Occurrence of haemoparasites in donkeys in Central Zone, Tanzania

By

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Tropical Animal Health in the Department of Veterinary Tropical Disease, Faculty of Veterinary Science, University of Pretoria

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DECLARATION

I hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

Student: ANTIPACHIUS ANTHONY MSOMI

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LIST OF ABBREVIATIONS AND ACRONYMS

ASPA	Arusha society for protection of animals
BC	Before Christ
BLAST	Basic local alignment search tool
CEAH	Center for epidemiology and animal health
CFT	Complement fixation test
DAD-IS	Domestic animal diversity information system
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EtOH	Ethyl alcohol
FAO	Food and agriculture organization
FAWAC	Farm animal welfare advisory council
ICZN	International commission on zoological nomenclature
IFAT	Indirect fluorescent antibody test
MAWO	Meru animal welfare organization
NBCI	National center for biotechnological information
NC	Negative control
NEWC	National equine welfare council
OIE	Office International des Epizooties
PBS	Phosphate buffered saline
PC	Positive control
PCR	Polymerase chain reaction
pH	Potential of hydrogen
rRNA	ribosomal ribonucleic acid
SPANA	Society for the protection of animals abroad
TAE	Tris base acetic acid
TAHUCHA	Tanzania humane charity
TAPO	Tanzania animal protection organization
TAWESO	Tanzania animal welfare society
URT	United Republic of Tanzania
USA	United State of America
UV	Ultra violet

ABSTRACT

Occurrence of haemoparasites in donkeys in Central Zone, Tanzania

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The aim of this study was to explore the occurrence and magnitude of haemoparasites and establish the risk factors associated with infection in donkeys of central zone, Tanzania. Donkeys are early-domesticated equines that have been a beast of burden for many years. Despite their significant role in developing countries like Tanzania, donkeys face a number of health and managerial constraints. Haemoparasite infections are among health constraints in donkeys, which may compromise their health and draught power efficiency. A cross-sectional study was conducted between October 2017 and October 2018. The study aimed to establish the occurrences of haemoparasites in donkeys in Central Zone, Tanzania. Blood samples were collected from the randomly selected 384 adult donkeys at Nala Livestock market in Dodoma. Age, sex and body conditions of selected donkeys were recorded and the blood samples were collected directly from jugular vein into EDTA tubes for blood smears preparation and molecular examination. Microscopic and molecular examination of the blood samples were done at Sokoine University of Agriculture. Stained blood smears were examined under microscope. Multiplex PCR for the detection of *Babesia caballi* and *Theileria equi* was conducted. Positive PCR results were sequenced, edited using genius software and subjected to NCBI nucleotide BLAST for analysis. All 384 examined slides under microscope were negative against *Babesia Theileria* and *Trypanosoma*,. The PCR results revealed 98 (93.2%) of 104 tested samples were positive against *Theileria equi* whereas the same samples tested negative against *B. caballi*. This study revealed a high occurrence of *T. equi* in donkeys in study area and that donkeys may be important carriers of the parasites. There is a need for further study on the epidemiology, pathogenicity and antiprotozoal resistance status of the prevailing protozoan parasites.

CHAPTER 1 INTRODUCTION

1.1 Background

Among the early domesticated equines, donkeys were assumed to be animals of burden for many years. Donkeys belong to the family *Equidae*, genus *Equus*, species *africanus* and subspecies *Equus africanus asinus,* according to the International Commission on Zoological Nomenclature (ICZN) (2003). They are said to be the first domesticated equids and to be used as working beast since 3000 BC in Egypt (Nowak 1999). As per world Food and Agriculture Organization (FAO) report of (2016), the world's donkey population is estimated to be 44 million, while in Tanzania they are estimated to be approximately 595,160 as per parliamentary budget for the Ministry of Livestock and Fisheries report (2017/2018).

Regardless of the increase in mechanization throughout the world, donkeys still have a noticeable role in the transport and agricultural sector. They are working as park and draught animals in many developing countries including Tanzania. They have been useful to women to perform domestic obligations timely in order to allow them to engage in other income generating activities (Marshall & Ali 2004). Donkeys are also important in the smallholder farming system, especially in rural communities as they are used for transportation of people, goods, farm implements and harvests to and from the farms (Swai & Bwanga 2008). In general, donkeys have a tangible role in the agricultural system especially in resource compromised societies located in rural and urban areas. Animal diseases are some of the factors that affect the well-being and production of donkeys (Svendsen 1997). In addition to that, unaffordable farm implements, inadequate skills and knowledge on the proper use of harnessing equipment and poor animal husbandry, are among the challenges hindering the use of draught animals in Tanzania (Tanzania National Livestock Policy 2006).

Donkeys suffer from inbreeding, poor nutrition, inadequate health and veterinary support services, bacterial, viral, fungal, protozoal and helminth infections, trauma and colic (Segwagwe et al. 1999, Swai & Bwanga 2008, Getachew et al. 2008). The common disease conditions that affect donkeys includes babesiosis, anthrax, rabies, tetanus, strangles, African horse sickness, colic, endoparasites, ectoparasites as well as Dourine (Joan 2015). Although these diseases have been affecting donkeys in different parts of the world, there is limited information on their occurrence in Tanzania.

In Tanzania, many donkeys have been working to the point of collapse due to illness caused by parasitic infestations, injuries, fatigue and starvation (Swai & Bwanga 2008). Lack of proper harnessing equipment and continuous beating usually create injuries and wounds which are left untreated due to inadequate veterinary care and carelessness of the owners

(https://www.donkeys/1210-help-for-donkeys-in-tanzania).Furthermore, housing systems are one of the managerial challenges which may compromise the health of the donkeys in developing countries such as Tanzania. Most of the donkeys are kept in open housing systems, sometimes without any shelter to protect them from harsh weather conditions. In areas where donkey houses exist, they have earthen floors with no routine cleaning of manure or beddings. Donkeys are perceived as disease resistant and hardy animals by the general communities and animal health policy makers (Swai & Bwanga 2008).

Haemoparasites such as *Theileria, Babesia* and *Trypanosoma* remain the most common parasitic infections of the donkeys. The most prevalent haemoparasites of donkeys are *T. equi* and *B. caballi* which are exclusively intraerythrocytic protozoa. They cause a disease condition known as Equine piroplasmosis (Asefa et al. 2011). *Theileria equi* and *B. caballi* parasites have a worldwide distribution and epidemiologically closely associated. They are also transmitted by common tick vector that belong to the genus *Hyalomma, Rhipicephalus* and *Dermacentor* (Asefa et al. 2011). On the other hand, *Trypanosoma* are another group of haemoparasites of donkeys that are transmitted by tsetse flies or sexually in the case of Dourine.

Their distribution is said to be restricted to tsetse infested areas (Friedhoff et al. 1990). These haemoparasites cause various degrees of damage based on species and parasite burden, nutritional and the immune status of the affected donkey (Asefa et al. 2011). However, donkeys require food, access to water and shelter, companionship and routine health checkup and veterinary care to prevent them against illnesses (Joan 2015). Despite their ever-increasing numbers and importance to the economy and livelihoods of the rural poor, knowledge about the health problems affecting donkeys especially haemoparasite infections in Tanzania is limited.

1.2 **Problem Statement and study justification**

Recently, there has been an increased demand for donkey meat trade in China (https://www.nytimes.com/2018/01/02/science/donkeys-africa-china-ejiao.html) that resulted into establishment of donkey abattoirs in central and Lake zones of Tanzania. Also, there is high demand for donkey production needed as working animals in Tanzania. All these have drawn attention for the donkey health and well-being. However, haemoparasites in donkeys have not been extensively studied, even though they are amongst the leading causes of health problems affecting these animals. Hence the need to investigate the occurrence of haemoparasites infecting donkeys in Tanzania.

1.3 Hypothesis

The null hypothesis is that, donkeys in the central zone of Tanzania are not infected with haemoparasites which significantly contribute to their poor condition and performance. There are also a number of risk factors that predispose donkeys to haemoparasites in the central zone of Tanzania.

