EPIDEMIOLOGY AND SPECIES CHARACTERIZATION OF MANGE IN CHEETAHS, THOMSON'S GAZELLES AND DOMESTIC ANIMALS IN THE MASAI MARA ECOSYSTEM

A thesis submitted to the University of Nairobi in fulfillment of Doctor of Philosophy degree in Veterinary Epidemiology

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DECLARATIONS

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

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DEDICATION

This thesis is dedicated to my wife Caroline Wangu and my children Duncan Gakuya and David Wachiuri

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TABLE OF CONTENTS

LIST OF TABLES

Table…… Page

LIST OF FIGURES

Figure……Page

LIST OF ABBREVIATIONS

CITES	Convention on International Trade in Endangered Species
Dps	genetic Distance based on Proportion of Shared allelles
F	F- statistic
gDNA	Genomic Deoxyribonucleic Acid
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg
IUCN	International Union for Conservation of Nature
KWS	Kenya Wildlife Service
LE	Linkage equilibrium
ºC	Degrees centigrade
PDS	Participatory Disease Search
PRA	Participatory Rural Appraisal
ul	Microlitres
χ^2	Chi square statistic

ABSTRACT

Mange is a contagious skin disease caused by one or a combination of several species of mites and is spread by direct contact with diseased animals or from various objects, which have been in contact with the diseased animals. The various species of mites affect domestic animals, humans and wildlife and mange is a disease of zoonotic importance.

A study was carried out in Masai Mara ecosystem to determine the spatial distribution and prevalence of mange, establish the level of knowledge of the disease dynamics of mange among pastoralists and wildlife officers, phenotypically and genotypically characterise the mange mites and determine relationship between the specific species found in various animal hosts.

For the spatial distribution study, a cross-sectional survey was used to collect data of mange-like skin lesions over a period of 2 years in cheetahs, Thomson's gazelle, sheep, goats, cattle, dogs and other wild animals. The study area was divided into 8 study blocks, 3 within the protected area and 5 in the community ranches. Global Positioning System (GPS) co-ordinates and dates of observation of all individual animals and/or herds observed to have clinical signs of mange were recorded. Maps of distribution were generated using Arc View software for Global Information System (GIS) mapping. Infected animals were observed in 6 out of the 7 study blocks where the study was carried out. There was a higher concentration of infected animals along the boundaries of

protected areas and community ranches where there is significant interaction between wildlife and domestic animals. There was a higher concentration of infected animals in dry than in wet season.

In the prevalence study, a cross-sectional survey was used to collect data of mange-like skin lesions over a period of 2 years in cheetahs, Thomson's gazelle, sheep, goats, cattle, dogs and wildebeest. Purposive random sampling method was used to get the sampling units. Sampling of domestic animals was based on study blocks closer to the protected areas or where cheetahs were known to occur, while Thomson's gazelle sampling was based on study blocks within the protected areas and in community ranches where they occur. Due to their low numbers and very large home ranges, cheetahs were sampled opportunistically. The prevalences of mange-like skin lesions were: cheetah 12.77% (n=47), dogs 4.67% (n=279), Thomson's gazelle 0.81% (n=10,788), sheep 0.76% (n=6,699), cattle 0.09% (n=2,311), goats 0.09% n=1,174) and wildebeest 2.00% (n=50). The factors that can affect prevalence of mange in the study area were identified as geographical location (study blocks), climatic season and time of sampling. There was a higher prevalence in study blocks that had high wildlife/livestock interaction, in dry than wet climatic season and in the year 2007/2008 than 2008/2009. However, it was only among Thomson's gazelles that the difference between prevalence in dry and wet season and between the first and second year of sampling was significantly different ($P = 0.0001$).

The study to establish the level of knowledge of the disease dynamics was conducted using pre-tested questionnaires. Fifty six pastoralists and 30 wildlife officers responded. Ninety three (93%) percent of pastoralists and 99.7% of wildlife officers stated that they had come across mange while 23.3% and 66.1% of the pastoralists and wildlife officers knew that the disease is caused by mites. Up to 70% of respondents in the 2 groups thought that the disease is transmitted from domestic to wild animals and vice versa. Over 80% of respondents from both groups confirmed that they had seen infected animals. Sixty eight (68%) percent of pastoralists confirmed they normally treated and initiated control measures against mange, with application of acaricides being most preferred. Pastoralists identified fungal diseases, sheep and goat pox, papillomatosis and phosensitization as skin diseases that can be confused with mange while wildlife officers identified lumpy skin disease, fungal infections and giraffe ear infection. This study concluded that there was a lot of information about mange among pastoralists and wildlife officers within the ecosystem but more studies using Participatory Rural Appraisal (PRA) approach were required to determine if the disease they identified was mange.

Phenotypic characterization studies were conducted on 78 samples collected from different species of wild and domestic animals with skin lesions. The clinical picture in the field was that of alopecia, pruritus, acute dermatitis, suppurative encrustation, skin roughening and poor body condition. Mites were identified in the laboratory through microscopy. The positive samples with respect to animals showing skin conditions were as follows: cheetahs 100%

(8/8), Thomson's gazelle 80% (8/10), sheep 52.9% (27/51), dogs 11.1% (1/9), wildebeest 100% (5/5) and lions 100% (2/2). Samples from cattle goat and impala were negative. *Sarcoptes scabiei* was isolated from all the animals except sheep where *Psoroptes communis* was isolated. The study revealed that sarcoptic mange is the commonest mange affecting wildlife in the Masai Mara ecosystem.

Genotypic characterization study was undertaken through use of molecular techniques. Deoxy-ribonucleic acid (DNA) was extracted from individual mites isolated from the various host species and subjected to Fluorescent-based polymerase chain reaction (PCR) analysis of microsatellite DNA. There was genetic diversity of mites of *S. scabiei* isolated from the same host species with the highest diversity observed in wildebeest and cheetahs and lowest in Thomson's gazelle and lion. Genetic similarities were observed between mites isolated from cheetahs and wildebeest, cheetah and Thomson's gazelle, cheetah and lion, and lion and wildebeest. The similarity was attributed to gene flow of mites as a result of predator-prey relationship and host-taxon-derived effect for cheetah and lion. The study showed that there is intra-host differences and inter-host genetic similarity of *Sarcoptes* mites among wildlife in Masai Mara ecosystem.

In conclusion, the spatial distribution of mange infected animals was found to be related to areas with close interaction between wild and domestic animals and climatic seasonal changes. The prevalence of mange in free-ranging

cheetah and Thompson's gazelle is reported for the first time in literature. S*arcoptes scabiei* was found to be the commonest cause of mange in wild animals while *P. communis* was the commonest in sheep within the ecosystem. There was also intra-host genetic differences and inter-host genetic similarities of *Sarcoptes* mites among wild animal hosts in Masai Mara ecosystem. There was also detection of host-taxon effect in *Sarcoptes* mites of genetically related species. This study has shown a *Sarcoptes* gene flow in predator/prey system, which is of pivotal interest for sarcoptic mange control and wildlife conservation.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Mange is a contagious skin disease caused by one or a combination of several species of mites. It is spread by direct contact with diseased animals or from various objects, which have been in contact with the diseased animals (Siegmund *et al*., 1973). There are various species of mites that affect domestic animals, humans and wildlife (Pence and Uckermann, 2002; Kahn *et al.,* 2005). It is a disease of zoonotic importance (Bornstein et al., 2001; Fischer *et al.*, 2003; Kahn *et al.,* 2005).

The world cheetah (*Acinonyx jubatus*) population is estimated at not more than 12,000, most likely fewer than 9,000, and is declining. The species is listed as endangered and placed in Appendix 1 by Convention on International Trade in Endangered Species (CITES) to enhance promotion and greater regulation of inter-continental and regional cross-border trade and utilization of the species (Mulheisen and Knibbe, 2001). The International Union for Conservation of Nature (IUCN) classifies the African sub-species as endangered and the Asiatic sub-species as critically endangered in the annually reviewed 'threatened' animal categories of the IUCN Red List programme which shows the species global conservation status.

The cheetah is now extinct in many areas within its historic and geographic range and is highly endangered where it remains (Weber and Rabinowitz,

1996). They once occurred in the whole of Africa, excluding, the tropical lowland forests, but are currently found mainly in Namibia and Tanzania protected areas. In Kenya the cheetah population is estimated to be less than 1000 individuals with Gros (1998) estimating the population to be 793. Among the major factors thought to have brought about the decline in numbers are diseases, with mange being placed among the leading causes of death (Weber and Rabinowitz, 1996). Other factors include predation and competition with other predators, tourist pressure, conflict with pastoralists and habitat loss (Ngoru and Mulama, 2002).

The Thomson's gazelle (*Gazella thomsoni*) is the most important prey animal to the cheetah in Masai Mara/Serengeti ecosystem (Hayward *et al.,* 2006) with reports of up to 90% of prey animals taken by cheetahs in the Serengeti being Thomson's gazelles (Cheetah News, 2002). Thomson's gazelles have also been reported to suffer from mange (Pence and Uckermann, 2002; Personal observation). Other wild animals in Africa that are affected by mange include lions (*Panthela leo*), impala (*Aepyceros melampus*), wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*), eland (*Taurotragus orynx*), kudu (*Tragelaphus strepsiceros*) (Pence and Uckermann, 2002), mountain gazelle (*Gazelle gazelle*) (Kurtdede et al., 2007) and mountain gorillas (*Gorilla gorilla berengei*) (Kalema *et al*., 1998). These animals interact closely with cheetahs either as prey or sharing the same range. Among domestic animals, cattle, sheep, goats, dogs, cats, horses and pigs are affected by mange (Kahn *et al.,* 2005).

The existing cheetah population in Kenya occurs in small-scattered populations with the Mara region believed to support a large cheetah population hence offering one of the best prospects for cheetah conservation (Ngoru and Mulama, 2002). Pastoral communities inhabit these areas and there is a lot of interaction between wildlife and livestock at the wildlife/livestock interphase. The natural resources are shared between wild and domestic animals and disease transmission occurs at this interphase.

Due to the fact that mange is regarded one of the major causes of decline in the cheetah population and the fact that it affects the cheetah's major prey (Thomson's gazelle) and domestic animals in contact, it is important to study the epidemiological dynamics of this disease among these animals. The causative organisms (mites) might be host specific or cross-infect all or some of these animals. This study is expected to identify the mange mites causing the disease, their epidemiology, and determine the level of knowledge of mange among pastoralists and wildlife officers. The results will facilitate formulation and implementation of concrete control measures especially among the cheetahs.

1.2 Objectives

1.2.1 Overall objective

To determine the epidemiology of mange parasites among the cheetahs, Thomson's gazelles and domestic animals sharing the same range in Masai Mara ecosystem and characterize the species of mange involved.

