PREVALENCE AND GENETIC CHARACTERIZATION OF *Anaplasma phagocytophilum* IN OLIVE BABOONS AND VERVET MONKEYS IN LAIKIPIA COUNTY, KENYA.

A thesis submitted in partial fulfilment of the requirements for the degree of Masters in Veterinary Epidemiology and Economics (MVEE).

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DECLARATION

I declare that this thesis is my original work and has not been presented for award of degree in any other university.

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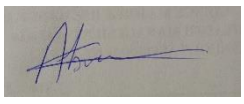
Signature:  Date:28th October 2021

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LIST OF ABBREVIATIONS

PCR Polymerase chain reaction

DNA Deoxyribonucleic acid

RNA Ribonucleic acid

NHPs Non-human primates

IL-8 Interleukin 8

HGA Human Granulocytic Anaplasmosis

rRNA Ribosomal ribonucleic acid

EIDs Emerging infectious diseases

BLAST Basic Local Alignment and Search Tool

SARS Severe acute respiratory syndrome

HIV/AIDS Human immunodeficiency virus/ Acquired immune deficiency syndrome

Msp Major surface protein

SIV Simian immunodeficiency virus

AAALAC Association of Assessment and Accreditation of Laboratory Animal Care

CDC Centres for Disease Control and Prevention

SIVsm Simian Immunodeficiency Virus

TAE Tris-acetate-EDTA.

ABSTRACT

The current threat to health, the economy and security globally is the rise in frequency of emerging infectious diseases (EIDs). Among EIDs, are zoonotic tick-borne infections which include rickettsial diseases such as anaplasmosis. The aim of this study was to investigate the presence of *Anaplasma phagocytophilum* in olive baboons and vervet monkeys in Laikipia County, Kenya. *Anaplasma phagocytophilum* is an obligate intra-erythrocytic microorganism with a wide host range and is the cause of anaplasmosis which is an emerging infectious zoonotic disease.

Among wildlife species, non-human primates (NHPs) are often proprietors to different microbial agents of zoonotic potential as they play an important role in zoonotic spill-overs, as either reservoirs or amplifiers. Certain factors facilitate the spread of these diseases such as interaction of these animals in a high risk interface area. Laikipia County is a potential interface because of its great diversity of vegetation types, rich wildlife biodiversity and a significant human and livestock population.

The objectives of this study were to determine the prevalence, genetic characterization and the risk factors associated with the occurrence of *Anaplasma phagocytophilum* in olive baboons *(Papios Anubis)* and vervet monkeys (*Chlorocebus pygerythrus)* in Laikipia County, Kenya.

A total of 146 blood samples collected from olive baboons and 18 from vervet monkeys from Mpala Research Center and Ol jogi Conservancy in Laikipia County were subjected to nested and conventional Polymerase Chain Reaction (PCR) to screen for the presence of *Anaplasma* species. *A. phagocytophilum* was confirmed by sequencing using gene targeting 16S rRNA. There was also detection of *Anaplasma platys* and *Candidatus anaplasma.* This is the first detection of *A. phagocytophilum* in olive baboons and vervet monkeys in Kenya.

Phylogenetic analysis of the study isolates showed homology of Kenyan *A. phagocytophilum* with isolates from Japan, South Korea, France, China, South Africa and Denmark. All the *A. phagocytophilum* Kenyan isolates clustered into one clade. The isolates from Japan, South Korea, France, China, South Africa and Denmark belonged to clade I but had recent common ancestor with the Kenyan isolates.

The study found an overall prevalence of 18.3% for *Anaplasma* species with 26 and 4 of the positives being from olive baboons and vervet monkeys respectively. No risk factors were identified for *A. phagocytophilum* because of the limited number of positive samples. Prevelance for anaplasma infection in olive baboons was 17.8% and 22.2% in vervet monkeys while 27.9% was recorded in Mpala and 3.3% in Ol jogi.

Conclusively, this study provides valuable information on the endemnity of *A. phagocytophilum* bacteria in olive baboons and vervet monkeys in Kenya. Future research is needed to establish the prevalence and public health implications of zoonotic *A. phagocytophilum* isolates and the role of nonhuman primates as reservoirs in the region.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Anaplasmosis as an emerging zoonotic tickborne infection

With the frequency of emerging infectious diseases (EIDs) being on the rise, these diseases are proving to be a significant threat to health, the economy and security globally (Heymann *et al*., 2015). Despite this, our understanding of the mechanisms underlying their emergence still remains incomplete (Allen *et al*., 2017). Among EIDs, are zoonotic tickborne infections which include rickettsial diseases such as anaplasmosis, ehrlichiosis, Q fever and Lyme disease among others (Nakayima *et al*., 2014). These infections have in the recent years hgained significance on a global scale because of an increase in understanding of their zoonotic potential and the public health implications globally (Vesco *et al.,* 2010).

What the pandemics have proven is that, emerging diseases are mainly of animal origin, particularly wildlife (Jones *et al*., 2013). For instance, Severe Acute Respiratory Syndrome- Coronavirus 2 (SARS-CoV-2) which has been shown to relate phylogenetically to (SARS-like) bat viruses pointing to bats as the possible primary reservoir (Shereen *et al*., 2020). Others include, SARS-like coronaviruses of bats in China Li W *et al*.,(2005) and Simian Immunodeficiency Virus (SIVsm) from Sooty mangabeys *(Cercocebus atys*) (Hahn *et al.,*2000). The complex interaction among wildlife, livestock and human populations contributes to their emergence (Brierley *et al.,* 2016). This is because they maintain them in circulation as most pathogens only gain infectivity following spill-over to different animal species like man and domestic animals (Wang & Crameri, 2014).

Among wildlife species, non-human primates (NHPs) are often proprietors to different microbial agents some which have zoonotic potential. Primates are closely related to man phylogenetically and ecologically (Locatelli & Peeters, 2012). A large number of shared

pathogens listed as emerging in humans have been isolated from wild NHPs (Jones *et al.*, 2008). However, certain factors such as forested tropical regions experiencing land-use changes and those with a high wildlife biodiversity facilitate the spread of these diseases to livestock and man (Allen *et al.*, 2017). Others include adoption of new technology in farms, climate change, travel and encroachment into new habitats (Jones *et al*., 2013).

Among the rickettsial diseases, this research focused on *Anaplasma phagocytophilum*, a pathogenic bacterium of zoonotic potential often spread among wildlife and livestock (Ismail *et al*., 2010). In man it causes human granulocytic anaplasmosis (HGA) and according to a recent Centres for Disease Control and Prevention (CDC) report, the cases of human HGA have risen from 350 cases in 2000, to 5,762 cases in 2017 globally since it was first reported.

Anaplasma phagocytophilum infection in man and animals is widely distributed in the United States of America, Africa, Southern Europe, South and Central America, the Middle and the Far East (Dumler *et al.*, 2001; Severo *et al.*, 2015). The incidence has shot to 6.1 cases per million persons from 1.4 cases per million persons in 2010 and 2000 respectively in the United States (CDC, 2019). Afonso and Goydadin (2018) detected a 22.7% prevalence in Eastern France in the horseshoe bat (R*hinolophus hipposideros*). Feral cats in Massachusetts had a prevalence of exposure of 9.7% and that of acute infection being 6.9% (Galemore *et al*., 2018). A 17.2% infectivity of *Anaplasma* species (assigned to *A. phagocytophilum*) in brazilian brown brocket deer (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*) was reported in Brazil (Silveira *et al.*, 2011).

In Africa, six species have been reported including *Anaplasma marginale, A. ovis* and *A. centrale* (Inokuma *et al*., 2001; Kocan *et al*., 2004; Renneker *et al*., 2013), *A. bovis* Atif (2016), *A. platys* Ramos *et al.*,(2014) and *A. phagocytophilum* (Dumler, 2012). Similar to

Africa, Europe has the same species (Aguirre *et al*., 2004; Dreher *et al.,* 2003; Mylonakis *et al.,* 2004) while Asia has six of what is in Europe (Inokuma *et al,*. 2001**).**

Infections of *A. phagocytophilum* has barely been reported especially in Africa. However, its exposure through antibody detection have been reported widely among wild animal species with 13% seroprevalence in free- ranging NHPs in Zambia (Nakiyima *et al*., 2014). A 2.7% prevalence was reported in cattle in Ethiopia (Teshale *et al.,* 2018). In Kenya, it is ranked among the most constraining diseases in the beef and dairy cattle industries because of the economic implication felt from the cost of treatment, prevention and control (Wesonga *et al*., 2010). In Kenya, only one study reports the infection in *Rhipicephalus maculatus* ticks (Mwamuye *et al.,* 2017).

Surveillance on zoonotic pathogens circulating in wild animals has proven to aid in rapid detection and prevention of potential spillovers. Through the One health approach, it is important to prioritise disease investigation and surveillance on key wild animals especially those in close contact with humans and livestock (Bean *et al.,* 2013). While *Anaplasma* in NHPs has been reported in some countries, its importance in NHPs in Kenya is not yet known. Therefore, this study aimed to establish the presence of *Anaplasma phagocytophilum*, genetically characterising it and identifying its potential risk factors in olive baboons and vervet monkeys in Laikipia County, Kenya.

1.2 Hypotheses

Anaplasma phagocytophilum is present in olive baboons and vervet monkeys in Laikipia County, Kenya and is genetically diverse.

1.3 Objectives

1.3.1 General objective

To determine the prevalence and genetic characterization of *Anaplasma phagocytophilum* in olive baboons and vervet monkeys in Laikipia County Kenya.

1.3.2 Specific objectives.

1. To determine the prevalence of *Anaplasma phagocytophilum* in olive baboons and vervet monkeys in Laikipia County, Kenya.
2. To genetically characterize *Anaplasma phagocytophilum* in olive baboons and vervet monkeys in Laikipia County, Kenya.
3. To determine potential risk factors for *Anaplasma phagocytophilum* in olive baboons and vervet monkeys in Laikipia County, Kenya.

1.4 Justification

It is widely believed that the main reservoirs of disease-causing pathogens in man, farm and companion animals are wild animals (Baneth, 2014). Non-human primates are closely related to man phylogenetically, they inhabit most of the ecosystem and are implicated in most of the listed emerging human pathogens such as retroviruses, filoviruses, foamy virus, coronaviruses amongst others (Jones *et al.,* 2008; Nunn & Altizer, 2006; Shereen *et al.,* 2020),

Interaction between animal and man facilitates the urban cycle of zoonotic transmission through tourism, farming near wildlife conservancies and occupation of the wildlife movement corridors. Laikipia is a perfect area where this interface is seen. It has the largest livestock keeping community in Kenya, the Maasai, group ranches both private and public and wildlife conservancies. Here, there is contact among man, livestock and wildlife.