1.4 Aim and objectives

1.4.1 Aim

The aim of this study was to explore the occurrence and magnitude of haemoparasites and establish the risk factors associated with infection in donkeys of central zone, Tanzania.

1.4.2 Objectives

(I) To determine the occurrence of haemoparasites in donkeys
(ii) To establish the risk factors associated in haemoparasites infection in donkeys

1.5 Benefit of the study

Since this is a first study in Tanzania, the data obtained will establish baseline information on haemoparasites that affect donkeys and their importance. This information can be used in designing strategies for the control and for informing the direction of further studies on haemoparasites in Tanzania.

CHAPTER 2 LITERATURE REVIEW

2.1 Origin and scientific name of donkeys

According to the zoologist Linnaeus (1785) and International Commission on Zoological Nomenclature (ICZN) (2003), donkey or ass is a domesticated animal that belong to the family Equidae, genus *Equus*, species *Equus africanus* and subspecies *Equus africanus asinus*. Before the amendment made by International Commission on Zoological Nomenclature on the scientific naming of donkeys, it was traditionally called *Equus asinus asinus.* Such naming followed the code of priority used for the scientific naming of animals. The current scientific name of the donkey is *Equus africanus asinus.* This name was introduced after giving wild species priority when wild species and domestic species are considered subspecies of one another (International Commission on Zoological Nomenclature 2003). The modern donkey's ancestors are believed to be the Somalian and Nubian subspecies of African wild ass which may be put in the category of endangered species (Clutton 1999, Beja-Pereira 2004).

The donkeys are estimated to have been used as working beast for 500 years. Their population worldwide is estimated to be 44 million. The large percentage of this population is said to be in developing countries where they are basically used as beasts of burden or draught animals. As described by Rossel et al. (2018) and Nowak (1999), genetic-based research indicated the origin of donkeys is in the African continent. They were first domesticated in Egypt about 3000 BC before their spread around the world. As pointed out by Clive (2007), it is believed that in the modern world, the donkey was first introduced in one of the Caribbean islands called Hispaniola on ships by the second voyage of Christopher Columbus in 1495.

Worldwide, breeds and types of donkeys are estimated to be close to hundred and eighty-nine and mainly used as a companion and working animals as described by Domestic Animal Diversity Information System (DAD-IS) of (FAO) (2014). History revealed for the first time that a German nobleman was seen accompanied with by a donkey in 1862 when he was attempting to climb the top of Mount Kilimanjaro in northern Tanzania Wilson (2013). Different geographic and climatic conditions in different countries have resulted in the creation of diverse types of donkeys (Jonas 2005). According to Grinder et al. (2006), wild donkeys inhabit arid and semi-arid regions with hillocks which they prefer to use as observation posts.

Some types of wild donkeys exist in the world including *Equus assinus somaliensis,* also known as the Somali. These donkeys are found in Somalia, Ethiopia, Kenya, Tanzania and other parts of East Africa. The Somali (*Equus assinus somaliensis*) are said to be the only existing African

wild assinus and are closely related to domestic donkeys (Grinder et al. 2006). Nubian Assinus (*Equus assinus africanus*) are found in Egypt, Sudan, Eritrea and some parts of Ethiopia. They have genetic relatedness to the domesticated donkeys and have a slightly redder coat compared to that of the Somali ass (Beja-Pereira et al. 2004). As pointed out by (Groves 1974), another type of wild donkey is Kiang (*Equus asinus kiang*), mostly found in India and Nepal. Kiang have physical features of well-marked black strip on the dorsal aspect. They also have pure white markings that extend dorsally, separating the colored areas into shoulders, flank and haunch blocks. The main coat colour is red which intercept with white colour to form red-white markings on the legs, ears and faces. Onager (*Equus onager*) are found in Iran, Syria and North Saudi Arabia. Kulan (*Equus hemionus*) is another type of donkey found in Central Asia. Also, some feral donkeys are found in South-west parts of the United States of America (Jones 2008, Groves 1974). Apart from domestic donkeys, there is a number of feral donkeys in different parts of the world. The latter have been intensively used as beast of burden, but recently replaced by advancement of technology in mechanization.

In 1980's, large population of feral donkeys were found in Australia and California in the United States of America (Beja-Pereira et al. 2004). However, for domesticated breeds, the breeding of donkeys was conducted with a particular purpose, such as to develop animals suited for certain duties and environment. This gradually resulted in many trait variations. With the passing of time, the environment and the working plan of the donkeys has changed. This made the donkeys to be used in areas where they have never been used before (Jonas 2004).

2.2 Characteristics, behavior and breeding of donkeys

A male donkey is called Jack, a female donkey is called jennet and a young donkey is called foul (oxforddictionaries.com). Sometimes the jack can mate with female horse to produce a hybrid offspring called mule. On the other hand, hinny is a hybrid offspring produced when a horse stallion is bred with a female donkey. In many cases, 99.9% of mule and hinnies are sterile in nature. The terms Molly and John are used to describe a female mule and a male mule respectively.

Depending on nature of husbandry and breed, donkeys' size differs considerably. The height at wither ranges from 79 to 160 centimetres, and live weight from 80 to 480 kilograms. The life span of the donkey varies with nature of management. In resource compromised countries, life expectancy ranges from 12 to 15 years and in developed countries may range from 30 to 50 years (Government of Alberta Agricultural and Rural development sector 1990, The donkeys 2012). Donkeys are said to be very calm, friendly, bright, vigilant and brave. They are very adaptive to

the harsh environment and special regions for survival. Domesticated donkeys and feral horses in arid areas are dependent on each other. Sometimes they form harems in contrast to wild donkeys which are independent and do not form harems. These arid areas are very hot sometimes during the day, temperature may rise up to more than 50˚C. Donkeys normally feed on pasture, crop residues and sometimes concentrate if available. During feed scarcity, they may consume whatever available feeds and search for water for up to 6 km.

They have a good food utilization efficiency of up to 95% by breaking down inedible vegetation and extracting moisture from consumed food. The wild donkeys consume about 30% of forbs, 61% of browse, 4% grass and 5% of others such as balk, twigs or roots (Grinder et al. 2006). The social structure of the donkeys depends on the availability of pastures, nutritious vegetation in the environment and water or scarcity of such conducive environment. In case of social organization in the wild, donkeys stay solitary, in small or large groups or even herds. It is also observed that 5 % of the population stay privately, 28 % in a group of 2-6 individuals, 30 % in groups of 7-20 individuals and 36 % in herds of 21-60. The ratio of males in each group differs from 1 in a small group up to 25 males in herds with the rest being females and their youngs (Orhan et al. 2012). The wild donkeys may form a heard or stay in separate fashion only coming together in large groups in non-breeding season. For breeding purpose, Jack may establish a home and dominate the large breeding area (Jane 1997). In social life, whether in wild or domesticated, donkeys usually select the strongest one among them to be their leader. In case of danger, the leader will be responsible to fight allowing others to escape to a safer place.

In the herd, they normally groom one another in the same way as primates do. However, donkeys don’t like to be kept alone, although one donkey can live happily with goats or sheep. In general, they are recognized as intelligent animals (Mike’s donkeys 2015). Donkeys are said to have the ability of recognizing areas even after 25 years of absence for such area.

They are neither forced nor frightened to do something and have a well-developed sense of self-preservation. Also, during training, the donkey trainer needs to show actions or words that ensures their protection. Donkeys can properly hear a distant sound and cool themselves by using their large ears. The audibility capacity of donkeys is high as they can hear a voice up to 96 km away in a bared land like desert. The donkeys protect themselves through kicking, biting or striking when their threatened (Mike’s donkeys 2015). Since they have no waterproof fur, donkeys don’t like rain. (http://www.hartshorsemanship.com).