1.2.2 Specific objectives

i. To determine the spatial distribution of mange among cheetahs, Thomson's gazelles and domestic animals in the Masai Mara Ecosystem
ii. To determine the prevalence of mange among cheetahs, Thomson's gazelles and domestic animals
iii. To establish the level of knowledge of the disease dynamics of mange among pastoralists and wildlife officers in the study area
iv. To identify the mange mites in cheetahs, Thomson's gazelles and domestic animals in the study area, using phenotypic characteristics.
v. To genotypically characterize the mange mites and determine the relationship between the specific species found in various animal hosts in the study area

1.3 Justification of the study

The cheetah population in Kenya and generally in the rest of the world is on the decline. Cheetah is one of the flagship species that attracts tourists to the

country hence its conservation status is high. Among the factors thought to contribute to the decline are diseases, with mange being one of the commonest.

The key prey of the cheetah is the Thomson's gazelle. From personal observations and reported studies this species is highly affected by mange. The cheetahs and Thomson's gazelles share the same ranges with pastoralist Maasai's domestic animals in Masai Mara ecosystem. The domestic animals are also affected by mange.

Although the literature from Kenya and elsewhere reports cheetah to be inflicted mostly by Sarcoptic mange, studies have not been done to investigate if there is transmission of parasites between the cheetahs, Thomson's gazelles and domestic animals in Masai Mara ecosystem where cheetahs are known to be affected by mange and the interaction with domestic animals is very high. The various varieties of *Sarcoptes* mites are morphologically indistinguishable but are known to be host specific experimentally and can only be distinguished genotypically. This also applies to other types of mites. Genotypic characterization can determine if the mites affecting the various species are similar or different.

The prevalence and partial distribution of mange in Masai Mara ecosystem has not been previously determined and the study gives clear indications of these parameters. Further, the seasonality or lack of seasonality of prevalence and

distribution was determined. Control measures can be focused on the season with higher probability of outbreaks.

The interventions in case of infection have been focused on cheetahs where the infected individuals are treated. Very little focus has been put on control of disease focusing on Thomson's gazelles and domestic animals sharing the same ranges. This probably is due to lack of knowledge on whether the mites, are host specific or non-specific. By determining the host specificity of the mites control measures can be directed to the other animals that are more abundant than the cheetahs.

This study aimed at identifying the mange mites responsible for mange outbreaks in cheetahs and other animals in Masai Mara ecosystem, understanding the epidemiology of mange in this ecosystem and coming up with recommendations on the most effective control measures.

CHAPTER TWO: LITERATURE REVIEW

2.1 Mange

Mange is a contagious skin disease caused by one or a combination of several species of mites. It is an ecto-parasitic dermatitis characterized by encrustation, alopecia, and pruritus of the skin (OIE, 2007) and is spread by direct contact with diseased animals or from various objects, which have been in contact with the diseased animals. The development of clinical mange depends on the mode and the place of the infection and the susceptibility of the host (Siegmund *et al*., 1973).

Mites belong to the order Acarina, in the phylum Athropoda and class Arachnida. The order Acarina includes mites and ticks that affect animals and man. This order comprises arachnids with mouthparts set off from the rest of the body on the false head (capitulum) and in which the body segmentation is greatly reduced or absent. The mites are quite small, most species being either microscopic or under 1mm in length. Parasitic mites mostly feed on blood, lymph, living and dead epithelial cells or feathers. Their mouthparts are adapted for either piercing or chewing (Margaret and Russell, 1978).

Different species of mites affect domestic animals (Siegmund *et al*., 1973; Mugera, *et al*., 1979; Blood and Radostitis, 1989; Ngoru and Mulama, 2002; Kahn *et al.,* 2005), humans (Mugera *et al*., 1979; Kahn *et al.,* 2005) and wildlife (Burgess, 1994; Mugera, *et al*, 1979; Mwanzia *et al*., 1995; Laurenson,

1995a; Kalema *et al*., 1998; Pence and Uckermann, 2002; Ngoru and Mulama, 2002; Williams *et al*., 2008). Mange is a disease of zoonotic importance (Kahn *et al.,* 2005; Ljunggren *et al*., 2006).

2.1.1 Sarcoptic mange

2.1.1.1 General

Sarcoptic mange, called scabies in man and mange in animals, is a common, widespread, highly contagious, mite-caused skin disease of mammals (Bornstein *et al.*, 2001). The disease is spread by direct contact or indirectly through formites (Kahn *et al.,* 2005). The disease has potential to cause huge economic loss due to reduced production and increased mortality in wildlife (Bornstein, 1995; Bornstein *et al*., 2001; Dagleish *et al*., 2007). In addition, it is an emerging/re-emerging infectious disease, which globally threatens human and animal health (Fthenakis *et al*., 2000; Daszak *et al*., 2000). In most animal species, it is characterized by intense pruritis followed by development of small papules and vesicles, which lead to acute dermatitis and eventually formation of scabs and crusts (Siegmund *et al*., 1973; Blood and Radostitis, 1989; Scott *et al.,* 2001; Pence and Uckermann, 2002; Fitzgerald *et al*., 2004; Ljunggren *et al*., 2003; Ljunggren *et al*., 2006; Kahn *et al.,* 2005; Williams *et al*., 2008). The disease causes loss of body condition and even death in severely affected animals (Fain, 1978; Yeruham *et al*., 1996). In humans it has been reported that bacterial colonization of the burrows in the epidermis formed by *Sarcoptes scabiei* leads to serious disease conditions such as septicemia, renal damage,

rheumatic fever and as a consequence rheumatic heart disease (Fischer *et al.*, 2003).

The causative mite, *S. scabiei*, or itch mites are known to infest a wide range of mammalian hosts including more than 100 species belonging to 27 families and 10 orders (Bornstein *et al*., 2001). This mite is a member of suborder Sarcoptiformes, family Sarcoptidae (Bornstein *et al*., 2001). Sarcoptidae are the "burrowing mites" that include the genera *Sarcoptes*, *Notoedres* and *Knemidocoptes* (Fain, 1978). The mite probably originated as a human parasite and man spread the infection to domestic animals (Fain, 1978). Various wildlife species are infected, often from contact with their domestic counterparts (Pence and Uckermann 2002). The mites are morphologically identical (Fain, 1978; Fain, 1991) and the question as to whether those infecting different hosts belong to different species or whether they are, in fact, monospecific, has been a subject of on-going, debate for many years. A generally acceptable answer has not yet been found, but there is general agreement that differences in biological characters, especially in host specificity, exist within this genus (Zahler *et al*., 1999; Berilli *et al*., 2002: Gu and Yang, 2008). Experimental transfer between hosts of different species is commonly unsuccessful (Arlian *et al*., 1984; Arlian *et al*., 1988a; Arlian, 1989), hence the mite is described as a single species with variable sub-species that are predominantly host specific.

Sarcoptes scabiei mites are named according to the main host they infest such as *S. scabiei var bovis* in cattle, S. *scabiei var canis* in dogs*, S. scabiei var ovis* in sheep, *S. scabiei var suis* in pigs, *S. scabiei var vulpes* in red foxes, etc (Mugera, *et al*., 1979; Blood and Radostitis, 1989; Pence and Uckermann, 2002; Bates, 2003). This mite has a characteristic oval, ventrally-flattened and dorsally-convex, tortoise-like body. The body (idiosoma) surface is covered with fine striations; the dorsal idiosoma has fields of several stout septae, and the adult female has fields of numerous cuticular spines, which are taxonomically important features (Fain, 1978; Pence *et al*., 1975). An adult *S. scabiei* measures 0.3-0.5mm in length, roughly circular in shape without distinctive head and has 4 pairs of short legs with females almost twice as long as the males (Kahn *et al*., 2005). The legs bear suckers on un-jointed pedicels on the first two pairs of legs in the male and female and on the fourth pair in the male but the remaining legs end in long filiform projections, which trail far behind the body. The third and fourth pairs of legs are short and do not extend beyond the body contour. On the dorsal surface are transverse grooves, small spines and few hairs. The dorsal part of the female bears three anterior and six posterior spines on each side of the midline (Mugera, *et al*., 1979).

Sarcoptic scabiei burrows into the skin, through the stratum corneum to the stratum granulosum and stratum spinosum, where they consume live cells or tissue fluids oozing into the burrows (Van Neste, 1988: Arlian *et al*., 1988b). Part of the burrowing is accomplished by the action of the cutting mouthparts, chelicerae and gnathosoma and the cutting hooks of the legs (Arlian *et al*.,

1988b; Burgess, 1994; Pence and Uckermann, 2002). Pence and Uckermann (2002) reported that mechanical disruption and ingestion of cells and tissue fluids by the mites in the skin contribute to the pathogenesis of sarcoptic mange. The excretions and secretions of living mites may have an irritant and allergic effect. A massive amount of antigenic material is released in the skin including dead mites, molten skins of living adult and immature mites, and eggshells. Thus a large part of the pathogenesis of sarcoptic mange is a manifestation of hypersensitivity to the mites. Both type I and type IV hypersensitivity have been demonstrated in dogs (Bornstein *et al*., 2001) and pigs (Davis and Moon, 1990).

The initial lesions in a sarcoptic mange infestation, their subsequent progression and the concordant clinical signs vary considerably among many different host species that may be infected (Pence and Uckermann, 2002). The course of the disease and the clinical symptoms is determined by the immunological state of the host. Specifically, this depends on whether or not the host species is immunologically naive (Ngoru and Mulama, 2002), or, if it has been previously exposed to the mite or whether it is allergic or can evoke hypersensitivity reaction (Pence and Uckermann, 2002). Whether immunologically compromised or not, the lesions begin as non-pruritic patches of erythematous papules grading into seborrheic dermatitis. Mites may not be particularly abundant in the skin at this point. After several weeks the lesions in the immunologically competent animal become intensely pruritic and there is extensive hyperkeratosis, alopecia and dermal inflammation. The skin becomes

greatly thickened, wrinkled and hairless. Mites are rarely seen in these advanced lesions. Eventually the infected animal becomes listless, dehydrated, emaciated and most may die from the infection (Blood and Radostitis, 1989; Pence and Uckermann, 2002; Kahn *et al*., 2005). In naive or immunologically compromised hosts, the lesions are those of encrusting dermatitis without pruritis. The dermatitis becomes extensive, often covering most of the body. The thickened dry crusts on the surface fissure become hemorrhagic or are pyodermic. Mites are present in large numbers. The debilitated host becomes emaciated and often succumbs to the infection (Pence and Uckermann, 2002).