The socioeconomic implications resulting from loss of production in livestock, cost of treatment, control and potential impingement of manpower due to disease also prompts the need to investigate pathogens in NHPs especially those around the human settlements (Nakayima *et al*., 2014). After all, a precursor to disease emergence in man and domestic animals is often attributed to wildlife maintaining pathogens and subsequent spill-over to livestock.

With limited information on *A. phagocytophilum* in Olive baboons and Vervet monkeys in Kenya, the recent rise in cases of HGA, persistence of Anaplasmosis in livestock despite vigorous tick control measures in livestock herds Granquist *et al*., (2010), justifies the study further. Epidemiological evidence may facilitate a better understanding of the mechanisms which the pathogen is spread in host populations. Therefore, this study aimed to investigate olive baboons and vervet monkeys potential as reservoir for *A. phagocytophilum*

CHAPTER TWO

2.0 LITERATURE REVIEW

Emerging infectious diseases (EIDs) of zoonotic origin constitute a recurrent threat to global health. Seventy five percent of them are considered to have originated from animals or products of animal origin (Allen *et al*., 2017). Interactions between man, wildlife and livestock can potentiate cross-species transmission and spill-over of diseases from reservoirs to new hosts species resulting in emergence of regional epidemics or global pandemics (Jones *et al.,* 2008). Therefore, a One Health approach should be applied at the human-animal interface by undertaking epidemiological surveillance and developing strategies on prevention and control of emerging and re-emerging zoonotic diseases (Wang & Crameri, 2014).

2.1 *Anaplasmosis*

Anaplasmosis is a tick-borne disease. It is caused by an alpha-proteobacteria from the genera *Ehrlichia;* an obligate and intracellular bacterium. *Ehrlichia canis* was first detected clinically as a disease in dogs in 1935 in Algeria (Donatien and Lestoquard, 1935). Thereafter, it gained acknowledgement as one of the important diseases in the tropics and subtropics in dogs and other canids (Granick *et al*., 2009). The knowledge of the existence of *A. phagocytophilum* has been there for over 200 years. However, it became a disease of focus after the first case of HGA was reported in 1986 (Maeda *et al.,* 1987).

2.2 Taxonomic classification

Anaplasma hemoparasites belong to the family of *Anaplasmataceae,* order of *Rickettsiales,* class *Alphaproteobacteria and* genus *Anaplasma* (Dumler *et al*., 2001). Some *Anaplasma* species previously belonged to *Ehrlichia* genus. However, with the advancement in molecular techniques from recent genetic analyses of 16S rRNA genes, surface protein and

groESL genes they have been reclassified (Dumler *et al.,* 2001). This resulted in the inclusion of *A. phagocytophilum* into the genus whose members are only pathogens with host specificity to ruminants such as *A. marginale* (Kocan *et al*,. 2004). Therefore, *Anaplasma* species include *Anaplasma marginale, A. phagocytophilum* (formerly *E. phagocytophilum), A. centrale, A. bovis* formerly *E. bovis, A. ovis,* and *A. platys* (formerly *E. platys)* (Kocan *et al*., 2003)

2.3 Morphology

Anaplasma phagocytophilum is an obligate intracellular bacterium which is gram-negative and about 1.3µm in size. In shape, it is pleomorphic coccoid to ellipsoidal and is enveloped by two membranes for most of the members of the family (Rikihisa, 2011). It often occurs in morulae which are tightly packed formulations and in hematopoietic cells in domestic animals (Dumler *et al*., 2001). They appear as inclusions mainly seen in neutrophils (Rikihisa, 2011). There are two types of the bacterial cells on microscopic examination; dense and reticulate (Rikihisa, 2011). On the outer membrane surface of *A. phagocytophilum*, there are major antigenic membrane proteins which include p44 and msp2 that are responsible for its virulence (Quan Lin, 2006). Genomically, *A. phagocytophilum* has only one annular genome with 1471282 base pairs and 1264 protein genes with no known plasmids (Felsheim, 2006).

2.4 Host range

Anaplasma phagocytophilum has a wide host range including horses (*Equus caballus*) Dzięgiel *et al*., (2013), cats (*Felis catus*) Little (2010), sheep (*Ovis*), cattle (*Bos Taurus*), goats (*Capra aegagrus hircus*) Woldehiwet (2010), dromedary camels (*Camelus dromedaries*) and llama (*Lama glama*) (Bahrami *et al*, 2018). A number of wild animals have been noted as reservoirs of the bacteria (Kocan *et al.*, 2015). For instance, in horseshoe bat

(*Rhinolophus hipposideros*) Afonso and Goydadin (2018), Galemore *et al*., (2018), brazilian brown brocket deer (*Mazama nemorivaga*) and marsh deer (*Blastocerus dichotomus*) Silveira *et al.,* (2011) and yellow baboons (*Papio cynocephalus*) (Nakayima *et al*.,2014). In Europe, its' establishment in red foxes (*Vulpes vulpes*), small mammals and wild boars (*Sus scrofa*) is vague (Stuen *et al.,* 2013). Raccoon (*Procyon lotor*) and the gray squirrel (*Sciurus carolinensis)* have also been implicated Carlyon *et al*., (2003) with humans being accidental hosts (Bakken *et al*., 1994).

2.5 Transmission and life cycle

Ixodid ticks are important in maintenance of *A. phagocytophilum* (Zaid *et al*., 2019). However, other tick genera such as Rhipicephalus, Dermacentor, and Amblyomma are the main vectors of *Anaplasma* and/or *Ehrlichia* bacteria in different parts of the world (Rar & Golovljova, 2011). The ticks lodge on the skin using their mouth parts which lacerate the skin and takes a blood meal through their hypostome. The tick acquires *Anaplasma* while feeding on blood from an infected host and transmits it to other hosts as they take the next blood meal since saliva from salivary glands and midgut contain the parasite (Zhi *et al*., 2002).

Anaplasma then accesses the bloodstream after the tick bite reaching the intracellular environment. Lipid rafts, which are signalling platforms are needed for the parasite to enter and cause infection intracellularly (Xiong and Rikihisa, 2010). It then multiplies strictly within the cell membrane-derived vacuole which is in the cytoplasm of the mammalian host cell, colonizing it (Hosseini-Vasoukolaei *et al.,* 2014). Vertebrates are the main reservoirs for *Anaplasm*a bacteria (Hosseini-Vasoukolaei *et al.,* 2014). For *A. phagocytophilum* to enhance its infection and multiplication it has been shown to affect the glucose metabolic pathways by interfering with the metabolism of carbohydrates (Cabezas-Cruz *et al*., 2017).

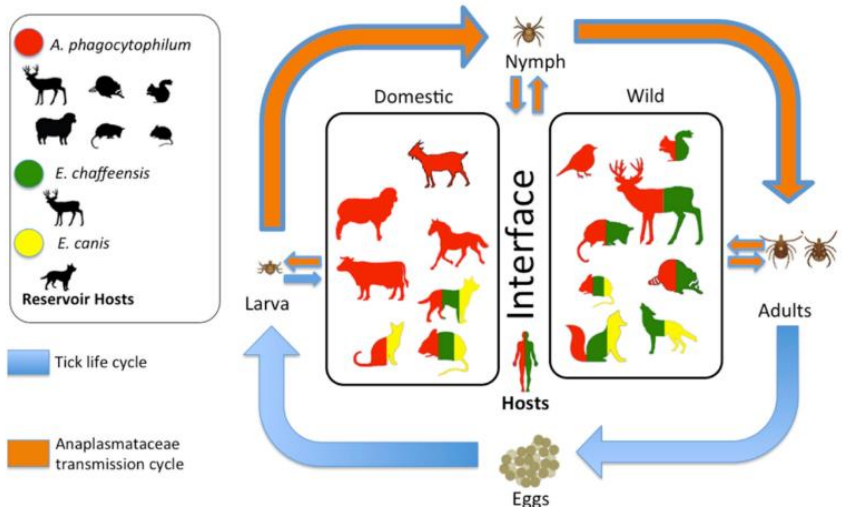

Figure 2. 1: Transmission and life cycle of Anaplasma

Source: Rojero-Vázquez *et al*., 2017).

2.6 Strain variation

Regarding strain variation, there appears to be existence of serological cross-reactivity in strains of *A. phagocytophilum* with 42- to 49 kDa proteins antigens being highly produced and expressed on the outer membrane of the bacteria (Wuritu *et al.*, 2009). The encoding of these proteins is by the p44 gene family which is the primary antigenic protein of *A. phagocytophilum*. It is also highly polymorphic (Barbet *et al*., 2006). A slight level of variation is seen in the nucleotide sequences of the 16S rRNA, groESL, gltA, ank, and msp2 genes (Lin *et al.*, 2004).

The strains clearly differ and not all appear to be capable of infecting humans or mice or to cause persistent infections (Massung *et al*., 2003). There is also a difference in the host infectivity of *A. phagocytophilum*. For instance, Foley *et al*., (2009) reported variation on host infectivity of *A. phagocytophilum* strains from wild rodents and Stannard *et al*., (1969) reported that strains infectious to equines were non-infectious to ruminants.

2.7 Disease in animals

In the natural cycle of *A. phagocytophilum*, a number of animals are believed to serve as reservoirs for this bacterium (Dugat *et al.*, 2015). The high infection rates seen in wild ruminants mainly Toe deer (*Capreolus capreolus*) in Germany resulted in the suggestion that they may be the substantial wild reservoir hosts (Overzier *et al.*, 2013).

Among the even-toed ungulates, infection is mostly seen in sheep and cattle, but it can also be detected in deer, reindeer (*Rangifer tarandus*), and goats (Atif, 2016). Most cases present with symptoms which include fever, anorexia, diarrhoea, leukopenia and thrombocytopenia (Dumler *et al.*, 2001). In cattle, it causes lowered milk yield, weight loss, coughing and reproductive symptoms (M'ghirbi *et al*., 2016). In immunocompromised sheep, *A. phagocytophilum* is able to survive and propagate for several months (Granquist *et al*., 2010). Other signs include, congested oral mucous membranes, digestive disturbances, malaise, swelling of facial area, lacrimation, mucoid nasal discharge and syncope (Center for Food Security and Public Health, 2013)

Infected animals have a higher susceptibility to secondary infections (Atif, 2016). Yellow baboons experimentally infected with *A. phagocytophilum* have been shown to develop fever, lethargy, anaemia, thrombocytopenia and neutropenia (Nakiyima *et al*., 2014). There has been confirmation of *A. phagocytophilum* in lesser horseshoe bat guano, four insectivorous bat species Leschenault's Rousette (*Rousettus leschenaultia*) in India and South Africa respectively (Afonso & Goydadin, 2018; Banskar, 2016; Dietrich, 2017). However, the bacterium hasn't been proven to cause disease in the bats.