The conception rate of donkey is relatively lower than horse ranging from 60% to 65% (Twins and Donkeys 2015). Despite the fact that, jennies come on heat within nine to ten days after parturition, they still have low potency potential. This may be due to incomplete involution of their

reproductive tracts. Therefore, in contrary to the mares, jennies have to wait for one or more estrous cycles before rebreeding. Due to strong social bond and being protective against their offspring, some jennies may not come on heat with their fouls adjacent to them, as a result donkey breeder don't expect to get a foul each year (Rachau 2017). The gestation period of jennet is about twelve months. That period may vary ranging from eleven to fourteen months. They usually give birth to single foul. The possibility of giving birth of twin fouls is about 1.7 % of all pregnancies. In some cases, donkeys may interbreed with other members of the family Equidae and produce hybrid offspring. The mating of Jack with mare produce hybrid offspring called mule. The mule is relatively strong and used mostly as working and riding animal in different countries. According to the government of Albert agriculture and rural development sector (1990), mule production has made possible through raising of large breeds of donkeys such as Mammoth Jack. If donkeys interbred with zebra, the hybrid offspring produced is called zonkey (Rachau 2017).

2.3 Donkey ppopulation and ddistribution

According to Blench (2000) and Starkey & Starkey (1997), the donkey populations in the world have kept on increasing from 37 million in 1961 to 44 million in 1997. Such tremendous increases may have been influenced by a number of factors. These factors include; increased human population, improved economic development and social stability, especially in the resource compromised nations, demand for range land, unaffordable fare and inadequate, reliable means of transport as well as increased awareness of keeping donkeys as pets.

Despite the steady increase of donkey population worldwide, large regional differences still exist with noticeable regional increase or decreases. In the last fifty years, donkey populations in the African continent have increased by 60% from 8.5 million in 1949 to 13.7 million in 1996. The high donkey population increase occurs in semi-arid and mountainous areas in countries such as Ethiopia, where the population is estimated to be 5 million compared to Sahelian countries where the population is said to be 2.5 million (Starkey 1995). Based on donkeys' population estimates, the African continent has four distinct regions. The first one is the Northern part of Africa which comprises countries like Libya, Tunisia, Algeria, Egypt and Morocco. The second is west and central Africa which comprises countries such as Cameroon, Mauritania, Burkina Faso, Nigeria, Benin, Ghana, Guinea Bissau, Gambia, Cape Verde, Guinea, Mali, Niger, Chad, Togo and Senegal. The third region is Southern and Eastern Africa which comprise countries like Botswana, Angola, Kenya, Ethiopia, Comoros, Djibouti, Lesotho, Eritrea, Malawi, Mozambique, Namibia, Somalia, South Africa, Swaziland, Uganda, Tanzania, Zambia, Zimbabwe and Sudan. Northern east and northern west parts of Africa seem to have a relatively large donkey population than the Southern part (FAO 1997, Starkey 1995).

In America and Caribbean countries, the population of donkeys has increased from 2.7 in 1949 to 3.3 million in 1996 with a small population seen in Central America. However, Brazil is only country with the largest donkey population in South America. Argentina and Chile have the smallest population size of donkeys of all and have been decreasing gradually while in the Caribbean the population has been stable especially in Haiti and Dominican Republic (FAO 1997). The Middle Eastern countries in Asia continent have long history of using donkeys. However, the variable population trends of the donkeys in these countries have been observed. The tremendous decline of the donkey population has been noticed for instance, in countries such as Turkey from 1.7 million in 1949 to 800,000 in 1997, Iraq from 1 million in 1949 to 164,000, in 1997. The gradual decrease has also been observed in Lebanon, Israel and Jordan. However, in other countries like Iran, Afghanistan, Yemen, Saudi Arabia and Syria, the population has increased slightly and remains stable in some countries (FAO 1997). As donkeys' historical information is concern, Europe, especially northern parts of European states had the large donkey population. In Tanzania, there is no adequate information about the exact number of donkeys present in the country, although in a few years ago donkey population was estimated to be 250,000, and recently the population is said to have increased to half a million and are mostly domesticated in the central, northern East, Lake zone as well as north Western parts of Tanzania (Starkey & Mutagubya 1992).

2.4 Uses of donkeys

For about 5000 years ago, donkeys have been used as a working animal especially in underdeveloped countries where almost 96% of the entire donkey population is found. They are perceived to offer the cheapest labour after human labour. Among other activities performed by donkeys, they offer transportation of people and agricultural inputs to and from the farm as well as land cultivation for crop production (Pearson et al 1999). Donkeys can also be used for ridding, threshing, water up lifting from the wells, grinding of grains and other related activities. Donkeys have limited traditional restrictions allowing them to be used by both sexes unlike with oxen where in some traditions, women are not allowed to use oxen especially in agricultural activities (Sosovele 2000). Societies using subsistence farming are mostly associated with the use of donkey as working animals (World Bank 2009). In Tanzania, donkeys have been of much help in many societies. For instance, women in Barabaig societies have been using donkeys for households and farm activities. Such activities include collecting firewood, fetching water, transportation of goods to the market place. They are also used for cultivation, carrying of grains and often weeding (Sosovele 2000).

This has been providing women opportunity to be involved in other socio-economic activities and subsequent improvement of their family livelihood. However, land tenure system practiced in

many societies in Tanzania, may have contributed to unsustainable use of the donkeys. This has been associated with high demand for land and existence of unequal access to it for different social groups such as women, pastoralists and poor farmers, and hence hinders possible initiatives for development (Ndagala 1996, Sosovele & Kulindwa 1997). The donkeys are no longer used as beast of burden in developed countries, instead they are used to perform other activities. Such activities include, to guard other animals like sheep, riding of children, tourists, or disabled people due to their loving and generous in nature. They are also used as pets as well as sire mules (Olsen 1995, Dohner 2007).

Due to their calming effect to the nervous horses, sometimes are pastured or put together with horses in stable and in case introduced in mare and foul, it can help to support the foul after has weaned from its mother. Starkey (1997) described that, in some countries, donkeys are raised for meat or milk production. Despite the relatively low milk yield, the price of donkey milk is high compared to milk from the cattle.

In a country such as Italy where equine meat is highly consumed, the average price for one litre of donkey milk is 15 euro (Il Prezzo Del Latte Di Asina 2009). Donkeys also have been used in war fare. Many years ago, during the world war, Italian, Australian, New Zealand armies used donkeys to carry out protective gears and wounded shoulders. In harsh condition, they have been slaughtered and consumed (Simpson 2012). Also, in countries with political instability and continuous social conflicts such as Afghanistan and others, donkeys have been used to carry explosives which may cause massive destruction and subsequent loss of human life (Evans 2009, Ganor 1991).

The high demand of donkey products in China has led to a dramatic decrease in donkey population, resulting into increased awareness pressure on the importance of the donkey in most African countries. In Kenya and Tanzania, there are well-established abattoir for slaughtering and packaging donkey meats and hides ready to be transported to China (Leithead 2017). However, this has raised a great concern on animal welfare because of a number of reported brutal killing cases of donkeys which is illegal according to animal health management policy (Leithead 2017).

2.5 Donkey hhusbandry

Unlike horses, donkey hooves are elastic and are naturally resistant to fast wear. However, routine clipping of them is quite important to avoid possible and subsequent permanent damage. Sometimes shoes are necessary and usually are smaller than horse shoes (Government of Albert agriculture and rural development sector 1990). As described by Taylor (1997), most of the time

in their natural environment, donkeys keep on consuming fodders and scrubs with poor nutritional values. Smith & Stephanie (2008) pointed out that, since donkeys are hind gut fermenters with tough digestive system, ingested food materials like roughage is properly digested by microbial action in large intestine and caecum. In contrast to the horses, the donkeys have efficient food digestibility despite no marked anatomical difference in their digestive system and may need relatively low food compared to the horse of the same weight and height. The amount of dry matter content required by the donkey per day is only 1.5 percent of its body wait compared to up to 2.5 percent required in horse (Smith & Pearson 2005). This may be due to relatively longer gut retention time and different intestinal microflora (Smith & Pearson 2005).