2.1.1.2 Distribution, epidemiology and transmission of sarcoptic mange in wildlife

Sarcoptic mange has been reported from many species of wildlife worldwide. Some of the most notable hosts include canids in North America (Pence *et al*., 1983; Little *et al*., 1998), red foxes and other canids in Europe (Morner, 1992; Gortazar *et al*., 1998), red foxes and dingos in Australia (MacCarthy, 1960; Hoyet and Manson, 1961), pampas foxes in South America (Deem *et al*., 2002), chamois and a variety of other ungulates in Europe (Rossi *et al*., 1995; Fernandez *et al*.,, 1997), felids in Europe (Morner, 1992; Ryser-Degiorgis *et al*., 2002) and Africa (Young, 1975; Laurenson, 1995a), wild boar in Europe (Ippen, *et al*., 1995), marsupials especially wombats in Australia (Skerratt *et al*., 1998), mountain gazelle in Turkey (Kurtdede *et al*., 2007) and a range of ungulates, primates and canids in Africa (Zumpt and Ledger, 1973; Young, 1975; Mwanzia *et al*., 1995; Kalema *et al*., 1998; Williams *et al*., 2008)

The epidemiology of mange in wildlife populations is not well understood and seems to differ between different areas of the world and animal species. In North America epizootics are mainly reported in wild canids mainly red foxes, coyotes, gray wolves and red wolves (Todd *et al*., 1981; Pence *et al*., 1981; Pence *et al*., 1983; Pence and Windberg, 1994). In Europe epizootics have mainly been reported in red foxes and chamois (Morner, 1992; Fernandez *et al*., 1997). In Africa small epizootic outbreaks have been reported in several of the larger bovid ungulates in South Africa (Zumpt and Ledger, 1973). In Tanzania an epidemic affecting Chimpanzee in Gombe National park was reported in 1997 (Williams *et al*., 2008**).** Although the disease can cause devastating short-term mortality in African species of great ape, cat and antelope, an epizootic does not generally affect long- term population dynamics due to high wildlife populations in large generally continuous ecosystems, although endangered and threatened species are vulnerable to its effects (Pence and Uckermann, 2002)

There is paucity of literature on prevalence of mange in the wild. Todd *et al*., (1981) reported a prevalence of 20% in trapped or hunter-killed coyotes in Alberta in 1972 to 1975 and 11% in gray wolves poisoned during wolf reduction programmes in 1972 to 1978. In southern North America, Pence and Windberg (1994) evaluated the effect of an epizootic in 1975 to 1991 on coyotes in south Texas. They examined 1489 coyotes and found that 80% were infected during the peak in 1980. They also noted that the epizootic had little

long-term effect on the coyote population in spite of coyotes experiencing approximately 70% mortality during the peak. In South America, Deem *et al*., 2002, observed characteristic gross lesions consistent with mange in 19 of 94 observations of free-ranging pampas foxes in Gran Chaco, Bolivia.

Transmission of *S. scabiei* among wildlife occurs both by direct and indirect contact. Larvae and nymphs frequently leave their burrows and wander on the skin (Arlian and Vyszenski-Moher, 1988), which may harbour hundreds to several thousands of mites/cm^2 (Zeh, 1974; Arlian *et al*., 1988c). Some may become dislodged from the host and fall off (Arlian and Vyszenski-Moher, 1988). Mites may survive in the environment for several weeks if conditions (microclimate) are optimal: that is, high relative humidity and low temperature prolong their survival time (Arlian *et al*., 1989).

2.1.1.3 Clinical signs in wildlife

In wildlife, clinical signs reported are often of generalized nature, obviously describing severely affected individuals. For example, Trainer and Hale (1969) describe the following clinical signs of sarcoptic mange in red foxes and coyotes: listlessness; emaciation; loss of fear of man; and hairless areas including muzzle, neck, shoulders, back, and sometimes the head and tail. Further, Pence *at al*., (1983) described the skin of a severely affected coyote as thickened, wrinkled, slate-gray in colour with numerous suppurative encrustations. In chimpanzee, Williams *et al*., 2008 reported hair loss, flaky, itchy skin in infected areas with some individuals showing noticeable weight

loss and general lethargy. Deaths were also reported in infants with more infections being noted in young and older ages. Clinical signs in other species such as lynx and chamois are similar to those described above (Pence *et al*., 1983**;** Morner, 1992**;** Ippen, *et al*., 1995; Rossi *et al*., 1995; Fernandez et al., 1997).

2.1.1.4 Control and treatment of sarcoptic mange

Sarcoptic mange occurs normally in animal populations as a widespread and common disease. Controlling the disease by reducing infected animals through hunting or culling may be counter-productive and result in more cases of mange because of high movement of animals into "animal free" areas (Lindstrom and Morner, 1985). Treatment of single infected wild animals is usually of little value in wild populations. However, where mange is having an impact on small, isolated, and threatened population (e.g., arctic fox or ibex), it may be worthwhile to capture, treat and release such animals. This has been done successfully with artic foxes in northern Sweden (Morner *et al*., 1988)

The drug of choice for treatment of sarcoptic mange is ivermectin. It has been used successfully in treatment of this condition in cheetahs (Pence and Uckermann, 2002; *Personal observation*.), mountain gazelle (Kurtdede et al., 2007), dogs (Kahn *et al*., *2005*) and cattle (Blood and Radostitis, 1989). Topical treatments using lime-sulfur, phosphet and amitraz have also been used successfully in dogs (Kahn *et al*., 2005).

2.1.2 Psoroptic mange

Psoroptic mange or scabs is a skin disease of man and animals caused by an itch mite *Psoroptes communis*. The mite belongs to the family Psoroptidae whose members are characterized by presence of a dorsal shield and bell-shaped curuncles borne on the legs (Mugera, e*t al*., 1979). These mites may be as long as 0.8 mm and may be seen grossly or with aid of a hand magnifier (Margaret and Russell, 1978).

Psoroptes communis has a number of subspecies that are host specific such as *P. communis ovis* in sheep and *P. communis bovis* in cattle. Some subspecies such as *P. communis ovis* in sheep are known to infect cattle, horses and donkeys (Mugera, *et al*., 1979; Blood and Radostitis, 1989).

The disease is characterized by intense pruritis, which usually begins in the shoulders and the rump. Later papules, crusts, excoriation, and lichenfication are seen. Lesions may cover the entire body and secondary bacterial infections are common in severe cases (Kahn et. *al.,* 2005).

Systemic treatment with ivermectin, moxidectin and doramexin has proved successful (Kahn *et al*., 2005). Blood and Radostitis, (1989) reported that topical application of benzene hexachloride, diazinon and propetamophos, coumphos, phoxim and amitraz can be used. Other than the above drugs Kahn *et al*., *(*2005) also reported use of topical application of hot lime-sulfur and flumethrin.

2.1.3 Notoedric mange

Notoedric mange also referred to as feline scabies, is a highly contagious disease of cats caused by *Notoedres cati*, which can be opportunistic and affect other animals (Kahn *et al*., *2005*). It has been observed in a wild cheetah (Pence and Uckermann, 2002), free ranging lynx (Ryser-Degiorgis *et al*., 2002), hedgehogs (Gregory, 1981), rabbits (Margaret and Russell, 1978), raccoons (Scott *et al*., 2004), and in eastern fox squirrel and gray squirrel (Michigan Wildlife Disease Manual, 2001-2006).

Notoedres mange mites resemble sarcoptic mange but are somewhat smaller. The anus is located on the dorsal abdomen area rather than terminally as for sarcoptic mite (Margaret and Russell, 1978).

Notoedric mange infestation results in alopecia, first over the chest and shoulders, but progressing over the entire body. Pruritis is severe. In extreme cases nearly the entire body is bare and the exposed skin becomes thickened and dark (Michigan Wildlife Disease Manual, 2001-2006; Kahn *et al*., 2005). Advanced lesions give cats an appearance of old age because of the wrinkling of the skin of the face (Margaret and Russell, 1978).

In cheetahs, Pence and Uckermann (2002) reported successful treatment of this condition using ivermectin at multiple treatments spaced a month apart. In dogs selamectin and ivermectin have been used systemically. Topical application of amitraz and lime-sulfur can also be used (Kahn *et al*., 2005).

2.1.4 Demodex mange

Demodex mange also referred to as demodecosis or follicular mange is a chronic highly contagious skin disease of domestic and wild animals characterized by a high morbidity and low mortality. It is caused by *Demodex folliculorum*, so called because it inhabits hair follicles (Mugera, *et al*., 1979). This mite has subspecies that are morphologically indistinguishable and are named according to the predominant host they parasitise such as *D. folliculorum var. bovis* in cattle, *D. folliculorum var. canis* in dogs, *D. folliculorum var. cati* in cats etc. *(*Mugera, *et al*., 1979; Blood and Radostitis, 1989; Kahn *et al*., *2005*).

Demodecosis in cattle, sheep and goats is not severe but causes significant damage to hides and skins and rarely death due to secondary bacterial infection (Blood and Radostitis, 1989). However, the disease is severe in dogs and cats where it causes generalized lesions that are usually aggravated by secondary bacterial infections (pyodemodicosis) (Kahn *et al*., *2005*).

Demodex folliculorum is a cigar shaped mite with a short broad head and four pairs of short stumpy legs situated anteriorly and the body is transversely striated. The female is approximately 0.44mm long but the male is slightly smaller (Margaret and Russell, 1978; Mugera, *et al*., 1979).

Demodex folliculorum inhabits hair follicles, sebaceous glands and the epidermis where they reproduce (Margaret and Russell, 1978). In small

numbers these mites are part of the normal flora of skins of dogs and cause no clinical signs. Two clinical forms have been described in dogs (localized and generalized). In localized condition, lesions consist of areas of focal alopecia, erythema and/or hyperpigmentation, and comedones. Pruritus is generally absent or weak. A percentage of these cases, especially the diffuse localized forms, progress to generalized forms. The generalized form is a severe disease that is usually aggravated by secondary bacterial infection. Dogs have systemic illness with generalized lymphadenopathy, lethargy and fever when deep pyoderma, furunculosis or cellulites is seen. In cats there is similar clinical picture although in generalized disease, allopecia, crusting and secondary pyoderma of the whole body are seen (Kahn *et al*., 2005).

For generalized conditions, topical applications of amitraz and benzyl peroxide in dogs and lime-sulfur in cats have successfully cured the disease ((Kahn *et al*., 2005)). Mugera *et al.,* (1979) reported successful treatment with benzene hexachloride, asuntol, negasunt and neguvon. Systemic treatments with milbemycin oxime, moxidectin and ivermectin have also proved successful in dogs (Kahn *et al*., 2005).

2.1.5 Other mange mites

The other mange that affects animals but of unknown occurrence in Africa include Chorioptic mange (leg mange), Psorergatic mange (itch mange), Trobiculidiasis (harvest mite), Straw itch mite (forage mite), Octodectic

mange, Cheyletiellosis (Walking Dandruff) and Trombiculosis (Kahn *et al*., 2005).