2.8 Disease in man

Anaplasma phagocytophilum is zoonotic with a wide range of hosts (Dumler, 2012). Despite man being an accidental host, the bacterium still causes disease and is referred to as

human granulocytic anaplasmosis (HGA) (Bakken *et al.*, 1994). Clinical manifestation of HGA include pyrexia, shivering, headache, depression and myalgia (Dumler and Brouqui, 2004). In both animal and human hosts, it can cause influenza- like symptoms (Ismail *et al*., 2010). However, the gravity of the disease ranges from asymptomatic to death in extreme cases in man (Bakken *et al.,* 1994). In Morocco, dog owners were reported having been infected with *A. phagocytophilum* (Elhamiani Khatat *et al.,* 2017).

2.9 Diagnostic techniques

2.9.1 Direct microscopy

This is the simplest technique for detection and it involves observing a blood smear stained with Giemsa or Wright through a microscope from animals clinically showing the disease acutely (Ribeiro & Passos, 2002). For a positive smear, what is observed is a cluster of bacteria called morulae within cytoplasmic vacuoles in peripheral blood neutrophils for *A. phagocytophilum (*Dumler, 2005)

2.9.2 Immunohistochemistry

This diagnostic technique is seldom used to examine blood and tissues to detect specific antibodies because unfortunately, it might not be readily available commercially. There is limited information on this technique (OIE, 2008)

2.9.3 Serology

This method is often used to detect the presence of antibodies (exposure rates) especially for animals not exhibiting clinical signs and symptoms. For the epidemiological studies of *Anaplasma*, a number of serological tests have been employed despite the fact that they do not differentiate varied *Anaplasma* species resulting often in cross-reactivity among the species (Schotthoefer *et al*., 2013). Competitive enzyme-linked immunosorbent assay

(cELISA) often detects anti-major surface protein 5 (anti-MSP5) of *Anaplasma* species as it reliably establishes a carrier state (OIE, 2008).

Indirect fluorescent antibody (IFA) is another technique commonly used as it is highly sensitive and specific. However, the fact that there may be a reaction with other autoimmune antibodies renders it faulty (Dumler & Brouqui, 2004).

2.9.4 Molecular diagnosis

Polymerase chain reaction (PCR) has been the ultimate diagnostic tool for clinical and environmental cases of anaplasmosis because it is fast and easy to carry out (Dumler and Brouqui 2004). It is used for genetic detection and characterization of strains of *A. phagocytophilum* infection (Lee *et al*., 2018). This diagnostic technique focuses on amplifying nucleic acids specific for *Anaplasma* species. Genetic characterization by use of DNA markers and comparing it with the gene bank allows us to know the genetic variation, phylogenetic and molecular structure of the pathogen (Dumler *et al*., 2001). Different PCR assays are available and with this comes a lot of variation in their performances with regards to their sensitivity or specificity Yang *et al*., (2016), the ability to detect minute amounts of nucleic acid and specific nucleic acid fragments (Dumler and Brouqui 2004; Massung & Skater, 2003).

In this study, the aim was to characterize genotypes of *A. phagocytophilum*, hence the choice of p44 gene (major surface protein 2 (msp2) gene) assay. This assay has been shown to be very specific when detection of specific nucleic acid fragments is desirable as it enables distinction of closely related strains (Massung & Slater, 2003). However, this may negatively impact detection since msp2 genes are less sensitive Yang *et al.,* (2016) especially in cases where previous disease status of the patients is unknown (Barbet *et al*., 2006).

Another useful assay is the use of ribosomal RNA genes such as 16S rRNA which are conserved greatly among all bacterial species Massung *et al*., (2003) and are shown to be the most sensitive assay in detecting *A. phagocytophilum* (Yang *et al.*, 2016). This makes them very useful in molecular diagnostics. However, because 16S rRNA sequences are highly similar, it is not recommended since they are not sufficiently informative when distinguishing genotypes of *A. phagocytophilum* (Bown *et al.,* 2009).

These methods include conventional PCR which targets msp2 or 16S rRNA gene of *A. phagocytophilum* to identify animals that have been infected naturally and real-time PCR (qPCR) which was recreated to detect more than one species (Massung & Slater, 2003). Loop-mediated isothermal amplification (LAMP) assay is often used to rapidly detect *Anaplasma* using primers specific to 16S rRNA gene of this organism as it amplifies novel nucleic acid. It is cost-effective and a simple detection technique (Notomi *et al*., 2015). The cost efficiency and infrastructure available in developing countries like Kenya make molecular diagnosis the method of choice beside it being highly sensitive and specific.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study area was based on a larger project (USAID Predict II) whose aim was to collect more-targeted and longitudinal information to support development of interventions to mitigate the risk of spill over, amplification, and spread of zoonotic viruses with pandemic potential. The focus was on interfaces and locations considered as high risk, where man and animals share environment and novel diseases continue to be detected

The study area, Laikipia County (Figure 3.1) located in the Rift Valley of Kenya (005'North 36040'East), was picked as one of the locations after looking into risk factors such as the behaviour of humans, as well as the effects of population growth, change in land-use, and other factors that potentiates disease emergence and spread. It is a risk interface area because of interaction between man and animals as it is largely inhabited by pastoralists who keep large herds of livestock and the huge wildlife population.

Among the diverse range of wildlife in the area, NHPs constitute a high percentage with eight species found in the area. The sampling sites Mpala research Centre and Ol jogi Conservancy (Figure 3.1) were conveniently chosen since large populations of the target species; olive baboons and vervet monkeys, inhabit these sites.

Figure 3. 1: Map of the sampling sites

(Source: QGIS version 3.20.2)

3.2 Sample population

Olive baboons and vervet monkeys were chosen because they are closely related to man phylogenetically and are implicated in most of the listed emerging human pathogens. They also inhabit most of the ecosystem because of their social nature and adaptability and are often seen close to human settlements.

Figure 3. 2: Olive baboons (Right) and vervet monkeys (Left)

Source: Kenya Wildlife Service

3.3 Study Design

This study was cross sectional and it utilized samples collected during the USAID Predict II project.

3.4 Sample size determination

Some of the whole blood samples from wild olive baboons and vervet monkeys that had been collected by the larger project from Laikipia County were included in this study constituting the study's sample size of 146 olive baboon and 18 vervet monkey. Basic information about the samples were noted from records kept including; when and where the samples we collected, species collected from and the sex of animals sampled.

3.5 Ethical approval

Approval for this study was given by the Institute of Primate Research Institutional Scientific and Ethics Review Committee (ISERC): Ethical approval number ISERC/T01/18, 16th March 2018 (Appendix 1). Since the information collected for this study was not of a sensitive nature and non-invasive as banked blood samples were used, the ethical approval and guidelines received from this committee was deemed sufficient to conduct the study. The study followed laid biosafety guidelines on handling and processing of biological samples.

3.6 Laboratory analysis of samples.

3.6.1 DNA extraction

Whole blood samples stored in TRIzol reagent at -80^{o}c were retrieved and allowed to thaw at room temperature. Extraction of genomic DNA from each of the whole blood sample was done using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) following manufacturer's instructions. The incubator was heated to 56^{o}c and buffers AW1,

AW2 and AE were prepared prior. To extract DNA, 20µl of proteinase K was pipetted into 2ml micro-centrifuge tubes and100µl of anticoagulated whole blood added. The volume was adjusted to 220 µl with Phosphate Base Saline (PBS) into each tube. I added 200µl Buffer AL (without added ethanol), mixed them thoroughly by vortexing, and incubated at 56°C for 10 min.

To the samples 200µl of 96% ethanol was added, mixed thoroughly by vortexing and the mixture pipetted into DNeasy Mini spin columns placed in 2ml collection tubes. Centrifugation was done at 6000 x g (8000 rpm) for 1 min and discarded the flow-through and collection tubes. The columns were placed in new 2ml collection tubes, 500 µl Buffer AW1 was added and the last step repeated then 500 µl Buffer AW2 added, and centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membranes. The flow-through was discarded, collection tubes were placed in clean micro-centrifuge tubes and 100µl Buffer AE pipetted directly onto the DNeasy membranes. They were incubated at room temperature for 1 min, then centrifuged for 1 min at 6000 x g (8000 rpm) to elute. The eluted DNA samples were stored at -80^{o}c awaiting further analysis.

3.6.2 Nested polymerase chain reaction

The nested PCR technique was used to amplify 400bp fragment of *A. phagocytophilum* p44 gene using primer pairs: (Table 4.1). The final 25ul PCR for each reaction volume consisted of 18.25ul double distilled RNase/DNase free water, 2.5ul PCR buffer, 0.75ul 1.5Mm $Mgcl_2$, 0.5ul of 10um DNTPs, 0.5ul of 10um forward primer, 0.5ul of 10um reverse primer, 0.1ul of 2.5U Taq DNA polymerase and 2.0ul of the DNA template.

Using the first set of primers (p3709-p4257), the initial amplification thermo-cycling condition consisted of 40 cycles, each cycle consisting of initial denaturation process at 94^{o}c for 5 minutes, denaturation for 30 seconds at 94°C, annealing for 30 seconds at 52°C,

elongation for 1 minute at 72°C and final elongation at 72^{o}c for 5 minutes. For the nested PCR amplification using the second set of primers (p3761-p4183), 1ul of the product from the first amplification was used in a 25ul reaction mixture; the amplification consisted of 40 cycles, each of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C

Visualization using gel electrophoresis was done. The positive results were to be indicated by the appearance of the bands within the 300bp and negative, with absence of bands. All the negative samples were tested again to confirm that they were true negatives. They were subjected to a nested PCR and optimised thermo-cycling conditions after troubleshooting with the same primers as above.

3.6.3 Conventional PCR

All the negative samples were subjected to conventional PCR technique to amplify 345bp fragment of *Anaplasma* 16S rRNA gene using primer pair (Table 4.1). The final 25ul PCR for each reaction volume consisted of 18.25ul double distilled RNase/DNase free water, 2.5ul PCR buffer, 0.75ul 1.5Mm $Mgcl_2$, 0.5ul of 10um DNTPs, 0.5ul of 10um forward primer, 0.5ul of 10um reverse primer, 0.1ul of 2.5U Taq DNA polymerase and 2.0ul of the DNA template.