Types of food and feeding regimes for donkeys have been discussed in different school of thoughts. Some suggest to feed animals with straw especially from barley supplementing it with controlled grazing in summer or with hay during the winter in order to have nutritional requirements. Others suggest to feed donkeys on available grains and feeding on straw especially for those used as the beast of burden (Government Agricultural and Rural development sector 1990). For the donkeys to attain optimal performance, it suggested that, they should be exposed to eat a small amount of feed for a long period of time. When allowed to graze on fresh dryland pasture for an average of seven hours a day, the donkeys may meet their nutritional requirements. Up to 1:4 legume to glass ratio of hay or other dried similar glass, mineral supplement and clean fresh water are required to feed the working donkeys which have no access to grazing pastures (Aganga et al. 2000).

From the fact that, the donkeys are mostly used as working animals in resource compromised societies, there is no doubt that most of them are usually subjected to inadequate food and are over-worked. However, some initiatives have been made to ensure the welfare of the donkeys are considered especially in developed countries. For instance, in European countries some organizations such as the British Donkey Sanctuary, Farm Animal Welfare Advisor Council (FAWAC) aimed at working out the guidelines for equine welfare. The different organizations have been emphasizing the consideration of the five basic needs required to maintain donkeys' welfare. These basic needs include; freedom from hunger, thirst and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal patterns behavior as well as freedom from fear and distress. These organizations have been providing technical and financial support to most developing countries to promote donkey welfare which have been neglected for a long time. Also, some organizations took a milestone to establish donkey clubs like Italian Asino Club which was a community-based club which brought together donkey breeders, keepers and curious people who wanted to know these animals. The club prescribed the donkey charter with five rights of donkeys which were; right to hospitality, right to health, right to a fair job, right to an ethical treatment and right to travel (Waltraud et al. 2008).

SPANA a charitable animal welfare organization have been soliciting fund from different sources and use that fund to strengthen capacity in terms of training of animal welfare-based issues to animal health specialists and establishment of demonstration laboratories in training institute like what have been done at Sokoine University of Agriculture in Tanzania.

Despite the fact that donkeys have been playing a crucial role especially for pastoralist societies in arid areas, they have been given less attention in terms of management as compared to other livestock such as cattle (Marshall & Weissbrod 2011). Mutua (2004) revealed that, donkey husbandry is still a challenge as human health management is concern.

In resource compromised societies, donkey management, possession as well as how donkeys are used may be used as indicator to assess their living conditions and wellbeing. Traditionally, most donkeys are kept in the backyard or being tethered where they may have fed with grasses and left over from households. They are fed on grazing fields, stable with access to the drinking water point. Mineral supplement such as salts and licking blocks are often provided in this traditional husbandry of donkeys. However, an ideal donkey husbandry should provide shelter, appropriate feeding facilities with enough and supplemented feeds, continuous water access in order to improve their working efficient (Félix 2014). Although there is a fact that, some African countries and China have been traditionally consuming donkey meat, production and husbandry system differs compared to that farm used for production of livestock and poultry.

Felix (2014) also revealed that in African countries, the adopted backyard system in donkeys is mixed with the other group of livestock and either women or youth are taking care of them. On the other hand, some American and European countries such as Ukraine, Peru or Mexico have a relatively large donkey farms though no legal right to slaughter them, production is for export to either China or Canada where can be slaughtered and converted to different traditional dishes (Félix 2014). Although dietary requirements of donkeys have not been well elaborated, it is said that they depend on poor nutrient foods for their survival and grasses being their main nutritional feed and have the same ration requirement with horses (Félix 2014). However, a convenient daily ration in donkeys should be provided by considering of purpose, breed, age, physiological status, maintenance as well as production energy as animal husbandry is concern. The appropriate feed ration of donkey should consist of forage as the main source of nutrients, while others supplementing vitamins, protein, and minerals followed by the unrestricted access to water. It is also revealed that; male donkeys have been maintaining good body condition during the entire dry period compared to female donkeys when used as working animals. It is advisable that, legume to grass ratio should not be 1:4. Although during the dry period, hay and other supplements are good for donkeys, feed on silage is not advisable due to their elevated protein and moisture contents as well as low pH (Félix 2014).

Suitable houses for donkeys should be roofed, with proper ventilation, lighting as well as bedding materials aiming at improving their welfare. For animal identification, various methods are used for donkey identification some resembling those used in identifying livestock. These methods include, hot iron branding, tattooing, use of ear tags, use of coat color or body identification mark and use of names. Identification is an essential aspect in animal husbandry to ensure proper record keeping, avoid theft cases and in case of transportation from one area to another (Félix 2014). Despite the fact that, donkeys are perceived hard and their health paid less attention, they still suffer a number of infectious diseases, pathological conditions, metabolic disorders and physical injuries as animal health management is concern (Wambui et al. 2004, Fahmy 2004).

2.6 Status of donkeys in Tanzania

Donkey population in Tanzania has increased from 250,000, in 1992 to 595,160 in 2018 (Starkey & Mutabugya 1992, parliamentary budget for the Ministry of Livestock and Fisheries report 2018). This information is just by estimation as no census has ever been conducted on donkey population and distribution in the country. The large percentage of donkeys in Tanzania is said to be found in rural areas. Donkeys are used for various purposes in Tanzania. They are used as draft and pack animal, although some research has shown that they mainly used as pack animals carrying farm harvest and firewood, fetching water, transporting goods to and from the market (Starkey & Mutabugya 1992). At households, they provide manure that is used on crop production (Kelvin 2014). The public perception towards them is not good compared to other domesticated animals. They are perceived as hard and difficult to get sick animals, as a result they are neglected. There is limited information on health constraints of donkeys in Tanzania. The little available information revealed that gastrointestinal parasites, ectoparasites infestations, managerial aspects and cruelty treatment of animals may be the known health challenges affecting donkeys in the country (Kelvin 2004). Gastrointestinal helminths such as *Strongylus, Paramphistomum* and *Moniezia*, and ectoparasites such as mange mites, flies, ticks and lice are common parasites in donkeys although little has been done to treat these conditions. The donkeys in Tanzania are kept in open housing system with the earthed floor. Sometimes the houses are just enclosures surrounded by thorn shrubs with no drainage system. This has been exposing the animals to flies, and all possible adverse weather condition and affect their health.

The use of poor designed harnessing system, the prolonged period of work, long distance travel and beatings have been creating injuries and wounds to donkeys affecting their working efficiency and their welfare in general (Kelvin 2004). Recently, there has been a change of previously existed negative mindset of the public towards donkeys. This has been influenced by increased

recognition of donkey contribution in livelihood improvements, its recognition by the livestock sector and high demand of donkey products in China.

For this case also, the government has started to put emphasis on improvement of donkey management and welfare. Tanzania has provided opportunity to various non-governmental organizations established within the country and others from outside the country to join up efforts aiming at improving animal welfare especially donkeys. According to the Ministry of Livestock and Fisheries report (2017/2018), there are five registered animal welfare organization in Tanzania. These includes, Animal Welfare Society (TAWESO), Tanzania Humane Charity (TAHUCHA), Tanzania Animal Protection Organization (TAPO), Meru Animal Welfare Organization (MAWO) and Arusha Society for the Protection Of Animals (ASPA).The programs of these organizations have been associated with donkey welfare, dog and cat population control, veterinary outreach, humane education in primary schools and communities, welfare of farm animals in markets and in transport, animal shelter as well as policy and advocacy. From all these efforts, veterinary care has now started to be provided to donkeys so as to manage injuries and wounds, treat all possible illness using appropriate drugs. However, there is little known on haemoparasites of donkeys in Tanzania (Mpango Kabambe wa Mifugo Tanzania na Ofisi ya Taifa ya Takwimu 2017).