2.2 Diagnosis of mange

2.2.1 Phenotypic characterization

Phenotypic characterization of species of mange mites depends on positive identification on microscopic slides (Pence and Uckermann, 2002; Scott *et al*., 2004; Kahn e*t. al.,* 2005). Mites are quite small, most species being either microscopic or less than 1 mm of length (Margaret and Russell, 1978).

Deep skin scrapings examined under a microscope in 10% potassium hydrochloride (KOH) are very helpful where mites are abundant in the skin (Bowman, 1999; Pence and Uckermann, 2002; Fitzgerald *et al*., 2004). *Sarcoptes scabiei* is easily identified based on the presence of idiosomal denticles and club-shaped setae (Pence and Uckermann, 2002). Notoedric mange mite is similar to Sarcoptic and can only be differentiated through having a dorsally placed anus. Differentiation of sarcoptic, psoroptic and chorioptic mange mites depends on the positions of suckers on the long un-jointed stalk of different pairs of legs in adult females and males (Margaret and Russell, 1978). Demodectic mites have a distinct appearance, with a non-hairy elongated body with very short pairs of legs placed anteriorly and transversely striated abdomen (Margaret and Russell, 1978).

Phenotypic characterization has been unable to differentiate mites of the same species infesting different hosts. For example, *S. scabiei* is known to infest a wide range of mammalian hosts but morphological studies have failed to identify any significant differences between populations of mites (Fain, 1978). Morphological differences which focus on the structure of the posterior dorsal field of scales, the shape of the dorsal propodosomal shield, and the presence or absence of ventrolateral scales, all in female mites (Fain 1978; Zahler *et al*., 1999), has been described. However, there is no agreement on how these phenotypic differences should be interpreted. According to one argument, these differences are regarded as appropriate in differentiating at least 10 species (Fain 1978). Another point of view acknowledges the wide morphological variation within the different mite populations which overlap each other, so that an unequivocal segregation into different species cannot be made, consequently mites of the genus *Sarcoptes* should be considered as only one, albeit heterogeneous, species (Pence et al., 1975; Fain 1978). Further, experimental transfer of mites between hosts of different species is commonly unsuccessful and hence *S. scabiei* is described as a single but variable species that is predominantly host specific (Arlian *et al*., 1984; Arlian *et al*., 1988; Arlian, 1989).

2.2.2 Sero-diagnostic methods

Sero-diagnostic tests, enzyme- linked immunosorbent assay (ELISA) can be used to support phenotypic diagnosis. Deep skin scrapings, even those including encrusted epidermis, can be negative (Hill and Steinberg, 1993). In

such cases the demonstration of specific antibodies in sera of suspected infected animals strongly confirm tentative diagnosis This assay, which demonstrates specific antibodies to *S. scabiei*, has been developed for dogs and foxes (Bornstein *et al*., 1995; Bornstein *et al*., 1996), pigs (Bornstein and Wallgren, 1997), lynx, and domestic cat (Bornstein *et al*., 1997). This test is used to verify whether an animal with skin lesions has been affected by *S. scabiei* or not. It is useful in diagnosing mange in animals with atypical or minute skin lesions and in conducting sero-epidemiological surveys.

2.2.3 Genotypic characterization

2.2.3.1 General

Molecular methods have revolutionalized insect systematics (Caterino and Sperling, 2000) and are increasingly being applied to mites and ticks (Najavas and Fenton, 2002). This involves phylogenetic studies. The vast diversity of mites makes them particularly suitable for these kind of studies and they represent a unique opportunity to test many evolutionary hypotheses. Such studies rely on robust phylogenies, but these are lacking for most groups of mites. This may be the reason why the mites have been overlooked by the wider community of evolutionally biologists (Cruickshank *et al*., 2001).

Sarcoptes mites lack free-living stages, and individual hosts, depending on their susceptibility and behaviour, are essentially ephemeral habitats providing patchy environments that hamper random mating. All mites on an individual host may in fact form an ‘infrapopulation’ that has a number of recurrent

generations. The number of generations is influenced by the short generation interval in this parasite (about three weeks), as well as by the infected host's life expectancy and susceptibility (Rosero *et al*., 2010). Other major determinants of gene flow between mites are the degree of host specificity, animal behaviour and geographical structure of host populations.

A number of different molecular markers have been used to characterize ticks and mites. All the markers have their unique problems but by choosing the marker most appropriate for a certain task these problems can be minimized before any sequencing is done. For example, ribosomal genes may be difficult to align but are likely to have more informative sites than protein coding genes (Cruickshank, 2002). Out of the markers reviewed by Cruickshank (2002), the second internal transcribed spacer of the nuclear ribosomal gene cluster (ITS2) and the mitochondrial protein-coding gene cytochrome oxidase together provide a powerful tool for studies of intraspecific variations and phylogenies of closely related species in ticks and mites. 18S rRNA and 28S rRNA are equally useful for phylogenetics at the other end of the taxonomic spectrum. According to Walton *et al*., 1997, microsatellite markers or simple sequence repeats have high levels of allelic variability demonstrated between individual mites and are suitable for epidemiological and taxonomical studies of both within and between host species. The authors identified fragment length polymorphism in 3 loci when resolved on polyacrylamide sequencing gel. Other authors (Walton *et al*., 1999; Walton *et al*., 2004a; Alasaad *et al*., 2008a) reported that studies on a central fragment of the 16S gene and the complete

CO1 gene in combination with microsatellite markers provided some support for a genetic differentiation of *S. scabiei*. These genetic markers demonstrated significant relationships between *S. scabiei* and mtDNA haplotypes and microsatellite allele's frequencies, and host species and geographical locations even at skin-scale level.

The most studied mite of human and veterinary importance is *S. scabiei*. Its importance as a pathogen of humans and animals is well documented, but this has not been reflected in the scientific literature, as very few basic studies employing molecular tools have been reported (Kemp *et al*., 2002). Further, little is known about the molecular interactions between this pathogen and its host and this is partly explained by the paucity of mite-derived materials including antigens (Ljunggren *et al*., 2003). However, much effort has been made lately and more efforts are in progress to increase the understanding of the parasite and the disease. The lack of parasite material and the absence of an in-vitro propagation system for *S. scabiei* make this parasite an excellent candidate for a molecular approach (Ljunggren *et al*., 2006). Several authors have reported developments in this area. Mattson *et al*., (2001) reported construction of an *S. scabiei* cDNA library and the identification of several antigens generated from mites isolated from red fox (*vulpes vulpes*). Immunoscreening of the library enabled Mattson *et al*., (2001) to clone a full-length cDNA coding for a 102.5 Kda protein. Sequence similarity searches identified the protein as paramyosin. They also designed a small paramyosin construct of about 17 kDa that included the N-terminal part, an evolutionary

variable part of the helical core, and the terminal part of the molecule. Ljunggren *et al*., 2006 on the other hand cloned a cDNA encoding a novel antigen of *S. scabiei* (Acari) cDNA library by immunoscreening with sera from *S. scabiei*-infected dog. The antigen was encoded by a 2157bp mRNA with a predicted open reading frame of 719 amino acids (molecular weight 79 Kda). Their sequence analysis identified the presence of an MADF domain in the N-terminus and downstream of this domain was a region of low sequence complexity. The antigen was named Atypical Sarcoptes Antigen 1 (ASA1) since the MADF domain normally occurs in proteins involved in transcriptional regulation. Ljunggren *et al*., (2003) analyzed over 1000 expressed genetic sequence tags (ESTs) of *S. scabiei*.

The average sequence read was 510 bp after editing and the overall sequencing success was 89%. The clustering of the sequences resulted in 76 clusters, comprising 36% of the ESTs. Many of the transcripts shared similarity with genes involved in basic metabolism and cellular organization. Walton *et al*., (1999), did molecular fingerprinting using three *S. scabiei*-specific single locus hypervariable microsatellite markers, with a combined total of 70 known alleles. Multilocal analysis of 712 scabies mites from humans and dog hosts in Ohio, Panama and Aboriginal communities in Northern Australia showed that genotypes of dog-derived and human-derived scabies cluster by host species rather than by geographical location. Fischer *et al*., 2003 used beadings of crusted scabies patients as a source of mites for the construction of libraries of

cDNA from *S scabiei var. hominis* in the bacteriophage λ vector λ ZAP express.

2.2.3.2 Microsatellite markers and primers

Microsatellites or simple sequences repeats have been described as both abundant and ubiquitous in the genomes of all eukaryotes (Tautz and Renz, 1984). These sequences consist of tandem repeats of short motifs such as di- or tri- nucleotides that are randomly dispersed throughout the genome. The domains are characterized by repeat length allelic hypervariability and consequently have been used as genetic markers in relationship studies within and between populations as well as for linkages (Goldstein and Clark, 1995; Tautz, 1989; Walton *et al*., 1997). So far, 18, 30 and 22 alleles for *Sarcoptes* microsatellites (Sarms) 1, 15 and 20 respectively have been recorded (Walton *et al*., 1999). By selecting primers from the unique sequence flanking the microsatellite, the polymerase chain reaction (PCR) can be used to amplify the repeat region that can then be sized on denaturing polyacrylamide gels (Weber and May, 1989).

2.3. Population and mange infestations in cheetahs

The world cheetah population is estimated to be no more than 12,000, most likely fewer than 9,000 and is continuously declining. The species is listed as endangered and placed in Appendix 1 by the Convention of International Trade in Endangered Species (CITES) and hence enhanced promotion and greater regulation of inter-continental and regional cross-border trade and utilization of the species (Mulheisen and Knibbe, 2001). The World Conservation Union

(IUCN) classifies the African sub-species endangered. Globally the major causes of decline are habitat loss and degradation, hunting, diseases and commercial markets for body parts (Weber and Rabinowitz, 1996).

Cheetah is now extinct in many areas within its historical and geographical range and is highly endangered where it remains (Weber and Rabinowitz, 1996). They once occurred in the whole of Africa, except the tropical lowland forests, but are currently found mainly in Namibia and Tanzania protected areas (Ellis, 2001). In Kenya the cheetah population is believed to be less than 1000 individuals with Gros (1998) estimating the population to be 793 individuals. The population occurs in small scattered areas and the Mara region is believed to support the largest cheetah population and hence of major conservation importance in Africa. Burney (1980) reported 61 individuals in the reserve and adjacent ranches. This declined to about 40 individuals in 2002 (Ngoru and Mulama, 2002), representing 34.4% decline.

According to Ngoru and Mulama (2002), the decline was as a result of diseases, tourist pressure, predation and competition with other predators, conflicts with pastoralists and habitat loss. The authors have further reported that, of concern among the diseases is *Sarcoptic* mange that leaves the animal weak and vulnerable to predation. The same disease was observed to affect Thomson's gazelles, lions, domestic goats and dogs as well as vervet monkeys. Out of the 40 individually identified cheetahs during the study period, 8 were observed to suffer from mange.