The thermo-cycling condition consisted of 40 cycles, each cycle consisting of initial denaturation process at 94^{o}c for 5 minutes, denaturation at 94^{o}c for 45 seconds, annealing at 52°C for 30 seconds, elongation at 72^{o}c for 45 seconds and final elongation at 72^{o}c for 5 minutes.

3.6.4 Gel electrophoresis

The PCR products were then electrophoresed using 1.5% agarose gel in the TAE buffer and thereafter stained using ethidium bromide dye. The amplicons were loaded in the gel after mixing with DNA loading dye then visualised using UV- illuminator. The size of the

amplicons were determined using DNA ladder ranging from 100 to 1000bp. The positive results were indicated by the appearance of the bands within the 345bp and negative, with absence of bands.

Below are the primer sets (forward and reverse) used in the study with their target genes, expected size of amplified amplicons and the annealing temperatures.

Target genes	Primers	PCR amplicons	Annealing temp	References
p44	p3709 5_-GCTAAGGAGTTAGCTTATGA p4257 5_AGAAGATCATAACAAGCATTG	400	52°C	Lin *et al*., 2002
	p3761 5_ CTGCTCTKGCCAARACCTC p4183 5_-CAATAGTYTTAGCTAGTAACC	300	52°C	Lin *et al*., 2002
16S rRNA	EHR16SD: GGTACCYACAGAAGAAGTCC EHR16SR: TAGCACTCATCGTTTACAGC	345	52°C	Parola *et al*.,2000

Table 3. 1: Target genes and their respective primer pairs

3.6.4 Purification of positive amplicons

The resulting positive PCR products were subjected to purification using Thermo Scientific GeneJET PCR Purification Kit#K0701, #K0702 Protocol. The centrifugation was done at room temperature in table-top micro-centrifuges at >12000 □g (10000-14000 rpm, following the manufacturer's instructions. A 1:1 volume of Binding Buffer to completed PCR mixture was added and mixed thoroughly. A 1:2 volume of 100% isopropanol was added since the DNA fragment was 345bp. Up to 800 µl of the solution from step 2 was transferred to the GeneJET purification columns and centrifuged for 30-60 seconds, then flow-through discarded. 700 µl of wash buffer diluted with the ethanol was added to the columns, centrifuged for 30-60 seconds, discarded the flow-through and placed into new micro-

centrifuge tubes. 50µL of elution buffer was added to the centre of the column membranes and centrifuged for 1 minute. The columns were discarded and purified DNA stored at -20°C.

3.6.5 Sequencing of purified PCR products

Ten purified PCR amplified fragments targeting 16S rRNA gene were sequenced. The Sanger sequence was performed using an ABI PRISM 377 Genetic Analyzer (Applied Biosystems, USA). This utilized forward and reverse primers of each PCR assay. Sequence assembly for forward and reverse primers was done using DNA Sequence Assembler v4 (2013), Heracle BioSoft (www.DnaBaser.com).

The sequences were matched to those deposited in the GenBank database using BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment of the sequences was done using BioEdit Sequence Alignment Editor (Hall, T.A. 1999). Confirmation of those species was established as the nearest BLASTn match with an identity of between 81 and 100% to those homologues found in the GenBank. Construction of phylogenetic tree was done using Muscle 3.8 using the neighbor-joining method and visualization of the trees with FigTree v1.4.4 (http://tree.bio.ed.ac.uk/)

3.7 Statistical analysis of data

The data was entered into a statistical software, R studio version 1.2.1335. Statistical significance was determined with a cut-off P-value of 0.05 for the analysis. Descriptive statistical analysis was done. Prevalence was calculated as a measure of the number with outcome of interest over the total sampled. Statistical association between the potential risk factors and the outcome was determined by using the Odds ratio (OR), Chi square and Fisher's exact test.

4.0 RESULTS

4.1 Polymerase chain reaction results for *Anaplasma phagocytophilum*

Molecular analysis nested PCR targeting *A. phagocytophilum* p44 gene yielded negative results for all the 164 whole blood samples olive baboons and vervet monkeys. Using conventional PCR and targeting *Anaplasma* species 16S rRNA gene (Table 4.1) to screen for *Anaplasma* species on all the 164 samples yield positive results. Visualization using gel electrophoresis shows amplicons at the specific band of approximately 345bp. Lane L is DNA marker, the first lane (N) is the negative control. The arrow indicates the position of the expected amplicons (Figure 4.1).

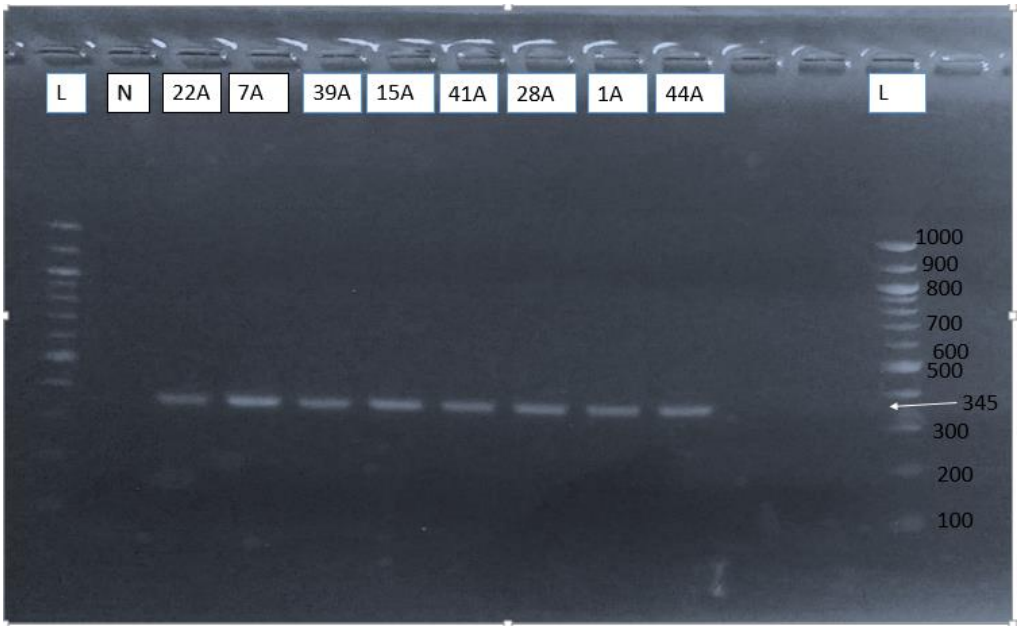


Figure 4. 1: Gel image from conventional PCR amplification of *A.phagocytophilum* using 16S rRNA gene primers

Thirty out of 164 blood samples were gel positive for the *Anaplasma* species; one from Ol jogi conservancy and 29, Mpala research centre.

Samples	**Species**	**Location**	**Samples**	**Species**	**Location**	**Samples**	**Species**	**Location**
7A	Baboon	Mpala	**N4**	Baboon	Mpala	**M33**	Baboon	Ol jogi

15A	Monkey	Mpala	**N28**	Baboon	Mpala	**O35**	Baboon	Mpala
M32	Baboon	Mpala	**015**	Baboon	Mpala	**O37**	Baboon	Mpala
39A	Monkey	Mpala	**016**	Baboon	Mpala	**O39**	Baboon	Mpala
22A	Baboon	Mpala	**41A**	Baboon	Mpala	**O41**	Baboon	Mpala
44A	Baboon	Mpala	**O59**	Monkey	Mpala	**O42**	Baboon	Mpala
28A	Baboon	Mpala	**O60**	Baboon	Mpala	**O44**	Baboon	Mpala
1A	Monkey	Mpala	**O61**	Baboon	Mpala	**O50**	Baboon	Mpala
64A	Baboon	Mpala	**O63**	Baboon	Mpala	**O54**	Baboon	Mpala
A28	Baboon	Mpala	**N1**	Baboon	Mpala	**O56**	Baboon	Mpala

Table 4. 1: Samples positive for Anaplasma species

4.2 DNA sequencing of positive samples

The PCR products from ten samples positive with the primer set EHR16S rRNA for *A. phagocytophilum* were sequenced. Out of all the samples, nine generated nucleotide sequences suitable for further analysis but one did not have enough DNA to allow sequencing. Bioinformatics analysis by BLAST method of three sequences of the EHR16S rRNA gene revealed homologues that were identical to *A. phagocytophilum*. Incidentally, another four of the sequences revealed homologues identical to *A. platys*, while one revealed homologues identical to *Candidatus anaplasma camelli*. They all shared 99% to 100% nucleotide similarity (Table 4.2).

Samples sequences	**Animal species**	**Homologous sequences**	**E values**	**Identity (%)**
7A	Olive Baboon	*A.platys*	7	98.53
41A	Olive Baboon	*A.phagocytophilum*	4	99.39
39A	Vervet monkey	*A.phagocytophilum*	3	99.39
22A	Olive Baboon	*A.platys*	3	100

44A	Olive Baboon	*Candidatus anaplasma spp*	1	99.38
28A	Olive Baboon	*A.phagocytophilum*	4	100
1A	Vervet monkey	*A.platys*	2	99.41
64A	Olive Baboons	*A.platys*	2	98.87

Table 4. 2: Results of *Anaplasma* species identified by BLASTn analysis using 16S rRNA sequences of the isolates from olive baboons and vervet monkeys

4.3 Results of nucleotides sequence alignment

4.3.1 Multiple sequence alignment of *A.phagocytophilum* with others from other regions.

Multiple alignment of *Anaplasma* nucleotide sequences of the *A.phagocytophilum* isolates revealed that all the sequences of samples from baboons were conserved expect the following: for our isolates, nucleotides at the first 3 positions and at position 174 differ with the rest of the sequences. At position 304, 39A differs with the rest and at the following positions 378, 381- 384,386-388 and 391, 39A and 41A differ with rest (Figure 2). (Appendix 5). The sequences of the *A. phagocytophilum* isolates from Kenya were identical to those from Japan, South Korea, France, China, South Africa and Denmark. The species isolates were from human, cattle, ticks, dogs and rodents. The accession numbers JQ622148.1, MF351963.1, KU559922.1, MG519284.1, KX810088.1, MH122888.1, MK814411.1, MK814407.1, MF582329.1, MK814412.1, MK271308.1, MH122891.1, AY776165.1, KT986058.1, KU513793.1, KR611718.1 obtained from the GenBank, are for *A.phagocytophilum* 16S rRNA sequences of isolates from Japan, South Korea, France, China, South Africa and Denmark. The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters representing the nucleotide A, C, G and T (Figure 4.2).

