights as a participant in this research, please contact the supervisor of the research (**Dr. M. Munyeme**) on +260 955 751 013 or mussomunyeme@unza.zm

In this survey there is no right or wrong answer. Please be frank and honest throughout.

Thank you.

RESPONDENT NUMBER _____

VILLAGE _____

GPS COORDINATES: _____ / _____

SECTION A: SOCIO-DEMOGRAPHIC INFORMATION

Instructions: Please tick in one bracket provided for each question next to the correct option of your choice or write in the space provided for specific answers.

1. What is your gender?
 1. Male
 2. Female

2. How old are you in years?
 1. 18-19
 2. 20-30
 3. 31-40
 4. 41-50
 5. Over 51

3. What is your educational status?
 1. None
 2. Primary level
 3. Secondary level
 4. Tertiary (College/University)

4. What is your occupation? Specify
 1. No particular job
 2. Farmer
 3. Fisherman
 4. Cattle herder
 5. Others specify _____

5. What is your marital Status?
 1. Currently married
 2. Widowed
 3. Divorced
 4. Separated
 5. Living with but not married
 6. Never married

6. How many children do you have? _____

7. How many dependents do you have? _____

SECTION B: LAND USE AND LIVESTOCK OWNERSHIP

Instructions: Please tick only in one of the three brackets provided next to each question or write in the space provided for specific answers.

8. Does your household own land for agriculture? Yes [] No []
9. Is the land enough for your agriculture? If not give reasons. _____

10. Do you cultivate for home use only?
1. Yes []
 2. No []
 3. Others (specify) _____

11. Does your household own any livestock?
1. Yes []
 2. No []
12. If your household **does not** have any livestock, is there a reason? Explain _____

If your answer to Q.12 is No, proceed to Q.27.

13. What type of livestock does your household own?
1. Cattle []
 2. Goats []
 3. Sheep []
 4. Other (specify) : _____
14. What are the livestock numbers of the animals indicated in Q.13?
1. Cattle: _____
 2. Goat: _____
 3. Sheep: _____
 4. Poultry: _____
 5. Others (specify): _____

If you do not own any cattle, please proceed to Q.27

15. Please indicate in table 1 below the current number of cattle in the household by age sex.

Table 1: For cattle owners only. Please indicate the cattle numbers in the table below

Age (years)	TOTAL
< 1 year	
1 to 5	
> 5	
TOTAL	

16. What management/grazing system do you use to keep your cattle? _____

17. Describe the cattle management/grazing system you have mentioned in Q. 16.

18. If you take out your cattle to graze, how far (Kilometers) do you go and how many days or months do you stay there as the animals graze? _____

19. In these areas of grazing, do your cattle come in contact with wildlife?

1. Yes []
2. No []
3. Other (specify): _____

20. Can you mention the areas where you take your animals to graze and in which months of the year do you visit the mentioned areas? _____

21. Why are you keeping cattle? (mark all those that apply)

1. Home consumption []
2. Commercial []
3. Security (as a bank) []
4. Tradition []
5. Other (specify) _____

22. Do you ever rely on cattle for draught power?

1. Strongly agree []
2. I agree []

- 3. Somehow agree []
- 4. Do not agree []
- 5. I don't know []

23. Do you use cattle for any of the following?

- 1. Pay for school fees []
- 2. Pay bride price []
- 3. Buy household commodities []
- 4. Traditional ceremonies []
- 5. Other (specify) _____

24. Does the number of cattle you have influence in any way your social standing in your community?

- 1. Yes []
- 2. No []

If yes, how? _____

SECTION C: HEALTH PROBLEMS OF CATTLE AND DISEASE AWARENESS

25. Have you ever had cattle health problems in your village in the recent past?

- 1. Yes []
- 2. No []
- 3. I don't know []

26. What kind of cattle health problems have you experienced? _____

YES	NO	NOT SURE
1	2	3

27. Have you ever heard of disease called Anthrax? [] []
[]

If your answer to Q27 is NO or NOT SURE, do not attempt to answer any more questions.