2.4. Mange infestations in other wild animals

The Thomson's gazelle (*Gazella thomsoni*) is the most important prey animal to the cheetah. The preference can possibly be attributed to its small size and can be easily captured (Ngoru and Mulama, 2002). The gazelle, which is less than 30kg, can be wrestled to the ground by a cheetah even if it starts rising again after initial capture. In Serengeti National Park, cheetah conceptions are believed to increase in the wet season because of availability of neonates and fawns of Thomson's gazelles whose population rises at this time. This could be due to improved female body condition and enhanced estrus, suggesting differential timing of breeding linked to fluctuating availability of preferred age-sex classes of prey (Laurenson, 1995b). Although, Thomson's gazelles are the preferred prey, cheetahs quickly respond to their scarcity by turning attention to other prey, although success rate of capture vary with species. Cheetahs have been known to prey on cape-hares, young impala and young wildebeests (Ngoru and Mulama, 2002). Thomson's gazelles have been reported to suffer from mange (Ngoru and Mulama, 2002; Pence and Uckermann, 2002). The other wild animals in Africa reported to be affected by mange include lions (*Panthela leo*), impala (*Aepyceros melampus*), wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*), eland (*Taurotragus orynx*) and kudu (*Tragelaphus strepsiceros*) (Ngoru and Mulama, 2002; Pence and Uckermann, 2002). These animals interact closely with cheetahs either as prey or sharing the same range.

2.5 Wildlife/livestock interphase

Wildlife/livestock interface occurs where wildlife and livestock share the same range. This is most common in ranching systems and in pastoral and agro-pastoral systems. The rangelands in Kenya where nomadic and transhumance pastrolism is practiced, shelter a great diversity of free-ranging wildlife species that often mixes with livestock (Bourn and Blench, 1999). Within these rangelands there has been increasing incidences of wildlife/livestock interface diseases such as bovine tuberculosis, rinderpest, anthrax and foot and mouth disease (Bengis *et al*., 2002). Major outbreaks of diseases in the interface have been associated with drought where due to limited water and food there is increased interaction between livestock and wildlife (Kock *et al*. 1999). This apparent increase in disease incidences is partly a result of the expansion of human and livestock populations into wildlife areas, with dramatically disturbed habitats and novel interactions, but also a result simply of increased awareness and better diagnostic and monitoring capacities (Jones *et al*., 2008).

CHAPTER THREE: SPATIAL DISTRIBUTION OF MANGE AMONG CHEETAHS, THOMSON'S GAZELLES AND DOMESTIC ANIMALS

3.1 Introduction

Although mange has been reported in cheetahs and other wildlife species in the Masai Mara Ecosystem (Mwanzia *et al*., 1995; Ngoru and Mulama, 2002) its spatial distribution across the range of Masai Mara ecosystem has not been determined. Previous observation studies of mange-like skin diseases in cheetahs were restricted to two community conservancies, Koiyaki and Lemek in Masai Mara ecosystem (Mwanzia *et al*., 1995). Over-time observations and sampling have been restricted to infected cheetahs reported for treatment although in 2002 attempts were made to capture and sample Thomson's gazelle by KWS, Veterinary Services Department where sarcoptic mange was isolated from 3 of them (*KWS veterinary report,* 2002).

The existing cheetah population in Kenya occurs in small-scattered populations. Masailand and the Northern districts of Kenya appear to offer the best prospect for cheetah conservation (Ngoru and Mulama, 2002). In these areas where pastoral communities inhabit, interaction between wildlife and livestock is high situation which creates a rich platform for disease transmission. There is illegal incursion of livestock into protected areas especially during the dry season which increases the interaction. It is therefore important to map out areas of greatest interaction of wildlife and domestic animals in relation to mange infestation.

Seasonality has been reported to play a role in distribution of sarcoptic mange in wildlife species (Christophersen, 1996) with more cases being observed in dry than wet season. Major outbreaks of other diseases in the interface have been associated with drought where due to limited water and food there is increased interaction between livestock and wildlife (Kock *et al*. 1999). It is prudent to determine if seasonality affects distribution of mange in the Masai Mara ecosystem.

This study was formulated to determine the spatial distribution of mange-like diseases across the ecosystem and the effect of seasonality on the distribution. This chapter discusses mange-like lesions observed during the study and confirmation of types of mites isolated is discussed in chapter 6 on phenotypic characterisation.

3.2 Materials and methods

3.2.1 Study area

The study was conducted in Masai Mara ecosystem in the larger Narok District (Fig. 3.1) which comprises Masai Mara National Reserve (NR), Mara Conservancy and surrounding community ranches of Koiyaki, Siana, Lemek, Olkinyei and Ol Choro Orogwa (Fig. 3.2). Masai Mara N.R. and Mara Conservancy form the protected areas of the Masai Mara ecosystem and are managed by Narok and Transmara County Councils respectively. A Maasai pastoral community whose livelihood is dependent on livestock rearing

surrounds the two game reserves. There is a lot of interaction between livestock and wildlife in the ecosystem.

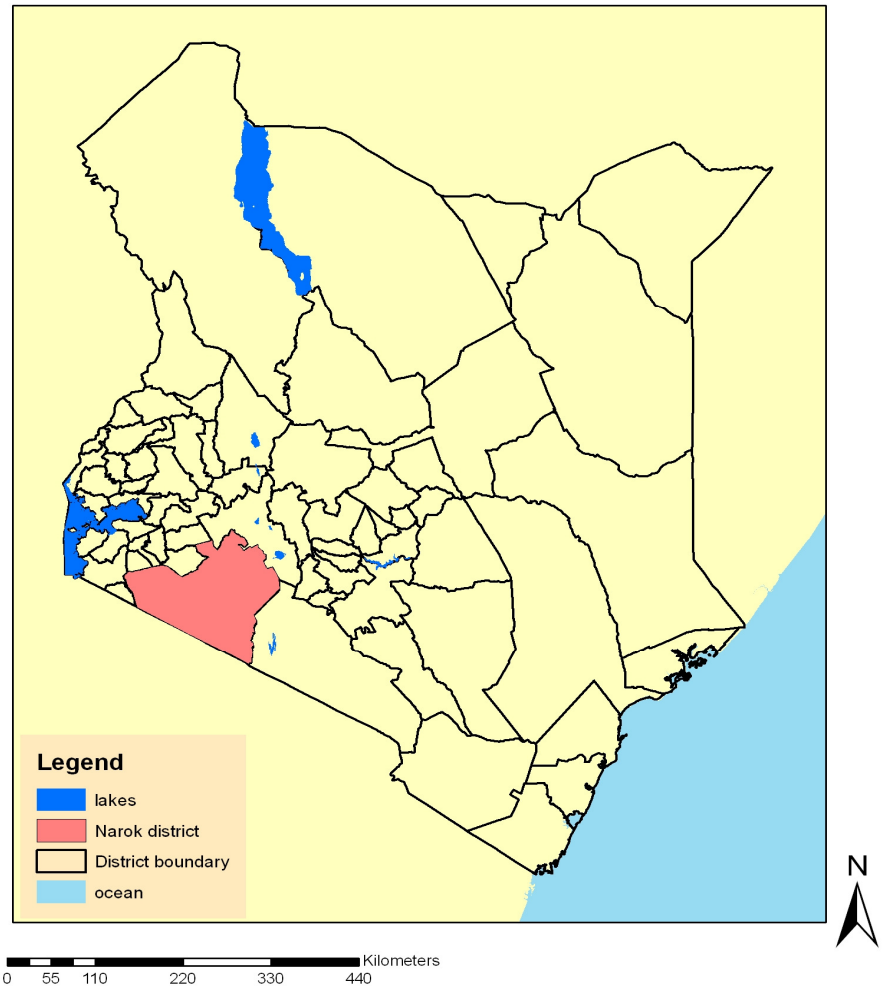


Figure 3.1:A map of Kenya showing the location and administrative boundaries of the larger Narok District

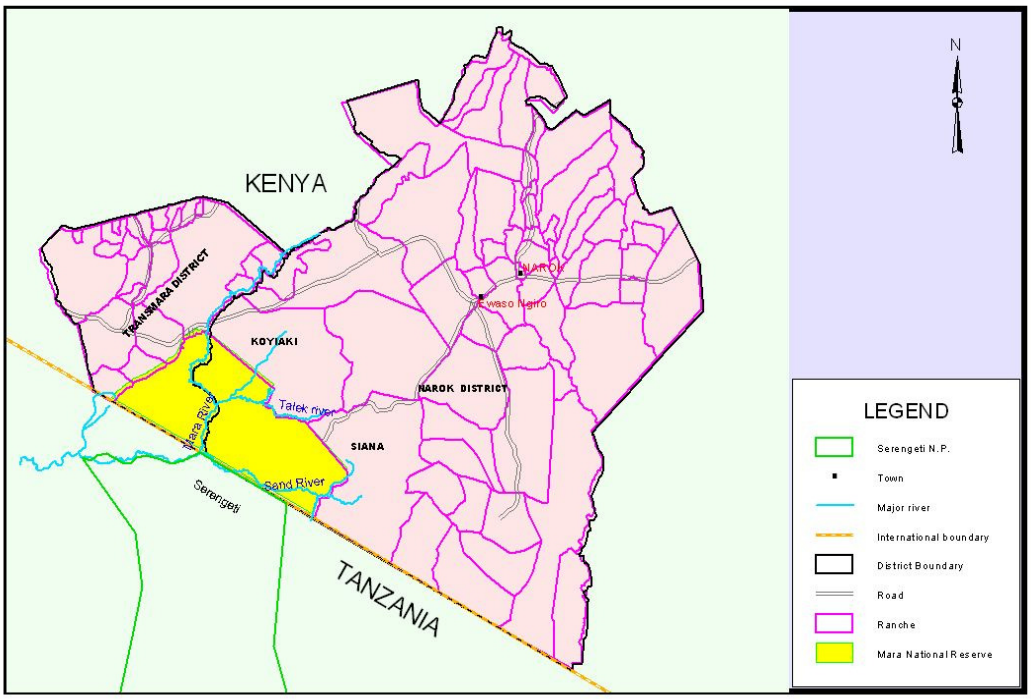


Figure 3.2: A Map of larger Narok District showing the protected areas of Masai Mara ecosystem and surrounding community ranches

Masai Mara N.R. is approximately 1510 Km^2 while Mara Conservancy is approximately 350 Km^2. Both reserves are located on the northern tip of the greater Serengeti National Park in Tanzania. The two reserves lie between $1^0$13' and $1^0$45' south and $34^0$45' and $35^0$25' east.

The Masai Mara Ecosystem was identified as the study area due to the following reasons;

i. Continuous reports and observations of cheetahs and Thomson' gazelles infestation with mange
ii. High wildlife/livestock interactions especially in community ranches
iii. Presence of cheetahs in community ranches

iv. Proximity to Tanzanian border. The area shares wildlife populations with Serengeti NP where cases of cheetah and other wildlife infestation have been reported.