2.7 Health problems affecting donkeys

2.7.1 Common diseases

Despite the fact that donkeys are resilient and tough, they still suffer different disease conditions that affect their health and subsequent working performance. The common disease conditions affecting donkeys can be categorized depending on their causative agents. Bacterial diseases such as tetanus, anthrax and strangle, viral diseases such as African horse sickness and rabies can affect donkeys (Joan 2015).

Gastrointestinal parasite infestations caused by nematodes, trematodes, cestodes and arthropods, ectoparasite and skin conditions such as mange, fungus, flies are also common (Joan 2015). Other disease conditions are caused by protozoan parasites such a piroplasmosis and dourine (Joan 2015). These disease conditions together with traumatic injuries and wounds not only have they been affecting health and welfare of donkeys but have also compromised their working efficiency and industry especially in developing countries including Tanzania (Joan 2015).

2.7.2 Haemoparasite infections

Haemoparasites such as *Babesia*, *Theileria* and *Trypanosoma* are responsible for causing health constraints in donkeys and hindering their performance in different parts of the word (Joan 2015). As described by Friedhoff et al. (1990), *T. equi* and *B. caballi* are the commonly occurring haemoparasites causing piroplasmosis. In contrast, *Trypanosoma* parasite infections, are not commonly encountered haemoparasites and are restricted only in tsetse infested areas. However, *T. equi* and *B. caballi* are epidemiologically closely related. They are transmitted by tick vectors of the family *Ixodidae*. The tick vectors are commonly known to belong to genus *Rhipicephalus, Haemaphysalis, Hyalomma* and *Dermacentor* (De Waal 1992).The use of blood-contaminated instruments and blood transfusion have also been identified as means for transmitting the haemoparasites in case of carrier state of piroplasmosis. *T. equi* may also be transmitted from jannet to offspring during pregnancy. *T. equi* infections are more prevalent compared to *B. caballi* although both infections are widely distributed throughout the world (Wise et al. 2013). However, the *T. equi* infection is more endemic throughout the African continent with a low prevalence of *B. caballi* reported in Arabic countries such as Sudan (Friedhoff et al. 1990). With the exception of Siberia, it has been revealed that, the parasites are endemic throughout Asia, including China. Infections cover large part of Europe and Asia while no infections have been reported in Australia (OIE 2009).

The life cycle of Equine piroplasma parasites involves three distinct stages that occur in host and ticks. They include; sporogomy as asexual reproduction stage in the salivary glands, merogomy as asexual reproduction stage in the vertebrate host and gamegomy as asexual reproduction with the formation and fusion of gametes in the tick gut. For *T equi* however, there is an additional stage in the life cycle by undergoing schizogony in the peripheral blood mononuclear cells to form a fourth stage (Ueti & Knowles 2018).

In general there are some variations in the life cycle of *T. equi* depending on the species of ticks involved. Usually the animal infected with *T. equi* or *B. caballi* develop protective immunity against the disease due to animal's carrier state of the pathogen (Rothschild 2013).There is no cross immunity between both parasites leading to possibility of the susceptible host to infected with one or both pathogens simultaneously (Maurer 1962). As described by Knowles (1994), both innate and adaptive immunity play an important role in the control of equine piroplasma parasites especially *T. equi.*

2.7.2.1 Diagnosis of haemoparasites

Piroplasmosis associated with *T. equi* have an incubation period ranging from 12 to 19 days and approximately 10 to 30 days when caused by *B. caballi* (OIE 2009). There is no pathognomonic clinical sing of piroplasmosis in donkeys in endemic areas. (Kouam et al. 2010). Clinical signs revealed by the donkey affected with that disease varies from mild to severe. In the mild form the affected animal shows generalized weakness with reduced appetite. In the severe form, the donkey shows high fever, jaundice, anemia, distended abdomen, labored breathing, rough hair coat, staggering gait and red urine which is not the case for trypanosomiasis. The chronic form trypanosomiasis, animal show progressed muscle wastage, but no observable sign as in piroplasmosis. Despite chronic infections, animals can look healthy and may keep on performing their usual roles while harbouring persistent parasites (Kouam et al. 2010).

Pathologically, the disease is associated with hematological changes such as hemolysis of infected erythrocytes leading to anemia regardless of the clinical form of the disease as described by Mahoney et al. (1977). Also, the disease reveals existence of distinct variations in erythrocytic parameters such as Mean Capsular Volume (MCV), Mean Capsular Hemoglobin (MCH) and Mean Capsular Haemoglobin Concentration (MCHC) in both horses and donkeys (Laus et al 2015). In addition, biochemical test, gross pathological examination and histopathological test have been also used for diagnosis of equine piroplasmosis in both horses and donkeys ((Laus et al. 2015)

Laboratory diagnosis is categorized into two categories, direct and indirect methods. Microscopic examination and molecular techniques such as polymerase chain reaction (PCR) have been considered as direct methods for haemoparasite diagnosis. Thin or thick smear are prepared, dried fixed and stained using Giemsa stain and examined under the microscope at 100x oil immersion for morphological identification of *T. equi* and *B. caballi*. In case of trypanosomiasis, the blood or lymph sample as a wet preparation is examined microscopically to observe motile trypanosomes waving between blood cells (Connor 2016). While microscopic examination is ideal to be used in acute cases accompanied with clinical signs, PCR is suitable in chronic cases in which parasite level in the blood is very low. In addition to that, a number of serological tests, such enzyme-linked immunosorbent assay (ELISA), compliment fixation test (CFT) and indirect fluorescent antibody test (IFAT) have been used as indirect methods for diagnosis of haemoparasites of equine (Reiter & Weiland 1989, Brüning 1996).

However, the health status of donkeys may be influenced by a number of factors such as living or working condition including environmental factors as described by OIE (2009). African horse

sickness, Surra, Equine infectious anemia, Dourine, Purpura hemorrhagic and plan/chemical poisoning are differential diagnosis of piroplasmosis in the donkey. At necropsy, for animals diagnosed with piroplasmosis intramuscular hemolytic associate lesions are observed. Jaundice of mucous membranes, thin and watery blood, enlarged with orange-brown discoloration of the liver, dark, friable and enlarged spleen, petechial hemorrhages may appear on the kidney (OIE 2009).

2.7.2.2 Treatment and control of haemoparasites

It is said that there is no drug that eliminates completely *T. equi* in infected animals. However, piroplasmosis in donkeys have been treated using the lower dose of antiprotozoal drugs such as imidocarb and diminazene aceturate. However, supportive treatment is said to be appropriate option (Joan 2015). Connor (2016) pointed out that, trypanosomiasis in the donkey is treated by the use of effective trypanocidal drugs such as isometamidium chloride. The latter is used for treatment as a chemotherapeutic and prophylactic drug for trypanosomiasis infection. The control of the disease depends on epidemiological factors. In areas where ticks are endemic, the appropriate and strategic use of various acaricide formulations is suggested.

The use of chemoprophylaxis drugs, insect repellant and insecticidal conjugated traps are some of the methods used to control trypanosomiasis in donkeys and other animals (Connor 2016). In some areas the disease may be introduced by carrier animals. In such instances, pre-testing of animals against haemoparasites of interest at the site to avoid the introduction of infected animals that can be the source of infection to naïve animals is recommended (Friedhoff et al. 1990).

CHAPTER 3 MATERIALS AND METHODS

3.1 Study area

The central zone is one of the administrative zones in Tanzania that constitute three regions namely Dodoma, Singida, and Manyara. Dodoma region is located at 6°10′23″S35°44′31″E, lies in the heart of Tanzania in the eastern-central part of the country, the main city being about 480 km from the coast. The region is semi-arid and receive a moderate rainfall throughout the year that range between 152.9 mm and 135.2 mm. The ambient temperature ranges between 25°C and 31°C. During the night, the temperature drops up to 17°C (https://www.com/weather/tanzania/dodoma). Dodoma region is bordered by a number of regions including Manyara, Singida, Iringa and Morogoro (Figure 1). However, sampled donkeys were obtained from Nala livestock market in Dodoma city brought from Manyara, Dodoma and Singida regions which defines the central zone of Tanzania. In Dodoma, there is a well-established Huacheng international abattoir at which about 40 donkeys are slaughtered per day.