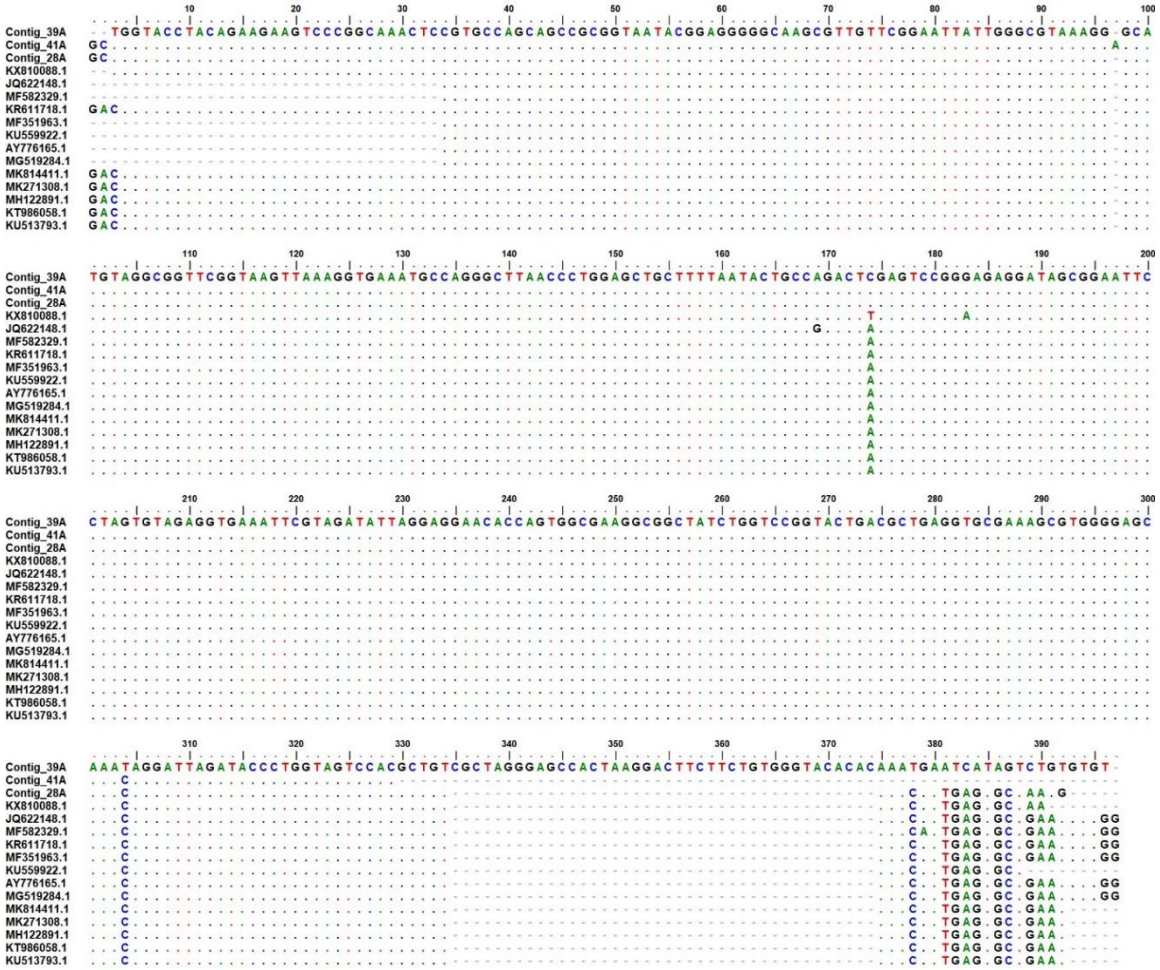


Figure 4. 2: A multiple sequence alignment of 16S rRNA gene fragments for comparison of *A. phagocytophilum* isolates from Olive baboons and vervet monkeys

4.4 Phylogenetic analysis

To generate the phylogenetic tree, neighbor joining bootstrapping method at 1000 replications was used. The phylogenetic tree was constructed by the Muscle3.8 software and visualization with FigTree v1.4.4 software.

4.4.1 Phylogenetic analysis of the Kenyan *Anaplasma phagocytophilum* isolates

A phylogenetic tree was constructed in order to further understand the evolutionary relationship of the *A.phagocytophilum* isolates from Kenya with those the rest of the world. The sequences of the *A. phagocytophilum* isolates from Kenya were identical to those from

Japan, South Korea, France, China, South Africa and Denmark. The species isolates were from human, cattle, ticks, dogs and mice. The accession numbers JQ622148, MF351963, KU559922, MG519284, KX810088, MH122888, MK814411, MK814407, MF582329, MK814412, MK271308, MH122891, AY776165, KT986058, KU513793, and KR611718 were all of *A. phagocytophilum* isolates (Figure 4.3). Phylogenetic analysis revealed that the isolates from Japan, South Korea, France, China, South Africa, Denmark and Poland belonged to clade I but have recent common ancestor with the Kenyan isolates clustered into one clade II.

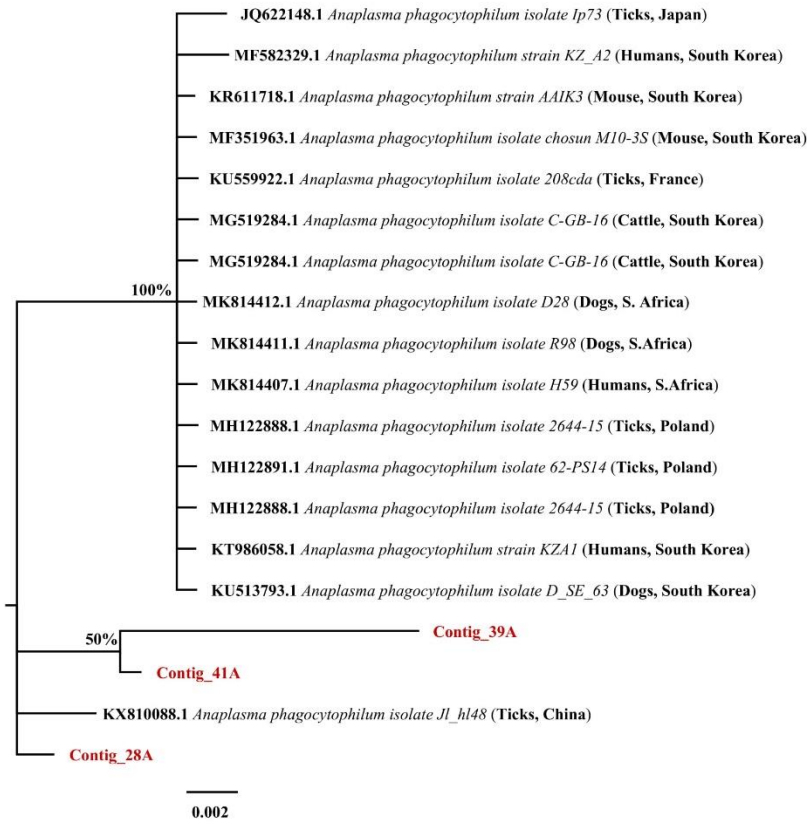


Figure 4. 3: Phylogenetic tree of the 16S rRNA gene from *A.phagocytophilum* isolated from Olive baboons and vervet monkeys in Laikipia County, Kenya and from other regions of the world.

4.5 Distribution of samples among olive baboons and vervet monkeys in Laikipia County

The samples included in this study were distributed as shown in the table 4.3.

Variables	Distribution	Number sampled
Sex	Male	87 (53%)
	Female	77 (47%)
Season	Wet	95 (57.9%)
	Dry	69 (42.1%)
Location	Mpala	104 (63.4%)
	Ol jogi	60 ((36.6%)
Species	Olive baboons	146(89%)
	Vervet monkeys	18 (11%)
Total		164

Table 4. 3: Distribution of samples

4.6 Descriptive statistics

To describe the distribution of *Anaplasma* species and the variation of its frequency among a set of variables identified as potential risk factors: sex, location, species and season without regard to causal or other hypotheses, descriptive statistics was done and summarized in the table 4.4.

Variables	Positive/examined	Prevalence %
Sex		
Male	17/87	9.6
Female	13/77	16.9
Season		
Wet	25/95	36.2
Dry	5/69	5.3
Location		
Mpala	29/104	27.9
Ol jogi	1/60	3.33
Species		
Olive baboons	26/146	17.8
Vervet monkeys	4/18	22.2

Table 4. 4: Prevalence of *Anaplasma* species

5.0 DISCUSSION

This study's primary aim was to determine the prevalence and risk factors of *Anaplasma phagocytophilum,* as well as carry out molecular characterization of the detected microorganisms from olive baboons and vervet monkeys in Laikipia County, Kenya. To the best of our knowledge, this might be the first study to investigate the molecular presence of *A. phagocytophilum* in olive baboons and vervet monkeys in Kenya and it proves that *A. phagocytophilum* is present in olive baboons and vervet monkeys' populations.

In this study, a total of 164 samples were tested and an overall prevalence for anaplasma infection was 18.3%. Having that this was a convenience sample from only two sites, comparison of this study with others may not be valid. Additionally, there is only one other available study on yellow baboons from Zambia which reported a 13% prevalence (Nakiyima *et al*., 2014). This proved to be a limiting factor to the current study as there is no sufficient data to draw comparison with.

Anaplasma organisms have been widely investigated, identified and characterized through a variety of techniques including molecular methods. In this study, primers targeting p44 and 16S rRNA genes of *A. phagocytophilum* were identified from previous studies (Lin *et al*., 2002; Parola *et al*., 2000) and used for detection and characterization of *A. phagocytophilum* species in olive baboons and vervet monkeys.

The primers selected for amplification of the p44 gene at approximately 400bp fragments did not yield any positive bands on PCR. Failure of the p44 gene to be amplified can be explained by the high variability of p44 gene, which includes variability among species, with resulting protein polymorphism and development of antigenic variations (Barbet *et al*., 2006; Dumler *et al*., 2005). Therefore, caution is advised in the use of msp2

(p44) genes for molecular typing and epidemiological analyses of different strains (Carter *et al*., 2001), as there is no sufficient data on how stable the different multigene loci are.

Conventional PCR targetting16S rRNA gene yielded positive results. Ribosomal RNA genes are conserved greatly among most if not all bacteria; therefore, in molecular diagnostics, pathogen identification and phylogenetic research they are important (Massung *et al*., 2003). The primers detected *A.phagocytophilum* with 16S rRNA gene fragment seen as distinct bands of 345bp indicating the presence of *A.phagocytophilum* in olive baboons and vervet monkeys.