3.2.2 Cross-sectional survey data collection

Observational data of mange-like skin diseases was collected over a period of 2 years (Nov 2007 – Nov 2009). Lesions were regarded as being mange-like skin if at least 3 of the following symptoms were observed in an animal; pruritus, alopecia, crust formation, skin roughening and poor body condition. An example of an affected cheetah showing some of the above symptoms is shown in Fig. 3.3.

Figure 3.3: A photo of a cheetah showing alopecia, crust formation, roughening of the skin; three of the five mange-like skin disease case definition skin symptoms

The study area was divided into 8 blocks, 3 in protected area and 5 representing each community ranch (Fig. 3.4). The animals observed were cheetahs, Thomson's gazelles, livestock (sheep, cattle and goats), dogs and other wildlife (incidentals). GPS co-ordinates of all individual animals and/or herds observed to have clinical signs of mange were recorded. The dates when data was collected were also recorded to enable seasonal analyses. The seasons were divided into dry (January to March and July to September) and wet (April to June and October to December) based on meteorological data. Pastoralists and wildlife officers were requested to report occurrence of skin diseases in domestic and wild animals.

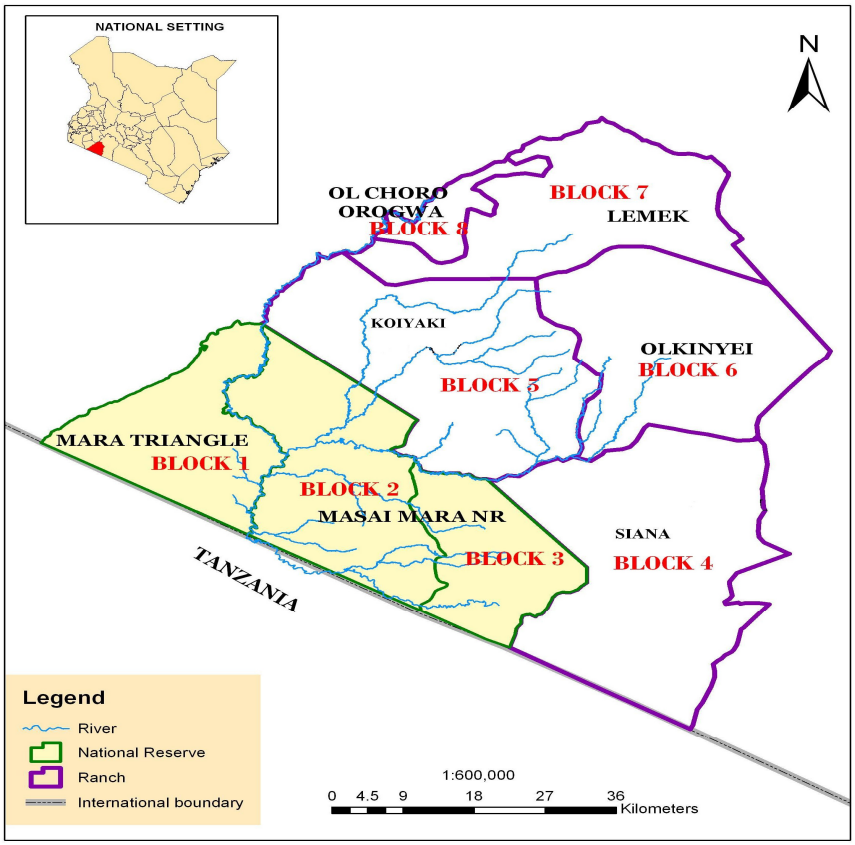


Figure 3.4: A map showing the study blocks

3.2.3 Data Analysis

The data was analyzed using Arc View software for GIS mapping. The analysed variables were animal species, climatic season and presence of mange.

3.3 Results

3.3.1 Observations

In general, study species were observed and followed up in all blocks except block 8 (Ol Choro Orogwa) (Fig. 3. 5). This was due to poor infrastructure of the block. There was greater concentration of observations in block 1 (Mara Conservancy), blocks 2 and 3 (Masai Mara N.R.), block 4 (Koiyaki) and block 5 (Siana) especially along the boundary of the protected areas (blocks 1, 2, 3) and adjacent community ranches (blocks 4 and 5) where there was high interaction among the study species.

3.3.2 Distribution of infected species with mange-like lesions

Infected animals were observed in six of the seven blocks studied (Fig. 3.6). There were no affected animals observed in block 6. There was higher concentration of infected animals in blocks 1, 2, 3, 4 and 5. There was also high concentration along the boundary of the protected areas (blocks 1, 2 and 3) and adjacent community ranches (blocks 4 and 5).

Infected cheetahs were observed in blocks 1, 3, and 5, Thomson's gazelles in blocks 2,3,5 and 7, sheep in blocks 4 and 5, dogs in blocks 4 and 5 and, wildebeest in block 1.

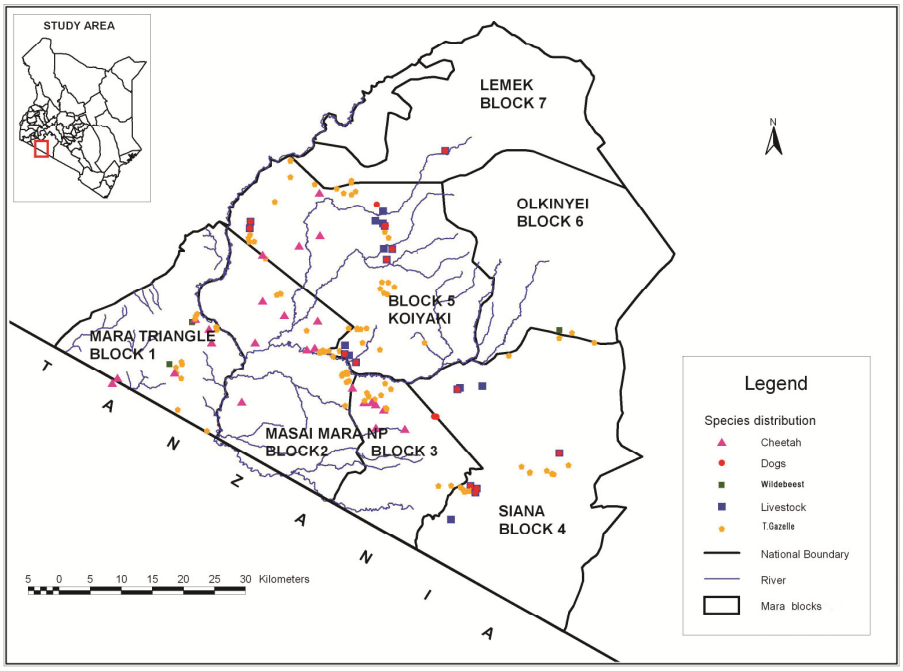


Figure 3.5: A map showing the distribution of sampling sites

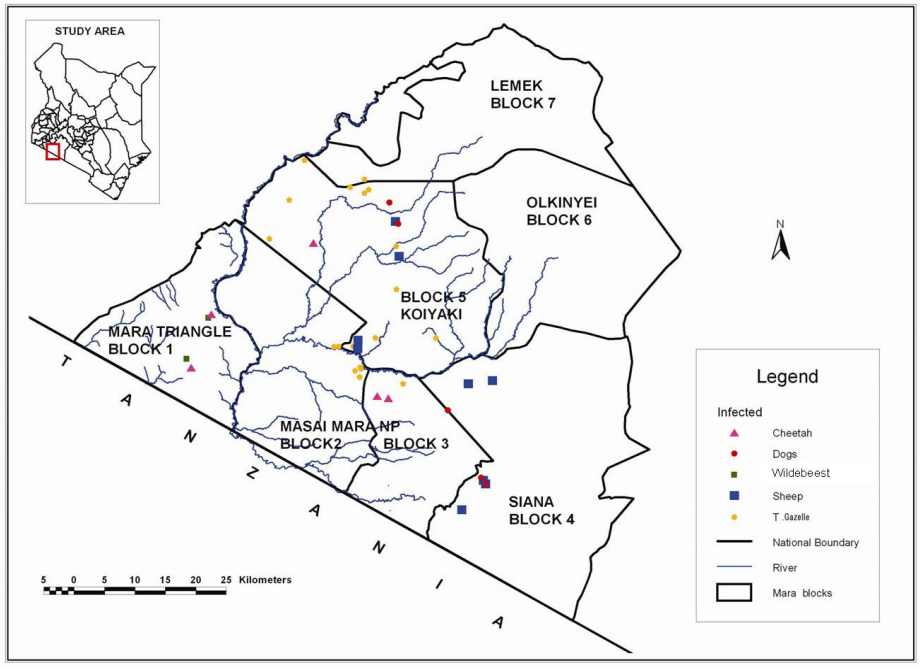


Figure 3.6: A map showing the distribution of infected animals of all species

3.3.3 Seasonal Distribution

Infected animals were observed in blocks 1, 2, 3, 4 and 5 during the dry season (Fig. 3.7) and in blocks 2, 3 and 5 during the wet season (Fig. 3.8). There was higher distribution of affected animals during the dry season.