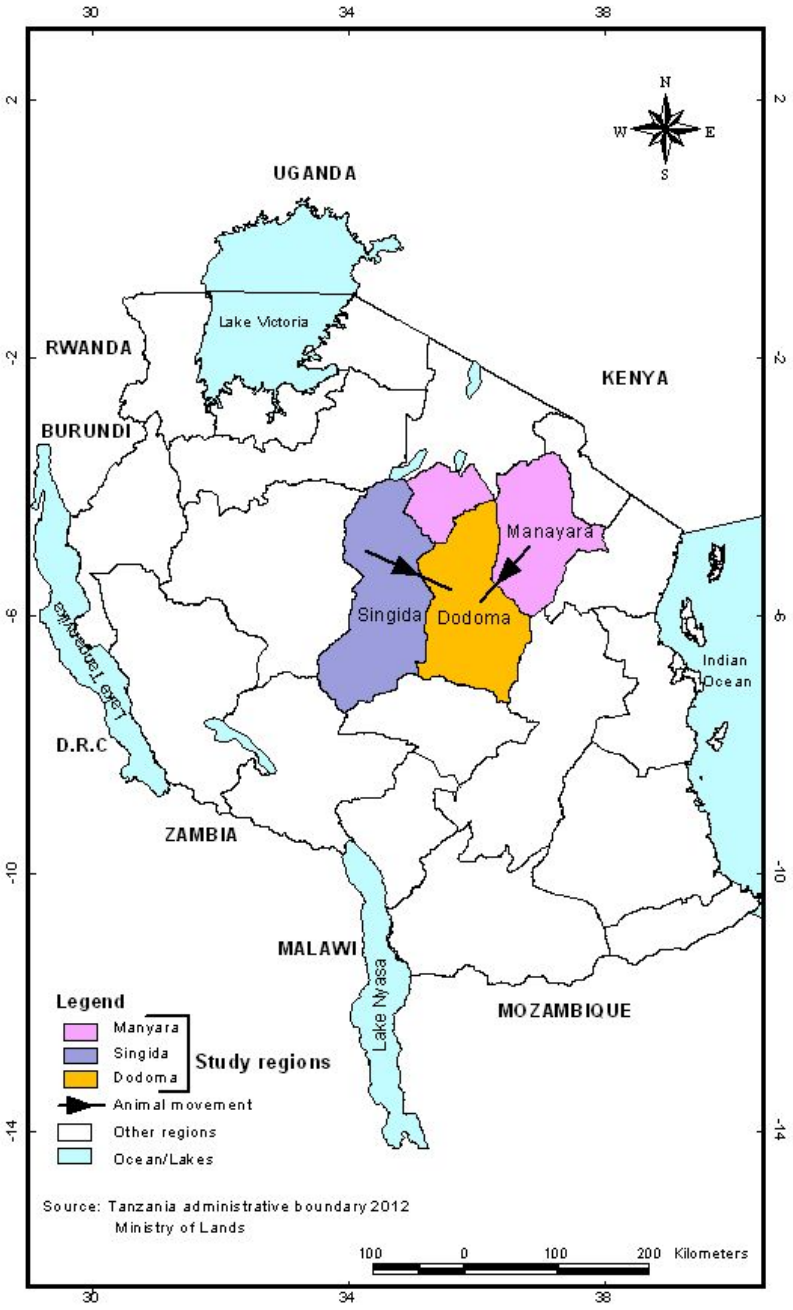

Figure 1: A map of Tanzania that show the central zone. Note that Dodoma region is a place where Nala livestock market is located.

3.2 Study design

A cross-sectional study was conducted to determine the occurrences of haemoparasites in donkeys and establish the possible risk factors for infection in donkeys of Central Zone, Tanzania. Sampling and laboratory analysis were done between October 2017 and October 2018. All the study donkeys were local breeds which are kept by small scale farmers who rarely get veterinary services and animal disease control programmes.

3.2.1 Sample size estimation

The sample size was determined using a formula as $n= Z^2 P(1-P)/d^2$ described by Daniel & Wayne (1999). Where n = sample size, Z= statistical correspondence to level of confidence intervals, P = expected prevalence and d = precision (corresponding to effect size). As there was no similar study, which has been conducted in the area, the 50% expected disease frequency was used to estimate the total sample size at the level of confidence of 95%. That, $n = 196*0.5(1-0.5)/0.05^2$, a total of 384 samples was collected for haemoparasite detection.

3.2.2 Study population and selection of study donkeys

A simple random sampling method was used to select the 384 study donkeys at the market. All the donkeys brought for sale at Nala livestock market during the study period formed the study population. On average, 135 donkeys were brought for sale at the Nala livestock market per market day. The selection of animals based on the region where they came from using information obtained from owners. A total of 128 donkeys from each region were selected for sample collection. The biodata of the selected animals like region of origin, sex, age, and body conditions were recorded. The body condition score was done based on criteria described by National Equine Welfare Council (NEWC) (2009). Evaluation of body fat to muscle relationship was done through observation. The body score was defined poor when emaciated, moderate when has some developed muscles and fat and ideal when has good muscle development and fats deposit around the neck and shoulders, withers, ribs and belly, back and loins as well as hind quarters as described by NEWC (2009). Aging was determined by dentition as described previously by Crane (1997). This was done by observing the replacement of temporary by permanent teeth.

3.3 Blood sample collection and handling

The selected donkeys for study were restrained in the crush facility available at the market place and halters. Sometimes the donkeys were restrained by using halter, chin hold, lift of fore leg and physical or manual holding. The restraining and handling of donkeys was performed by two veterinarians, field officers and animal owners. The blood samples were collected directly from the jugular vein into both plain and EDTA 5 millitres vacutainer tubes using vacutainer needle with holders. After blood collection, the animals were immediately released. The blood samples were stored in cool box with ice packs during the field work. Subsequently, the blood samples were transported under cold chain to Sokoine University of Agriculture laboratories for analysis.

3.4 Laboratory analysis of the blood samples

3.4.1 Microscopic examination

In the laboratory both thin and thick blood smears were made on the glass slide from EDTA tubes, dried in the air, fixed with absolute methanol for 30 seconds, dried and stained using 10% Giemsa stain solution in phosphate-buffered saline (PBS) for 30 minutes. Thereafter, the smears were washed with tap water and dried in the microscopic dry rake. The prepared smears were thoroughly examined for the presence of haemoparasites using the light microscope under 100x oil immersion. Haemoparasites were identified based on their morphological characteristics as described by Soulsby (1982) and Uilenberg (1998). These morphological features include, double pear shaped for *Babesia species* inside red blood cells, single cone like shape for *Theileria* species inside red blood cells, and elongated with kinetoplast, tail and the undulating membrane outside red blood cells for *Trypanosoma* species. A total of 384 slides was examined and the results recorded.

3.4.2 DNA extraction and Polymerase Chain Reaction (PCR)

3.4.2.1 DNA Extraction

To identify positive DNA of the parasites *T. equi* and *B. caballi*, in collected blood samples, molecular techniques were applied. DNA was extracted from 200 µL of blood using Macherey-Nagel kit (2016) as per protocol for DNA purification from whole blood. A total of 200 µL of blood sample was mixed with 25 µL of proteinase K. In the mixture, 200 µL lyses buffer B3 was added, vortexed vigorously and incubated in the water bath at 70 ºC for 30 minutes.