The results concurred with previous studies that detected *Anaplasma* species using the primers targeting 16S rRNA gene including in baboons and Rhesus macaques in Zambia Nakiyama *et al.,* (2014), *A phagocytophilum* in humans and dogs in Morocco Elhamiani Khatat *et al* (2017), and Teshale *et al (*2018) who used it to detect *Anaplasm*a in cattle in Ethiopia. This reinforced that 16S rRNA gene may be a good marker for detection of *Anaplasma*.

The above data confirms the relevance of the role played by olive baboons and vervet monkeys as hosts and reservoirs for *Anaplasma* organisms as shown by detection of *A. phagocytophilum, A. platys* and one *Candidatus anaplasma camelli.* This was indicative of exposure of these baboons to ticks infected by *Anaplasma* suggesting a possible co-infection.

Blast analysis of the 16S rRNA gene fragments sequenced in this study revealed nucleotides homologous to those of *A. phagocytophilum* for samples from ticks, cattle, humans, dogs and rodents. These findings indicated the infection of olive baboons and vervet monkeys with *A. phagocytophilum* in Kenya is consistent with that from yellow baboons in Zambia (Nakiyama *et al.,* 2014).

In the analysis of *A. phagocytophilum* 16S rRNA gene, this study found that the sequences of 16S rRNA were highly conserved not only between isolates from Africa but also between those of world-wide origin and agreeing with previous studies (Zhang *et al.*, 2013). Most of the Kenyan *A. phagocytophilum* isolates appeared to cluster in the same but unique clade compared to other isolates from Japan, South Korea, France, China, South Africa and Denmark suggesting a genetic relationship amongst them except for one (15A) (Appendix 6). This is consistent with a previous study using phylogenetic analyses based on 16S rRNA gene (Bekker *et al.*, 2002; Nakiyama *et al.*, 2014; Yang *et al.*, 2016).

Studies have reported genetic relatedness between *A. phagocytophilum* infecting animals and humans (Arraga-Alvarado *et al.*, 2014; Elhamiani Khatat *et al.*, 2017). This is also emphasized by a recent study in South Korea that identified strains of *A. phagocytophilum* infecting man (Lee *et al*., 2018). Like in the current study, the nucleotide sequences for *A. phagocytophilum* from baboons appear to be genetically related to those of human (MF582329.1) (Appendix 6). Genetic variation among the isolates across the different geographical regions and animal species may result from factors such as climatic difference, environmental factors resulting in selection pressure or mutation.

A total of 164 samples, 146 from olive baboons and 18 from vervet monkeys were included in this study all from Laikipia County: 36.6% from Oljogi (60/164) and 63.4% from Mpala (104/164). Eighty nine percent were olive baboons (146/164) and the rest (18/164) 11% vervet monkeys. Of the 164 animal samples, males were 53% (87) while females were 47% (77). Female Olive baboons were 75 while 71 male and for vervet monkeys 6 were female and 12 male. Most of the samples were obtained during the wet season 57.9% (95/164) with 42.1% (69/164) during the dry.

The overall apparent prevalence for *anaplasmosis* was 18.3% (30/164*100). There was 17.8% prevalence of anaplasmosis in Olive baboons and 22.2% in vervet monkeys. Prevalence of 27.9% was recorded in Mpala and 3.3% in Ol jogi. These sites were conveniently selected by the USAID Predict II project because of the abundance of these animals in the sites. A prevalence of 36.6% was reported during the wet season than dry (5.3%). Therefore, rainfall (humidity) might seem to be an important macroclimatic factor influencing seasonal variation in tick infestation (Vatsya et al. 2007).

A prevalence of 19.5% was reported in male than female (16.9%). Our findings were similar to other studies which reported higher prevalence in male animals than female (Azmat *et al*., 2018); Javed *et al*., 2014). This can be attributed to the fact that estrogen hormone which is higher in female animals has been shown to be protective against bacterial infections as a first line of defense. This is through its immunomodulatory effects on epithelial cells (Medina-Estrada *et al*., 2018**)**.

Several factors; sex, season, species and location have been identified as potential risk factors. However, in this study no risk factors were determined because of limited number of samples positive for *A. phagocytophilum.*

The presence of *A. phagocytophilum* in olive baboons and vervet monkeys in close proximity to humans (wildlife conservancies, ranches) raises the question: to what extent is this bacterial presence in these baboon and monkey blood a concern for humans? The study localities where the samples were collected is a research center and conservancy with researchers, tourists and local community members who either work or grazed their livestock within. Therefore, this study shows that there is a need for an extensive bio-surveillance of these pathogens with particular attention to these groups of people and their livestock to evaluate the risk of disease transmission in such communities.

This could serve as a good indicator of bacteria circulation in ecosystem and explain the persistence of anaplasmosis in domestic animals despite consistent control. Therefore, epidemiological surveillance of NHPs' pathogens is important in generating information that can generate actions developing strategies on prevention and control of emerging and re-emerging zoonotic diseases (Nakayima *et al*., 2014)

The study established the presence of *A. phagocytophilum* in olive baboons and vervet monkeys' populations in Laikipia, Kenya, the genetic relationship among the Kenyan *A. phagocytophilum* isolates with others from different regions of the world, and the determination of risk factors for *A. phagocytophilum* in the sampled olive baboons and vervet monkeys.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The following conclusions were made based on the findings of this study;

- This study confirms the presence of *A. phagocytophilum in* olive baboons and vervet monkeys in Laikipia County, Kenya.
- Olive baboons and vervet monkeys from Laikipia County are infected with various *Anaplasma* species including *A. phagocytophilum, A. platys and Candidatus anaplasma camelli* with *A. phagocytophilum* being genetically distinct. Therefore, these animals are likely to play a significant role in the maintenance *A. phagocytophilum*

.

6.2 RECOMMENDATIONS

The following recommendations were made;

1. The role of olive baboons and vervet monkeys in the maintenance of *Anaplasma* organisms should be investigated further to establish the public health implications of zoonotic *A. phagocytophilu*m isolates.
2. Expansive bio-surveillance studies with a larger sample size of these NHPs and including the communities and livestock in Laikipia county be done to determine the factors that enhance the presence of *A. phagocytophilum.*

7.0 REFERENCES

A.G. Bean, M.L. Baker, C.R. Stewart, C. Cowled, C. Deffrasnes, L.F. Wang, et al. **(2013),** Studying immunity to zoonotic diseases in the natural host — keeping it real Nat Rev Immunol, **13** pp. 851-861, 10.1038/nri3551

Afonso E, Goydadin A-C (2018). Molecular detection of *Anaplasma phagocytophilum* DNA in the lesser horseshoe bat (*Rhinolophus hipposideros*) guano. Epidemiology and Infection **146**,1253–1258.https://doi.org/10.1017/S0950268818001279

Aguirre, E., A. Sainz, S. Dunner, I. Amusategui, L. Lopez, F. Rodriguez-Franco, I. Luaces, O. Cortes, and M. A. Tesouro. (2004). First isolation and molecular characterization of *Ehrlichia canis* in Spain. Vet. Parasitol. **125:** 365–372.

Allen, T., Murray, K. A., Zambrana-Torrelio, C., Morse, S. S., Rondinini, C., Di Marco, M., Daszak, P. (2017). Global hotspots and correlates of emerging zoonotic diseases. Nature Communications**, 8(1**). doi: 10.1038/s41467-017-00923-8v

Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, Hegarty BC, Breitschwerdt EB. (2014) Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. **91**:1161–5.

Atif, F.A., (2016). Alpha proteobacteria of genus *Anaplasma* (Rickettsiales: *Anaplasmataceae*): epidemiology and characteristics of Anaplasma species related to veterinary and public health importance. Parasitology **143**, 659–685.

Azmat, M., Ijaz, M., Farooqi, S. H., Ghaffar, A., Ali, A., Masud, A. Zhang, H. (2018). Molecular epidemiology, associated risk factors, and phylogenetic analysis of anaplasmosis in camel. Microbial Pathogenesis, **123**, 377–384. doi:10.1016/j.micpath.2018.07.034

Bakken, J.S., Dumler, J.S., Chen, S.M., Eckman, M.R., Van Etta, L.L., and Walker, D.H. (1994): Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? **272:** 212–218.

Baneth G (2014): Tick-borne infections of animals and humans: a common ground. Int J Parasitol. **44**:591-6.

Banskar Setal. (2016) Microbiome analysis reveals the abundance of bacterial pathogens in Rousettus leschenaultia guano. Scientific Reports6, 36948.

Barbet, A. F., Lundgren, A. M., Alleman, A. R., Stuen, S., Bjo¨ersdorff, A.,Brown, R. N., Drazenovich, N. L. & Foley, J. E. (2006). Structure of the expression site reveals global diversity in MSP2 (P44) variants in *Anaplasma phagocytophilum*. Infect Immun*74*, 6429–6437

Bekker, C. P. ., de Vos, S., Taoufik, A., Sparagano, O. A. ., & Jongejan, F. (2002). Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of Ehrlichia ruminantium in *Amblyomma variegatum* ticks by reverse line blot hybridization. Veterinary Microbiology, **89**(2-3), 223–238.

Bown, K. J., et al. (2009) Delineating *Anaplasma phagocytophilum* ecotypes in co-existing, discrete enzootic cycles. Emerg. Infect. Dis.**15**:1948–1954.

Brierley, L., Vonhof, M. J., Olival, K. J., Daszak, P. & Jones, K. E. (2016). Quantifying global drivers of zoonotic bat viruses: a process-based perspective. Am. Nat **187**, E53–E64

Cabezas-Cruz, A., Espinosa, P. J., Obregón, D. A., Alberdi, P., & de la Fuente, J. (2017). *Ixodes scapularis* Tick Cells Control *Anaplasma phagocytophilum* Infection by Increasing the Synthesis of Phosphoenolpyruvate from Tyrosine. Frontiers in Cellular and Infection Microbiology, **7**. doi:10.3389/fcimb.2017.00375

Carlyon, J. A., and E. Fikrig (2003): Invasion and survival strategies of *Anaplasma phagocytophilum.* Cell. Microbiol. **5:**743–754.

Carter, S. E., M. D. Ravyn, Y. Xu, and R. C. Johnson. (2001). Molecular typing of the etiologic agent of human granulocytic ehrlichiosis. J. Clin. Microbiol. **39**:3398–3401.

Center for Disease Control and Prevention (CDC). (2019, February 19). Anaplasmosis. *Epidemiology and statistics*. Retrieved from https://www.cdc.gov/anaplasmosis/stats/index.html

Dietrich Metal. (2017). The excreted microbiota of bats: evidence of niche specialisation based on multiple body habitats. FEMS MicrobiologyLetters364, fnw284.

Donatien A, Lestoquard F. 1935 Existence en Algerie d'une Rickettsia du chien. Bull Soc Pathol Exot.;**28**:418–9.