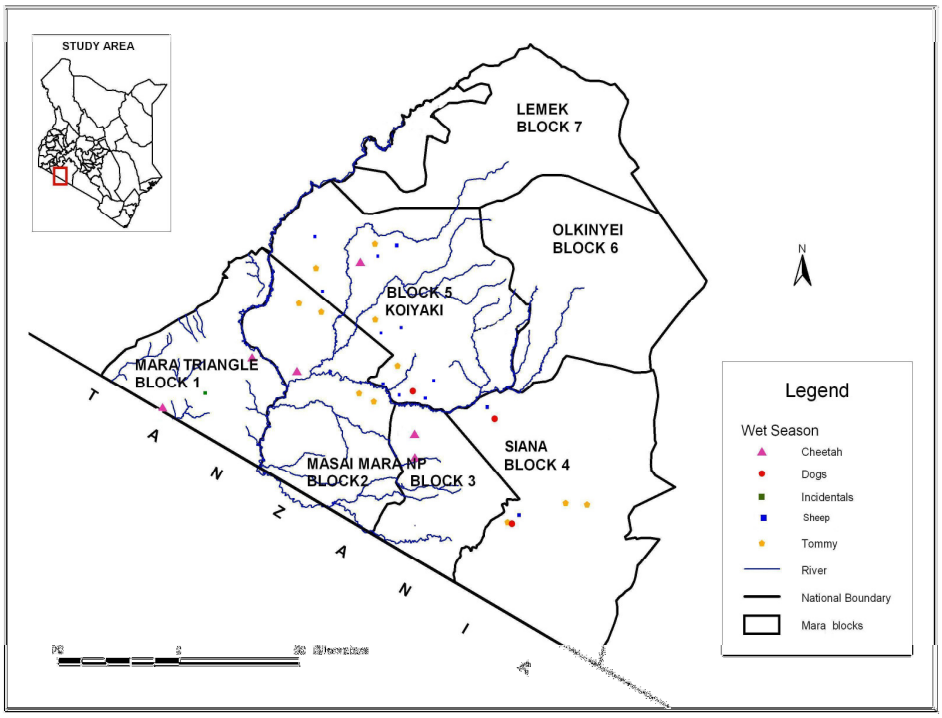


Figure 3.7: A map showing the distribution of infected animals during the dry season

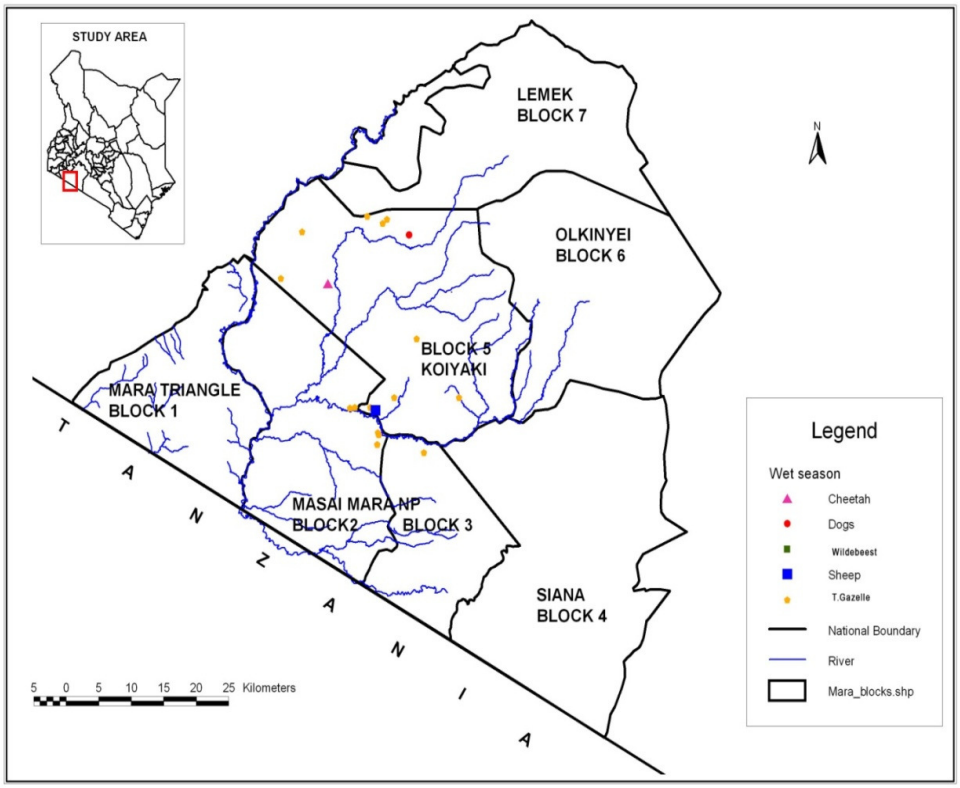


Figure 3.8: A map showing the distribution of infected animals during the wet season.

3.4 Discussion

The infected animals were distributed in most of the study area with higher concentration being observed along the boundary of the protected area and the community ranches. It is in these areas that there is high interaction between wildlife and domestic animals. Wildlife also moves to and from the protected areas to community ranches especially on the periphery of the protected areas. This observation agrees with Jones (2008) who reported that increase in disease

incidences is partly as a result of the expansion of human and livestock populations into wildlife areas.

Infected cheetahs were observed in protected areas (block 1 and 3) and in a community ranch (block 5). The occurrence of mange in cheetahs in Koiyaki community ranch (block 5) had been reported earlier by Mwanzia *et al*., (1995). However, Mwanzia *et al*., (1995) also reported the disease in cheetahs in Lemek community ranch (block 7) which was not the case in this study. No cheetah was located in this area during the study period. Cheetah population might have gone down significantly in Lemek community ranch due to increased shift to crop agriculture and increased settlement which are land-use changes, a factor attributed to decline in cheetah population in Masai Mara by Ngoru and Mulama (2002).

The infected cheetahs in block 3 were found close to the boundary of the Masai Mara NR and Koiyaki community ranch near Talek shopping centre. This is an area that also reported high number of affected Thomson's gazelles and sheep. It's an area where livestock illegally graze in the reserve. Thus the area has a high possibility of transmission of the parasites among the hosts. However, observation of affected cheetahs in Mara Conservancy (Block 1) is quite interesting in that there is normally very low likelihood of interaction of cheetahs and livestock in this area since illegal grazing of livestock is minimal or none existent as the management is very strict on illegal grazing. Further, no affected Thomson's gazelles were observed in this area. However, observation

of infected wildebeest was recorded in the area. Since cheetahs normally have very large home ranges (Ngoru and Mulama, 2002; Creel and Creel, 2002; Broomhall *et al*., 2003; Houser et. al., 2009), they could have picked the parasites far away from the conservancy. The cheetah could also have picked the parasites from wildebeest which normally serve as an alternative prey (Hayward *et al*., 2006). The cheetah could also have moved across the border of Mara Serengeti National Park where cheetah infestation with mange has been reported by Laurenson (1995a).

Infected Thomson's gazelles found in blocks 3 and 5 were very close to infected cheetahs in the 2 blocks. Since, Thomson's gazelle is the favourite prey species of cheetah, there is a possibility of cheetah getting infected during feeding. On the boundary of the 2 blocks and inside block 5 infected sheep were observed close to infected cheetah and Thomson's gazelles and the infection of either species could occur through shedding of mites in the environment. Interestingly no infected Thomson's gazelle was observed in block 4 where infected sheep were recorded.

Infected dogs were found in shopping centers of Aitong and Sekenani in block 5 and Ololoimutiak in block 4. The dogs scavenged in the shopping centers with a few returning to their manyatta's in the evening. Interestingly, no dog was observed affected within the manyatta and the grazing fields. There is high likelihood that there was transmission of the parasites among the dogs within the shopping centre, with minimal or no transmission to other dogs and to

sheep in the manyatta where some of the dogs spent their night. The likelihood of dogs transmitting the parasites to cheetahs and Thomson's gazelle is minimal since these species do not come close to highly settled areas such as shopping centers.

There was higher observation of infected animals among species and across study blocks in dry than in wet season. During dry season prey populations disperse in search of pasture and water making it quite difficult for cheetahs to hunt. This is in contrast to wet season when prey populations congregate and breed, increasing the chance of food availability for cheetahs. The dry season causes increased stress due to prey unavailability as cheetahs have to move long distances in such of prey thereby expending a lot of energy. This observation agrees with that of Malan *et al*., (1997) who reported that sarcoptic mange in most wildlife species shows sub-clinical signs. It is only when stressed that clinical signs are observed, and that of Christophersen (1996) who associated seasonality to spread of sarcoptic mange. It also concurs with Laurenson (1995b) who reported that in Serengeti National Park, cheetah conceptions increase in the wet season because of availability of neonates and fawns of Thomson's gazelles whose population rises at this time. This increases cheetah prey base leading to decreased stress.

In conclusion, this study shows that the distribution of infected species of animals is related to areas of close interaction between wild and domestic animals and stress due to climatic seasonal changes.

CHAPTER FOUR: PREVALENCE OF MANGE AMONG CHEETAHS, THOMSON'S GAZELLES AND DOMESTIC ANIMALS

4.1. Introduction

Mange has been known to affect cheetahs in the Masai Mara ecosystems for many years (Ngoru and Mulama, 2002; Mwanzia *et al*., 1995: KWS Veterinary Field Reports), even causing death (Fig. 4.1), but no cross-sectional study to estimate its prevalence has been carried out. This endangered species shares the same habitat with other wild and domestic animals that are known to be affected by mange and whose prevalence is not known. With only an estimated population of 61 cheetahs in the Masai Mara ecosystem (Burney, 1980), and mange being one of the leading causes of death (Weber and Rabinowitz, 1996), it is prudent that the prevalence of this parasitic disease in cheetah and other in-contact species is known so as to help in developing control strategies.

The number of observed mange cases has been reported to follow seasonality and geographical location of cheetahs and other wildlife species (Christophersen, 1996; *Personal observation*). It is important to understand the effect of these risk factors to mange prevalence in cheetah and other in-contact animals. The effect of time of sampling to prevalence of mange among the study species was also investigated.

In an effort to understand the epidemiology of mange in Masai Mara ecosystem this cross-sectional study was formulated to determine the prevalence of mange in study species across the ecosystem and the effect of geographical location,

climatic season and temporal factors to the disease prevalence. This chapter as in the earlier chapter discusses prevalence of mange-like lesions observed during the study and confirmation of types of mites isolated is discussed in chapter 6 on phenotypic characterisation.

Figure 4.1: A photo of a dead cheetah that was found to have succumbed to mange infection being watched over by its sibling

4.2 Materials and methods

4.2.1 Study area and blocks

The study area and study blocks were as discussed in section 3.2.1. A cross-sectional survey was undertaken to collect prevalence data.

4.2.2 Determination of prevalence

Observational data of mange-like skin disease among domestic animals, cheetahs, Thomson's gazelles and other wildlife species was collected over a period of 2 years (November 2007 – November 2009). Case definition of mange-like skin lesion was as described in chapter 3.

4.2.2.1 Sampling method

A purposive random sampling method was used to get the sampling units. Purposive sampling of domestic animals was based on study blocks (community ranches) closer to the protected areas or where cheetahs were known to occur while that of Thomson's gazelle was based on study blocks within the protected areas and in community ranches where they occur. Cheetahs due to their low numbers and very large home ranges for example 126 to 195 km^2 in Kruger National Park (Broomhall *et al*., 2003) and 241 to 849 km^2 in farm and conservation land of Botswana (Houser *et al*., 2009), sampling was purposive (opportunistic). Data on other wildlife species showing mange-like skin disease was also opportunistically collected.

Animals showing and those not showing signs of mange-like disease were recorded. The sample size of each animal species was determined using the formula below (Pfeiffer, 2002)

$$n = 1.96^2 \frac{P(1-P)}{d^2}$$

Where n = sample size

P = Probable prevalence (assumed to be 0.5 for Thomson's gazelle, livestock and dogs whose prevalence was unknown while for cheetahs P was estimated to be 0.1, close to what was reported by Todd *et al*., 1981 (11%) in gray wolves.

d = Desired precision (5%)

1.96 = Z value at 95% Confidence Level

For cheetahs (N=60) and domestic dogs (N=100) since the calculated n was greater than 10% of the total population size, n was corrected by applying the following formula for finite populations (Pfeiffer, 2002)

new n = 1/(1/n + 1/N)

The calculated sample sizes of the various species were; cheetahs (42), Thomson's gazelles (384), cattle, sheep and goats (384), and dogs (79).