28. If yes, where did you get the information? (Tick all that apply)

- 1. Health Institutions []
- 2. Government veterinary/Livestock officer []
- 3. Orally/Village []
- 4. Television/Radio/Newspaper []
- 5. Church []
- 6. Public meetings []
- 7. School/training []
- 8. Bar/Tavern []

YES NO NOT SURE
1 2 3

29. Do you know what causes anthrax? [] [] []

30. If your answer to Q.29 is yes, mention them.

- a) _____
- b) _____
- c) _____
- d) _____

YES NO NOT SURE
1 2 3

31. Do you know the clinical signs of anthrax? [] [] []

32. If your answer to Q.31 is yes, mention them:

- 1. _____
- 2. _____
- 3. _____
- 4. _____
- 5. _____

33. In which years have you seen anthrax outbreaks and indicate the number of outbreaks for each of the years you have you indicate? _____

	YES	NO	NOT SURE
	1	2	3
34. Have your cattle been affected by anthrax?	[]	[]	[]

If yes, in which year(s)? _____

	YES	NO	NOT SURE
	1	2	3
35. Do you recall the number of cattle in your herd (kraal) that were affected by anthrax?	[]	[]	[]

[]

36. If yes to Q.35, how many died of anthrax in your herd? Indicate the year in which the cattle died.

	YES	NO	NOT SURE
	1	2	3
37. Do you know the number of cattle that died in your village?	[]	[]	[]

38. If yes to Q.37, how many died including yours? Indicate when this happened (year)

39. How did you manage the disease?

- 1. Vaccination []
- 2. Treatment []
- 3. Quarantine []
- 4. Did nothing []

40. What did you do with the animals that died of anthrax?

YES NO NOT SURE
1 2 3

41. Did you or any of your family members suffer from anthrax? [] [] []

If yes, what were the symptoms? _____

YES NO NOT SURE
1 2 3

42. Did you or any of family members go to the clinic for treatment? [] [] []

D. IMPACT OF ANTHRAX ON LIVELIHOOD

YES NO NOT SURE
1 2 3

43. Are there any cattle movement restrictions by the authorities? [] [] []

44. How is the trend of cattle trade during and after anthrax outbreaks?

1. Increasing []
2. Constant []
3. Decreasing []
4. I don't know []

YES	NO	NOT SURE
1	2	3

45. Are you able to access a good market for your beef? [] [] []

46. How has the beef or live animal price become?

1. Still the same []
2. Increasing []
3. Reducing []
4. Fluctuating []
5. I don't know []

YES	NO	NOT SURE
1	2	3

47. Is there any impact of anthrax at your household? [] [] []

48. If yes to Q.47, what impact has it had?

1. Economic loss of cattle []
2. Reduced beef production []
3. Reduced draught power []
4. Increased production cost through:
 - i. Vaccinations []
 - ii. Treatment []
 - iii. Supplementary feed during quarantine []

49. How would you rate veterinary services in your area?

1. High []
2. Medium []
3. Low []
4. Nonexistent []

THANK YOU FOR YOUR SUPPORT AND COOPERATION!

THE END

APPENDIX 3: FIELD DATA ENTRY FORMS

**ANTHRAX FIELD SURVEY DATA ENTRY FORMS
CIRAD/RP-PCP & SCIDS PROJECTS
2014**

GENERAL DATA COLLECTION RECORDING SHEET – Form 01

Recording Officer _____

Area/strata: _____

Date: _____

GPS location: _____

District/Veterinary Camp area: _____

Species/Type of Sample	No of Animals affected	Ecological Information		Biological Data collected	
		GPS/Waypoints	Ecological assessment (Drainage/soil types etc)	General	Specific Biological Sample (i.e. Soil, blood, ear piece etc)

**ANTHRAX FIELD SURVEY DATA ENTRY FORMS
CIRAD/RP-PCP & SCIDS PROJECTS
2014**

RETRODICTIONARY (PARAMETERS)-RETROSPECTIVE DATA RECORDING SHEET – Form 02

Recording Officer: _____ **District/Area:** _____

GPS/Waypoint: _____

Year (under consideration)	Animal Cases		Any Human Contacts (Y/N)	No. of Individual animals Affected?	Estimated No. in whole Herd?	Human Cases			Comments (Physiological parameter)
	Species	Breed				Any Humans affected & (No in Brackets)	Sex	Age	

Comments on the Physiology can be any of the following i.e.:

Difficulty in walking	Abnormal posture	Abnormal body surfaces	Inability to breed	Lagging behind group	Recumbent	Dozing	Bleeding	
Limping	Bloody Discharge from nose, eyes, vagina, penis	Dead animals	Lack of appetite	Behavioural change	Poor hair coat	Difficulty in breathing	Not alert	Inactive
Diarrhoea	Abnormal colour of urine	Excessive salivation	Poor body condition	Blood in faeces	Loss of hair	Shivering	Inactive tail	

**ANTHRAX FIELD SURVEY DATA ENTRY FORMS
CIRAD/RP-PCP & SCIDS PROJECTS
2014**

CURRENT: SUPLEMNATRY MORTALITY RECORD DATA SHEET – Form 03

2014 OUTBREAKS

Recording Officer: _____ **Date:** _____

Animal Species	GPS	Actual or Suspected Cause of Death	Observations /Comments	Study Strata By		
				Village	Camp	District