Dreher, U. M., R. Hofmann-Lehmann, M. L. Meli, G. Regula, A. Y. Cagienard, K. D. Stark, M. G. Doherr, F. Filli, M. Hassig, U. Braun, K. M. Kocan, and H. Lutz. (2005). Seroprevalence of anaplasmosis among cattle in Switzerland in 1998 and 2003: no evidence of an emerging disease. Vet. Microbiol. **107:**71–79.

Dugat T, Lagrée A-C, Maillard R, Boulouis H-J and Haddad N (2015). Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. Front. Cell. Infect. Microbiol. **5**:61.doi: 10.3389/fcimb.2015.00061

Dumler JS (2012): The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol. Med. Microbiol.* **64**: 13–20.

Dumler, J. S. (2005): *Anaplasma* and *Ehrlichia* Infection. Annals of the New York Academy of Sciences **106:** 361-373.

Dumler, J. S., & Brouqui, P. (2004). Molecular diagnosis of human granulocytic anaplasmosis. Expert Review of Molecular Diagnostics, **4**: 559–569.

Dumler, J. S., A. F. Barbet, C. P. J. Bekker, G. A. Dasch, G. H. Palmer, S. C.Ray, Y. Rikihisa, and F. R. Rurangirwa (2001): Reorganization of the genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combination and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila.* Int. J. Syst. Evol. Microbiol. **51:**2145–2165.

Dzięgiel B, Adaszek L, Kalinowski M, Winiarczyk S (2013): Equine granulocytic anaplasmosis. Res Vet Sci **95**: 316–320.

Elhamiani Khatat, S., Daminet, S., Kachani, M. et al. (2017). Anaplasma spp. in dogs and owners in north-western Morocco. Parasites Vectors 10, 202 doi: 10. 1186/s13071-017-2148

Felsheim. Roderick F. (2006): "Transformation of *Anaplasma phagocytophilum*". BMC Biotechnology, **6**:42

Foley J. E., et al. (2009): Distinct ecologically relevant strains of *Anaplasma phagocytophilum* Emerg. Infect. Dis. **15**:842–843

Galemore, E. R., Labato, M. A., & O'Neil, E. (2018): Prevalence of *Anaplasma phagocytophilum* infection in feral cats in Massachusetts. Journal of Feline Medicine and Surgery Open Reports,

Granick JL, PJ Armstrong and JB Blender, (2009): *Anaplasma phagocytophilum* infection in dogs: 34 cases (2000-2007). J Amer Vet Med Assoc, **234**: 1559-1565.

Granquist et al., (2010) Variant -and individual dependent nature of persistent *Anaplasma phagocytophilum* infection Acta Veterinaria Scandinavica 52:25

Hahn, B. H. *et al.* (2000) AIDS as a zoonosis: Scientific and public health implications. *Science* **287**: 607-614

Heymann, D. L. *et al*. (2015). Global health security: the wider lessons from the West African Ebola virus disease epidemic. Lancet 385, 1884–1901

Hosseini Vasoukolaei, Nasibeh & Oshaghi, Mohammad & Shayan, Parviz & Vatandoost, Hassan & Babamahmoodi, Farhang & Yaghoobi Ershadi, Mohammad Reza & Zakkyeh, Telmadarraiy & Mohtarami, Fatemeh. (2014). Anaplasma Infection in Ticks, Livestock and Human in Ghaemshahr, Mazandaran Province, Iran. Iranian Journal of Arthropod-Borne Diseases. **8**.

Inokuma, H., Y. Terada, T. Kamio, D. Raoult, and P. Brouqi. (2001). Analysis of the 16S rRNA gene sequence of *Anaplasma centrale* and its phylogenetic relatedness to other *Ehrlichiae*. Clin. Diagn. Lab. Immunol. **8:**241–244.

Ismail N, Bloch KC and McBride JW (2010): Human ehrlichiosis and anaplasmosis. Clinics in Laboratory Medicine **30**; 261–292.

Javed, K., Ijaz, M., Ali, M.M., Khan, I., Mehmood, K., Ali, S., (2014). Prevalence and haematology of tick borne hemoparasitic diseases in equines in and around Lahore. Pak. J. Zool. **46**, 400 401-408

Jones-Engel L, May CC, Engel GA, Steinkraus KA, Schillaci MA, et al. (2008): Diverse contexts of zoonotic transmission of simian foamy viruses in Asia.

Kocan K.M., de La Fuente J., Blouin E.F. & Garcia-Garcia J.C. (2004): .*Anaplasma marginale* (Rickettsiales: *Anaplasmataceae*): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology **129**: S285–S30

Kocan, K. M., de la Fuente, J., Guglielmone, A. A., & Melendez, R. D. (2003). Antigens and Alternatives for Control of *Anaplasma marginale* Infection in Cattle. Clinical Microbiology Reviews, 16**(4),** 698–712.

Kocan, Katherine & de la Fuente, Jose & Cabezas-Cruz, Alejandro (2015): The genus *Anaplasma*: New challenges after reclassification. Revue scientifique et technique (International Office of Epizootics). **34**: 577-586.

Lee, S. H., Park, S., Lee, Y. S., Lee, H. K., & Hwang, S. D. (2018). Diagnosis and molecular characteristics of human infections caused by *Anaplasma phagocytophilum* in South Korea. Journal of Microbiology, **56(11),** 847–853. doi:10. 1007/s12275-018-8385-8

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005): Bats are natural reservoirs of SARS-like coronaviruses **310**:676-9.

Lin Q, Rikihisa Y, Felek S, Wang X, Massung RF, Woldehiwet Z (2004): *Anaplasma phagocytophilum* has a functional msp2 gene that is distinct from p44. Infection and Immunity **72**, 3883–3889.

Lin Q., N. Zhi, N. Ohashi, H. W. Horowitz, M. E. Aguero-Rosenfeld, J. Ruffalli, G. P. Wormser, and Y. Rikihisa. 2002. Analysis of sequences and loci of p44 homologs expressed by *Anaplasma phagocytophila* in acutely infected patients. J. Clin. Microbiol. **40**:2981–2988.

Little SE (2010): Ehrlichiosis and anaplasmosis in dogs and cats. Vet Clin North Am Small Anim Pract **40**: 1121–1140

Locatelli, S. & Peeters, M. (2012) Non-Human Primates, Retroviruses, and Zoonotic Infection Risks in the Human Population. Nature Education Knowledge 3(10):62

Maeda K, Markowitz N, Hawley RC, et al. (1987) Human infection with Ehrlichia canis, a leukocytic Rickettsia. N Engl J Med; **316**: 853–856. [PubMed: 3029590]

Massung RF, Priestley RA, Miller NJ, Mather TN, Levin ML (**2003):** Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. J Infect **188**:1757–6310

Massung RF, Slater KG. **(2003**): Comparison of PCR assays for detection of the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum*. J Clin Microbiol.;**41 (2):**717–22.

Medina-Estrada, I., Alva-Murillo, N., López-Meza, J. E., & Ochoa-Zarzosa, A. (2018). Immunomodulatory Effects of 17β-Estradiol on Epithelial Cells during Bacterial Infections. Journal of Immunology Research, 2018, 1–11. doi:10.1155/2018/6098961

M'ghirbi, Youmna & Béji, Marwa & Oporto, Beatriz & Khrouf, Fatma & Hurtado, Ana & Bouattour, Ali. (2016). *Anaplasma marginale* and *A. phagocytophilum* in cattle in Tunisia. Parasites & Vectors. **9**. 10.1186/s13071-016-1840-7.

Mwamuye, M. M., Kariuki, E., Omondi, D., Kabii, J., Odongo, D., Masiga, D., & Villinger, J. (2017). Novel Rickettsia and emergent tick-borne pathogens: A molecular survey of ticks and tick-borne pathogens in Shimba Hills National Reserve, Kenya. Ticks and tick-borne diseases, 8(2), 208–218. https://doi.org/10.1016/j.ttbdis.2016.09.002

Mylonakis, M. E., A. F. Koutinas, E. B. Breitschwerdt, B. C. Hegarty, C. D. Billinis, L. S. Leontides, and V. S. Kontos. (2004). Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. J. Am. Anim. Hosp. Assoc. **40:**174–184.

Nakayima J, Hayashida K, Nakao R, Ishii A, Ogawa H, Nakamura I, Moonga L, Hang'ombe BM, Mweene AS, Thomas Y, Orba Y, Sawa H, Sugimoto C (2014):Detection and characterization of zoonotic pathogens of free-ranging non-human primates from Zambia. Parasit Vectors **7**:490

Nunn, C. L. & Altizer, S. (2006) Infectious Diseases in Primates. Behaviour, Ecology and Evolution. Oxford, UK: Oxford University Press,

OIE 2008, World Organisation for Animal Health (OIE). Manual of standards for diagnostic tests and vaccines for terrestrial animals, 6th edition, **599**–10

Overzier E, Pfister K, Herb I, Mahling M, et al. (2013) Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), questing ticks (*Ixodes ricinus*) and ticks infesting roe deer in southern Germany. Ticks Tick Borne Dis b; **4**:320–328

Parola, Philippe & Roux, Veronique & Camicas, J & Baradji, Issa & Brouqui, Philippe & Raoult, Didier. (2000). Detection of Ehrlichiae in African ticks by polymerase chain reaction. Transactions of the Royal Society of Tropical Medicine and Hygiene. **94**. 707-8. 10.1016/S0035-9203(00)90243-8.

Quan Lin. (2006): "Analysis of Involvement of the RecF Pathway in p44 Recombination in *Anaplasma phagocytophilum* and in *Escherichia coli* by Using a Plasmid Carrying the p44 Expression and p44 Donor Loci". Infection and Immunity,**74** :2052-2062.

Ramos RA, Latrofa MS, Giannelli A, Lacasella V, Campbell BE, Dantas-Torres F, Otranto D. (2014) Detection of *Anaplasma platys* in dogs and *Rhipicephalus sanguineous* group ticks by a quantitative real-time PCR

Rar V, Golovljova I. (2011) Anaplasma, Ehrlichia, and ‘‘*Candidatus Neoehrlichia’’* bacteria: Pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infect Genet Evol; **11**:1842–1861.

Renneker S, Abdo J, Salih DE, Karagenç T, Bilgiç H, Torina A, Oliva AG, Campos J, Kullmann B, Ahmed J, Seitzer U. (**2013)** Can *Anaplasma ovis* in small ruminants be neglected any longer? Transbound Emerg Dis.; **60**: 105–112.

Ribeiro, M.F. B., Passos, L. M. F. (2002): Tristeza parasitaria bovina. *Cadernos Tecnicos*.