DNA binding was adjusted by the addition of 210 µL absolute ethanol to the sample mixture. Then, the mixture was transferred to nucleospin blood column and centrifuged at 11000 x g for 1 minutes. The first wash of silica membrane was done by the addition of 500 µL washing buffer BW. Thereafter, the sample mixtures were centrifuged at 11000 x g for 1 minute. The silica membrane was washed for the second time by using 600 µL of Buffer B5 and centrifuged at 11000 x g for 1 minutes. Drying of the silica membrane was performed by extra centrifugation at 11000 x g for 1 minutes. To obtain higher yield the DNA was eluted by adding 50 µL of preheated Buffer BE at 70ºC. Then, the mixture was incubated at room temperature for 1 minute and centrifuged at 11000 x g for 1 minute. The same procedure was repeated twice. The eluted DNA was stored at -80ºC until further analysis.

3.4.2.2 Polymerase Chain Reaction (PCR)

The multiplex PCR was performed for detection of *T. equi* and *B. caballi* as described by Alhassan et al. (2005) by using AccPower® Taq PCR PreMix kit manufactured by Bionner Corporation, Republic of Korea. The premix tube in the tubes contained Containing 1U Taq polymerase, 30 µM KCL, 250 µM dNTP, 10 µM Tris-HCL, 1.5µM Mgcl2. This was done by using two reverse primers; EquiR 5'-TGCCTTAAACTTCCTTGCGAT-3' for *T. equi* and CabR: 5'-CTCGTTCATGATTTAGAATTGC-3' for *B. caballi* as well as a common forward primer, UFP: 5'-TCGAAGACGATCAGATACCGTCG-3'. The targeted amplified gene was 18S rRNA at a region with 430 bp for *T. equi* and 540 bp for *B. caballi*. Then, in each premix tube 4 µL of 10pm/ µL primers, 16 µL of nuclease free water and 1 o µL f DNA was added to make a total volume of 25 µL. Then master mix of 106 samples was prepared and 25 µL of the mixture solution was dispensed in each PCR tube. The DNA amplification was done in a thermocycler machine (GeneAmp PCR® System 9700). Amplification conditions were initial activation at 96 ºC for 10 minutes followed by 36 cycles with denaturation at 96 ºC for 1 minute, annealing at 60.5 ºC for 1 minute and elongation at4 72 ºC for 1 minute. The final extension and hold were set at 72 ºC for 10 minutes and at 4ºC respectively.

3.4.2.3 Electrophoresis

The evaluation of PCR products was done in 1.5% agarose gel. Briefly, 1.5 g of agarose powder was mixed in 100 mL of 1xTAE buffer in a conical flask. The mixture was vigorously shaken and boiled using heat block with magnetic stealer to make clear solution. The gel was allowed to cool while adding 10 µL of EZ-Vision® In-Gel solution to facilitate staining of DNA. A volume of 5 µL DNA amplicon was added in the gel wells immersed in the electrophoresis tank containing 1xTAE. A total of 10 µL of a1000 kb molecular weight marker mix (Promega, Madison, USA) was loaded in a parallel track with the DNA amplicon. The reaction was carried out at 120 voltages for 1 hour. The distinct DNA bands of *T.equi* (430 bp) and *B. caballi* (540 bp) were visualized by using UV transilluminator. Positive controls were obtained from the Department of Veterinary Tropical Disease, Pretoria University and water was employed as negative control.

3.4.2.4 DNA purification and sequencing

Purification of obtained DNA amplicons was performed using ExoSAP DNA clean up protocol. In brief, ExoSAP clean up master mix was prepared using 1.5 µL of Exonuclease I, 1.5 µL of TSAP and 247.25 mL of nuclease free water to make a total volume of 250 mls. Then 5mL of the prepared master mix was added into each 20mL of the PCR product. The reaction mixture was subjected to the thermocycler machine (GeneAmp PCR® System 9700) and allowed to run under the following conditions; 37^0C for 25 minutes, 80^0C for 20 minutes and 4^0C as holding

temperature. Cycle sequencing was done using 5x sequencing buffer, Big Dye Terminator, primers, DNA template and Nuclease free water as reaction reagents.

The reaction was performed under the following conditions; initiation at 96 ºC for 1 minute, 25 cycles of denaturation at 96 ºC for 10 seconds, annealing at 50 ºC for 5 seconds, extension at 60 ºC for 4 minutes and 4 ºC as the storage temperature. Then, ethanol precipitation was done in the tubes, in which to each reaction mixture, 5 µl 125 Mm EDTA and 60 µl 100% EtOH was added followed by the vortex for 10 seconds. The reaction mixture was then incubated in dark at room temperature for 15 minutes, vortexed and centrifuged at 12000 g for 30 minutes. All the supernatants were removed following the addition of 60 µl 70% ethanol in the reaction mixture, vortexed for 10 seconds and centrifuged for 30 minutes.

Then Vacu-dry was performed for 45 minutes in the dark at room temperature followed by addition of 20 µl Hi-Di Formamide to re-suspend the DNA then loaded in the sequencing machine. Sequencing of PCR product was performed at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture using AB 3500 Genetic analyzer.

3.5 Data Analysis

Collected data from study animals and laboratory analyses were entered into a Microsoft Excel version 2007 spread-sheet and analysed in R. Studio software. The relationship between the prevalence of haemoparasites and risk factors such as age, sex and body condition were determined using the Chi-square test. The significant association was identified at p-value less than 0.05.

CHAPTER 4 RESULTS

4.1 Microscopic examination

Thin and thick blood smears were prepared from 384 donkey blood samples obtained from study areas. Microscopic examination of all slides was conducted to determine the presence of target haemoparasites (*Babesia, Theileria and Trypanosoma* species) based on morphological appearance. All examined slide revealed negative results against those targeted haemoparasites.

4.2 Polymerase chain reaction

Diagnosis of targeted haemoparasites was done and the amplification of positive DNA products revealed amplicons of 430 bp as indicated by the *T. equi* positive control band (Figure 2). Out of 104 samples that were tested by PCR; 98 (94.2%) were *T. equi* positive whereas the same sample revealed negative results for *B. caballi*

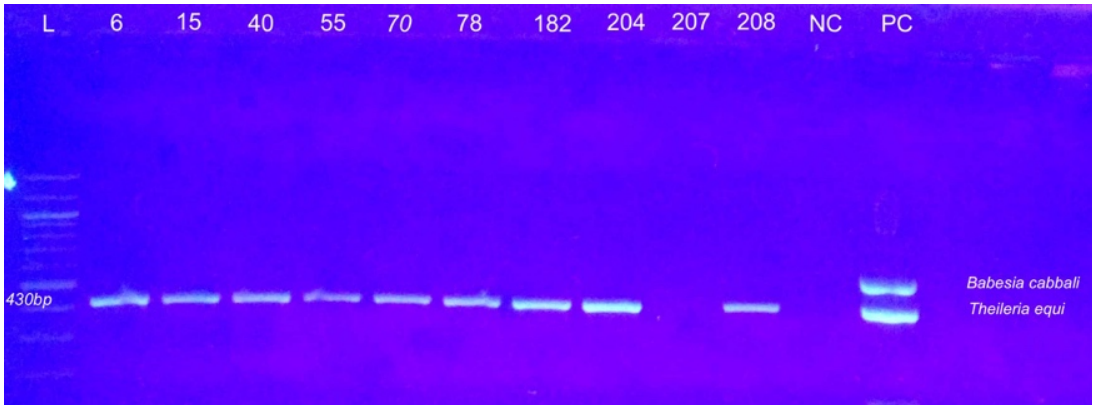

Figure 2: An agarose gel after performing Multiplex PCR using EquiR, CabR and UFP primers. A PCR product of approximately 430 bp for *Theileria equi* was amplified

Key: L = 1kb ladder, number (6, 15, 40, 55, 70, 78, 182, 204, 207, 208) = samples, PC= positive control, NC= negative control.