Rikihisa, Y. (2011). Mechanisms of Obligatory Intracellular Infection with *Anaplasma phagocytophilum.* Clinical Microbiology Reviews, **24(3),** 469–489. doi:10.1128/cmr.00064-10

Rojero-Vázquez, E., Gordillo-Pérez, G., & Weber, M. (2017). Infection of *Anaplasma phagocytophilum and Ehrlichia* spp. in Opossums and Dogs in Campeche, Mexico: The Role of Tick Infestation. Frontiers in Ecology and Evolution, **5**. doi:10.3389/fevo.2017.00161

Schotthoefer, A. M., Meece, J. K., Ivacic, L. C., Bertz, P. D., Zhang, K., Weiler, T., … Fritsche, T. R. (2013). Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. Journal of Clinical Microbiology, **51(7**), 2147–2153. doi:10.1128/jcm.00347-13

Severo, M. S., Pedra, J. H. F., Ayllón, N., Kocan, K. M., & de la Fuente, J. (2015). Anaplasma. Molecular Medical Microbiology, 2033–2042. doi:10.1016/b978-0-12-397169-2.00110-4

Shereen Adnan, M., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. (2020). COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. Journal of Advanced Research. doi:10.1016/j.jare.2020.03.005

Silveira, J. A. G., Rabelo, E. M. L., & Ribeiro, M. F. B. (2011). Molecular Detection of Tick-Borne Pathogens of the Family Anaplasmataceae in Brazilian Brown Brocket Deer (*Mazama gouazoubira*, Fischer, 1814) and Marsh Deer (*Blastocerus dichotomus*, Illiger, 1815). Transboundary and Emerging Diseases, **59(4),** 353–360.

Stannard A. A., Gribble D. H., Smith R. S. (1969): Equine ehrlichiosis: a disease with similarities to tick-borne fever and bovine petechial fever. Vet. Rec. **84**:149–150.

Stuen S, Granquist EG, Silaghi C (2013): *Anaplasma phagocytophilum* - a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol **3**: 31

T. Notomi, Y. Mori, N. Tomita, and H. Kanda. (2015)"Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects, "Journal of Microbiology,vol.53,no.1,pp.1–5,

Teshale, S., Geysen, D., Ameni, G., Dorny, P., & Berkvens, D. (2018). Survey of *Anaplasma phagocytophilum and Anaplasma* sp. "Omatjenne" infection in cattle in Africa with special reference to Ethiopia. Parasites & Vectors, **11**(1).

Vatsya S, Yadav CL, Kumar RR, Garg R. **(2007)** Seasonal activity of *Boophilus microplus* on large ruminants at an organised livestock farm. J Vet Parasitol. 21(2):125–128. [Google Scholar]

Vesco U, Knap N, Grindatto A, Avsic-Zupanc T, Labruna M, Estrada-Peña A, et al., (2010): A database on ticks and tick-borne zoonoses in the sub tropics: an example of inter-sectoral and institutional collaboration at international level.

Wang, L., & Crameri, G.S. (2014). Emerging zoonotic viral diseases. Revue scientifique et technique, 33 **2**, 569-81

Wesonga F.D., Kitala P.M., Gathuma J.M., Njenga M.J., Ngumi P.N. (2010): An assessment of tick-borne diseases constraints to livestock production in a smallholder livestock production system in Machakos district, Kenya. Livest. Res. Rural Dev; **22**

Woldehiwet Z (2010): The natural history of *Anaplasma phagocytophilum*. Vet Parasitol **167**: 108–122.

Wuritu, et al. (2009): Structural analysis of a p44/msp2 expression site *of Anaplasma phagocytophilum* in naturally infected ticks in Japan. J. Med. Microbiol. **58**:1638–1644.

Xiong Q., Rikihisa Y.. 2010. Flotillin-1 is required for *Anaplasma phagocytophlium* infection of mammalian host cells, Abstr. B-2538. Abstr. 110th Gen. Meet. Am. Soc. Microbiol., S

Yang, Jifei & Liu, Zhijie & Qingli, Niu & Liu, Junlong & Xie, Jingying & Chen, Qiuyu & Chen, Ze & Guan, Guiquan & Liu, Guangyuan & Luo, Jianxun & Yin, Hong. (2016). Evaluation of different nested PCRs for detection of *Anaplasma phagocytophilum* in ruminants and ticks. BMC Veterinary Research. 12. 10.1186/s12917-016-0663-2.

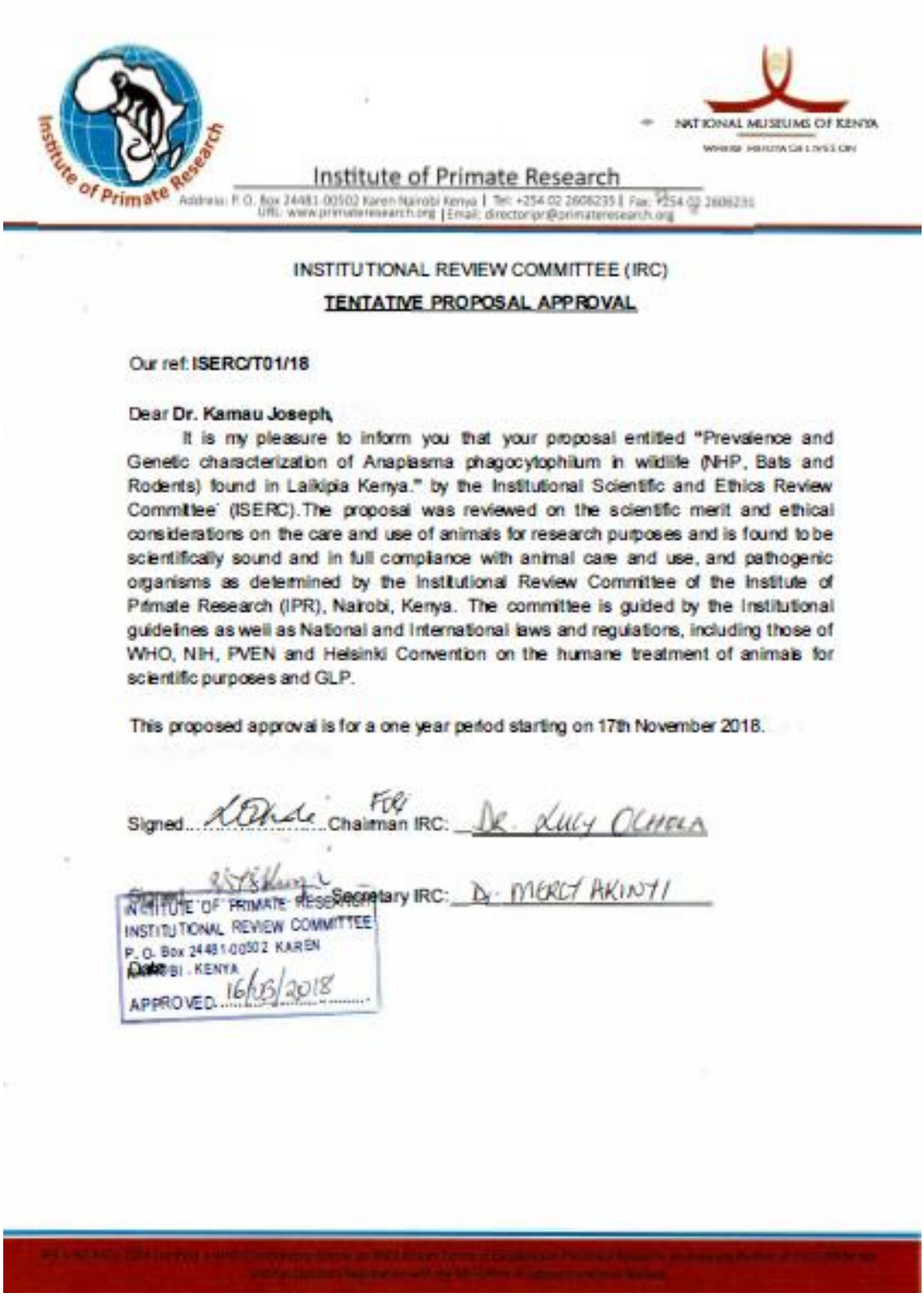
Zaid, T., Ereqat, S., Nasereddin, A., Al-Jawabreh, A., Abdelkader, A., & Abdeen, Z. (2019). Molecular characterization of *Anaplasma* and *Ehrlichia* in ixodid ticks and reservoir hosts from Palestine: a pilot survey. Veterinary Medicine and Science. doi:10.1002/vms3.150

Zhang L, Wang G, Liu Q, Chen C, Li J, Long B, Yu H, Zhang Z, He J, Qu Z, YuJ, Liu Y, Dong T, Yao N, Wang Y, Cheng X, Xu J (2013): Molecular analysis of *Anaplasma phagocytophilum* isolated from patients with febrile diseases of unknown aetiology in China. PLoS One, **8**:e57155–e57164.

Zhi N., et al. (2002): Transcript heterogeneity of the p44 multigene family in a human granulocytic ehrlichiosis agent transmitted by ticks. Infect. Immun. **70**:1175–118

APPENDICES

Appendix 1: Ethical approval



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INSTITUTIONAL REVIEW COMMITTEE (IRC)

TENTATIVE PROPOSAL APPROVAL

Our ref: **ISERC/T01/18**

Dear **Dr. Kamau Joseph,**

It is my pleasure to inform you that your proposal entitled "Prevalence and Genetic characterization of Anaplasma phagocytophilum in wildlife (NHP, Bats and Rodents) found in Laikipia Kenya." by the Institutional Scientific and Ethics Review Committee (ISERC). The proposal was reviewed on the scientific merit and ethical considerations on the care and use of animals for research purposes and is found to be scientifically sound and in full compliance with animal care and use, and pathogenic organisms as determined by the Institutional Review Committee of the Institute of Primate Research (IPR), Nairobi, Kenya. The committee is guided by the Institutional guidelines as well as National and International laws and regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposed approval is for a one year period starting on 17th November 2018.

Signed..... For Chairman IRC: Dr. Lucy Ochola

Signed..... Secretary IRC: Dr. Mercy Akinyi

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI. KENYA
APPROVED 16/05/2018

Appendix 2: Turnitin Originality Report

Thesis 2

ORIGINALITY REPORT

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1	wwwnc.cdc.gov Internet Source	1%
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3	Submitted to Imperial College of Science, Technology and Medicine	1%

I certify that this is a true copy of the Turnitin Originality Report.

Dr. Gerald Muchemi,

Lead Supervisor

Date: 9th November, 2021