4.3 Sequencing

A total of 11 PCR products of the samples were randomly selected out of 104 and sequenced. All sequenced PCR products showed similar results. The obtained sequences were edited and aligned by using genius software and subjected to nucleotide Basic Local Alignment Search Tool

(BLASTn) for analysis. The aligned sequences were 99% identical to *T. equi* 18S rRNA with accession no MG052915.1 in the GenBank

4.4 Risk factors for haemoparasites in donkeys

The association between *T. equi* prevalence and associated risk for sex and body condition was determined using Chi-squire test with p-value of 0.77 and 0.71 respectively. The age was not considered in the analysis because all donkeys were adults.

Table 1: Occurrence of *Theileria equi* by PCR method (n=104)

Risk factor	Category	Number of positive	Percent	P value
Age	Adult (n=104)	98	94.23	
	Young (n=0)	0	0.0	
Sex	Male (n=58)	55	94.5	
				0.77
	Female (n=46)	43	93.5	
Body condition score	Good (n=42)	39	92.9	
				0.71
	Moderate (n=53)	50	94.3	
	Poor (n=9)	0	0.0	

CHAPTER 5 DISCUSSION

During blood sample collection, donkeys were clinically healthy with no clinical sign associated with haemoparasite infections. However, the existence of chronic infection with the persistent low level of parasites in the blood has been previously reported to be possible (Allsopp et al 2007). Neglect, inadequate veterinary care and negative public perception towards donkeys especially related to managerial aspects, may have exposed them to tick infestations that transmit haemoparasites. The donkeys from which blood samples were collected may have chronic infections of *T. equi* due to tick infestations of which during sample collection, ticks were observed on donkeys. As described by Allsopp et al (2007), George et al. (2011), chronicity of parasite infestation may have been caused by a vertical mode of transmission as revealed in *T. equi.* The resulting newborn foals remain carriers throughout their life.

In the present study, no haemoparasites were detected in the donkey blood smears examined under microscope. This result may have been caused by the following: low sensitivity of smear test, no active circulating parasites in the blood, and the presence of very low level of haemoparasites in the blood. Such situations are mostly present during chronic Haemoparasite infections. On the contrary, a similar study conducted by Abedi et al (2015) in Iran revealed few positive results 4 (3.77%) out of 100 and six blood smears prepared from apparently healthy donkeys that were positive for *T. equi.* Another study conducted by Makibi et al. (2009) in Ethiopia revealed 2.5% donkeys of positive results of 400 samples collected from apparently healthy donkeys that were positive *for B. caballi.* The latter may have been associated with the presence of active circulating haemoparasites in the donkey blood in those areas.

These findings and the result variabilities observed agree with the previous studies on the prevalence of haemoparasites. They described the chances of detecting haemoparasites especially *T. equi* and *B. caballi* being high in acute cases that present clinical signs and relatively lower in chronic cases (Reiter & Weiland 1989, Brüning 1996). However, PCR and other serological assays such as such enzyme-linked immunosorbent assay (ELISA), compliment fixation test (CFT) and indirect fluorescent antibody test (IFAT) can be used for determination of the presence of parasite antibodies of haemoparasites in donkeys which will be an indicator of exposure to infection (Reiter & Weiland 1989, Brüning 1996).

Data from multiplex PCR showed that 98 donkeys out of 104 which is equal to 94.23% had the predictive band at approximately 430 bp for *T. equi*. This result confirms the presence of *T. equi* in donkeys in central zone Tanzania. The PCR technique exhibited high sensitivity for detection of *Theileria equi* regardless of low parasitaemia in the blood samples. The same results have

been observed in different studies conducted previously. In a study conducted in Brazil using the PCR method, DNA of *T. equi* were detected in 31. 81% of donkeys (Machado et al. 2011), in Iran, Abel et al. (2015) reported 50.94% of the same parasite in the examined donkeys using multiplex PCR. Low prevalence of equine piroplasmosis in donkeys may be attributed by low susceptibility to *T. equi* infection compared to horses that are more susceptible (Qablan et al. 2013, Balkaya et al. 2010). On the other hand, in some countries such as Brazil (Santos et al. 2011), Greece, (Kouam et al. 2010) and Spain (García-Bocanegra et al. 2013), researchers reported that mules were more susceptible than horse and donkeys.

These previous studies indicated that donkeys may be important carriers for *T. equi* infection in different countries. In the present study *B. caballi* was not detected in blood samples of donkeys. However, *B. caballi* infection and its geographical distribution is low worldwide compared to *T. equi* (Friedhoff et al. 1990). The p-values of 0.77 and 0.71 at 0.05 level of significance was obtained following analysis for association between the prevalence and associated risk of sex and body condition respectively. This result revealed no significant association between the prevalence of *T. equi* and associated risks in donkeys from the study area. On the other hand, age was not considered because all donkeys were adult. However, as indicated by Shkap et al. (1998), Moretti et al. (2010), pasture grazing as managerial practices mostly practiced in developing countries including Tanzania, may expose donkeys to tick infestation and the subsequent cause of equine piroplasmosis. However, there is no previous study that indicate the presence or absence of vectors of piroplasma parasites in central zone in Tanzania.

Conclusion and Recommendations

Recent studies have shown that there is increased spectrum of tick-borne diseases infecting domestic animals and human. This has given concern to veterinarian and physicians (Dantas-Torres et al. 2012). The present study conducted on donkeys haemoparasites in Central Zone, Tanzania revealed high occurrence of *T. equi* detected using PCR technique indicating that donkeys in the study area may be carrier of the parasites.

The parasites might be contributing to compromising donkey' health, welfare and their subsequent draught power. According to Kouam et al. (2010), the risk factors associated with occurrence of equine piroplasmosis may be both intrinsic and extrinsic factors. These factors include; age, sex, breeds equine species, host activity, altitude, grazing and climatic factors. On the other hand, the study revealed no significant association between observed prevalence of *T. equi* in donkeys and associate risk of body condition and sex in the study area. Therefore, the data obtained in this study proved the existence of one type of haemoparasites with no associated risk factors for their occurrence in donkeys in central zone in Tanzania.

Therefore, the following recommendations are suggested: regular and strategic tick control programmes should be implemented using appropriate acaricides and; the government should formulate and implement policies regarding management and health aspect of donkeys. Further research is needed to further determine the epidemiology, pathogenicity and the antiprotozoal resistance status of the prevailing protozoan parasites. There is also a need to ensure adequate political support to enhance reflection of animal traction development institution with emphasize to agricultural training and research institutions to have appropriate plans for the development of animal traction. Large scale epidemiological study for equine piroplasmosis in donkeys using serological techniques are required in Tanzania as it will give better picture of the prevalence and distribution of equine piroplasma parasites.

On social limitations, since donkeys are used by small scale and resource compromised farmers with no purchasing power for heavy and good agricultural implements such tractor, there is a need for liberalization of policies to favour not only the importation of tractors but also equipment and breast harnessing systems used for animal traction. Stakeholder engagement, routine public education on the use of donkeys to cultivate more land for food and cash crops production is recommended. Tanzania societies have to produce adequately to have enough food while having excess harvest to sell and earn money to improve their livelihood. In so doing the mindset of the farmers could change from cultivating small scale farm by using hand hoe to relatively medium to large scale farm using donkeys so long as the associated equipments are available and affordable.

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LIST OF ANNEXURE

Annexure 1: Proof of ethical clearance

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Occurrence of haemoparasites in donkeys in the oofcentral zone of Tanzania**
PROJECT NUMBER	**V120-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. MA Anthony**

STUDENT NUMBER (where applicable)	**U_17388598**
DISSERTATION/THESIS SUBMITTED FOR	**MSc (Tropical Animal Health)**

ANIMAL SPECIES	**Domesticated Donkeys (Equine)**	
NUMBER OF ANIMALS	**384**	
Approval period to use animals for research/testing purposes		**November 2017 – November 2018**
SUPERVISOR	**Prof. PJ Matjila**	

Conditions: The AEC has noted that this project will be completed in a facility outside of South Africa. Since the AEC has not inspected the facility, please note that we cannot comment on the quality of the facility other than what was provided in the study questionnaire

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 2 February 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15