All animals observed to have mange-like skin condition were recorded.

4.2.2.2 Factors influencing prevalence

Several factors that can have effect on mange prevalence in the study area were identified. These were climatic season (wet and dry) and temporal factors (year of sampling). The wet and dry season months are as described in section 3.2.2. The years were divided into November 2007 to October 2008 (2007/2008) and November 2008 to October 2009 (2008/2009)

4.2.2.3 Data management and analysis

The data was entered into Ms Excel program (Microsoft Operation, USA) and analysed using STATA version 11 (College Station, Tx USA). A t-test was used to compare proportions.

4.3 Results

4.3.1 Overall prevalence

The prevalence of mange-like disease for each species in the entire study area was as shown in Table 4.1. The prevalence during the study period was highest in cheetahs 12.77% (n=47) followed by dogs 4.66 (n=279). The lowest prevalence was 0.09% observed in cattle (n=2311) and 0.09% in goats (n=1174).

Table 4.1: Overall prevalence of mange in each wildlife and livestock species

Species	No. infected	Sample size (n)	Prevalence (%)
Wildlife			
Cheetah	6	47	12.77
Thomson's gazelle	87	10,788	0.81
Wildebeest	5	100	5.00
Domestic animals			
Sheep	51	6,699	0.76
Cattle	2	2,311	0.09
Goats	1	1,174	0.09
Dogs	13	279	4.66

4.3.2. Factors affecting prevalence in each species

4.3.2.1. Cheetahs

The different factors that were shown to affect prevalence of mange infestation in cheetahs are study blocks, climatic season and year of sampling as shown in Table 4.2 and Table 4.3. The prevalence per study block was highest in block 5 (40%) and lowest in block 2 (0%). Blocks 1 and 3 had similar prevalence of 18.2%. There were no cheetahs sighted in blocks 4, 6 and 7 during the study period. No statistical analysis was carried out on study blocks since all blocks had values less than 5 and even some had 0 values.

The prevalence was higher in dry season (14.28%) than wet season (8.33%). However, the two are not statistically different (P = 0.597). It was also higher in the sampling year 2007/2008 (17.39%) than in 2008/2009 (8.33%). As for seasonality, the two are not statistically different (P = 0.357). It is important to note that no infected cheetah was observed after January 2009.

Table 4.2: Prevalence of mange infestation in cheetahs according to study blocks

Study blocks	No. Infected	Sample size (n)	Prevalence (%)
1	2	11	18.2
2	0	20	0
3	2	11	18.2
5	2	5	40
Total	6	47	12.77

Table 4.3: Prevalence of mange infestation in cheetahs according to season and year of sampling

Factors		No. Infected	Sample size (n)	Prevalence (%)	Confidence Interval (CI) (%)	P -Value
Climatic season	Dry	5	35	14.28	6.27 - 29.38	0.597
	Wet	1	12	8.33	1.49 - 35.38	
	Total	6	47	12.77	-	
Time (year)	2007/08	4	23	17.39	6.98 – 37.14	0.357
	2008/9	2	24	8.33	2.31 – 25.84	
	Total	6	47	12.77	-	

4.3.2.2 Thomson's gazelle

The different factors that were shown to affect prevalence of mange infestation in Thomson's gazelle were study blocks, climatic season and year of sampling as shown in Table 4.4 and 4.5. The prevalence per study block was highest in block 7 (3.11%) followed by block 5 (2.13%) and lowest in block 1, 4 and 6 (0%). The prevalence of blocks 2 and 3 were 0.67% and 0.23% respectively. As in the case of cheetahs, no statistical analysis was carried out on study blocks since some blocks had 0 values.

The prevalence was significantly higher (P = 0.001) in dry season (1.64%) compared to wet season (0.16%). It was also significantly higher (P = 0.001) in

the year 2007/2008 (0.98%) than in 2008/2009 (0.13%). It was also noted that the prevalence dropped to near zero by April 2009.

Table 4.4: Prevalence of mange infestation in Thomson's gazelle according to study blocks

Study blocks	No. Infected	Sample size (n)	Prevalence (%)
1	0	1,668	0
2	18	2,669	0.67
3	6	2,563	0.23
4	0	788	0
5	48	2,252	2.13
6	0	366	0
7	15	482	3.11
Total	87	10,788	0.81

Table 4.5: Prevalence of mange infestation in Thomson's gazelle according to season and year of sampling

Factors		No. Infected	Sample size (n)	Prevalence (%)	Confidence Interval (CI) (%)	P -Value
Climatic season	Dry	77	4,688	1.64	1.31 – 2.05	0.0001
	Wet	10	6,100	0.16	0.09 – 0.30	
	Total	87	10,788	0.81	-	-
Time (year)	2007/08	84	8,556	0.98	0.79 – 1.21	0.0001
	2008/9	3	2,232	0.13	0.04 – 0.39	
	Total	87	10,788	0.81	-	-

4.3.2.3 Sheep

The different factors that affected prevalence of mange infestation in sheep were as shown in Table 4.6 and 4.7. The period prevalence per block was highest in block 6 (2.42%) and lowest in block 7 (0%). Blocks 4 and 5 had prevalences of 0.97% and 0.79% respectively. No sheep were observed in blocks 1, 2 and 3. As in the case of cheetahs and Thomson's gazelles, no statistical analysis was carried out on study blocks since some blocks had 0 values.

The prevalence was higher in wet season (0.88%) than dry season (0.46%). However, the two were not statistically different (P = 0.0726). It was also

higher in the year 2008/2009 (1.01%) than in 2007/2008 (0.60%) although the two were not statistically different (P = 0.0595). It was also noted that infected sheep were observed throughout the study period.

Table 4.6: Prevalence of mange infestation in sheep according to study blocks

Study blocks	No. Infected	Sample size (n)	Prevalence (%)
4	16	1,658	0.97
5	18	4,269	0.42
6	17	703	2.42
7	0	61	0
Total	51	6,691	0.76

Table 4.7: Prevalence of mange infestation in sheep according to season and year of sampling

Factors		No. Infected	Sample size (n)	Prevalence (%)	Confidence Interval (CI) (%)	P -Value
Climatic season	Dry	51	6,691	0.76	0.24 – 0.87	0.0726
	Wet	9	1,941	0.46	0.65 – 1.19	
	Total	42	4,750	0.88	-	-
Time (year)	2007/08	51	6691	0.76	0.4 – 0.89	0.0595
	2008/9	24	4,018	0.6	0.7 – 1.49	
	Total	27	2,673	1.01	-	-

4.3.2.4. Dogs

The different factors that affected prevalence of mange infestation in dogs were as shown in Table 4.8 and 4.9. In the 3 blocks 4, 5 and 7 where infected dogs were observed, the prevalences were 5.88%, 4.07% and 0% respectively. No dogs were seen in blocks 1, 2, 3 and 6. As in the case of cheetahs, Thomson's gazelles and sheep no statistical analysis was carried out on study blocks since some blocks had 0 values.

The prevalence was higher in wet season (7.69%) than dry season (2.86%). This observation is different from what is observed in the other animals. However the two prevalence were not statistically different (P = 0.053). It was also higher in the year 2007/2008 (5.64%) than in 2008/2009 (2.38%) although the two prevalence were not statistically different (P = 0.237). It was also noted that no infected dog was observed after April 2009.

Table 4.8: Prevalence of mange infestation in dogs according to study blocks

Study blocks	No. Infected	Sample size (n)	Prevalence (%)
4	6	102	5.88
5	7	172	4.07
7	0	5	0
Total	13	279	4.66

Table 4.9: Prevalence of mange infestation in dogs according to season and year of sampling

Factors		No. Infected	Sample size (n)	Prevalence (%)	Confidence Interval (CI)	P -Value
Climatic season	Dry	5	175	2.86	1.23 – 6.52	0.0653
	Wet	8	104	7.69	3.95 – 14.45	
	Total	13	279	4.66	-	
Time (year)	2007/08	11	195	5.64	3.18 – 9.82	0.237
	2008/9	2	84	2.38	0.65 – 8.27	
	Total	13	279	4.66	-	

4.3.2.5. Cattle, goats and wildebeest

Two cattle (n=2,311) and 1 goat (n=1,174) with mange-like disease were observed during the study period giving a prevalence of 0.09% in both species. Five wildebeest (n=100) with mange-like disease were also opportunistically observed during the study period giving a prevalence of 5.0%.

4.4. Discussion

Although mange has been known to affect cheetahs in the Masai Mara ecosystems for many years (Ngoru and Mulama, 2002; Mwanzia *et al*., 1995: KWS Veterinary Field Reports), there has been no study carried out to estimate its prevalence. It is only Ngoru and Mulama (2002) in a study they carried out on "Cheetah population status, problem and possible mitigation measures in

Masai Mara National Reserve and adjacent group ranches" who reported that 8 out of 40 cheetahs that were individually identified were infected with mange. The prevalence of 12.77% observed in this study is the first comprehensive report on prevalence in the literature of mange in free-ranging cheetahs in a specific area where mange is known to occur. The sample size (n=47) of cheetahs observed in this study is above that reported by Ngoru and Mulama (2002). It also represents over 75% of the 61 cheetahs estimated by Burney, (1980) to be residing in the Masai Mara ecosystem. The prevalence observed is almost similar to that reported by Todd *et al.,* (1981) of 11% in gray wolves in North America but differs with 20% reported in coyotes in North America (Todd *et al.,* 1981) and 20% in pampas foxes in South America (Deem et al., 2002). It is also worth noting that no cheetahs were observed in Lemek and Ol Kinyei group ranches that have undergone a lot of land-use changes especially in crop agriculture and human settlements factors that were noted to affect cheetah populations in Masai Mara (Ngoru and Mulama,2002).

Just like for the cheetahs, there are no previous reports of prevalence of mange in Thomson's gazelles. Thomson's gazelles have been observed to be affected by mange in the Masai Mara ecosystem (KWS field reports, *Personal observation*) but no study has been conducted to determine its prevalence. The prevalence of 0.81% reported in this study is of great significance especially due to the large number of Thomson's gazelles observed (n=10,788). This number represents almost a quarter of the estimated population of 40,000 Thomson's gazelles in the Masai Mara ecosystem (Ottichilo *et al.,* 2000) and a

significant proportion of the estimated population of 550,000 in Kenya and Tanzania where they occur (East, 1999). The Thomson's gazelle is the preferred prey species of cheetahs (Ngoru and Mulama, 2002; Hayward *et al.,* 2006) with reports of up to 90% of prey animals taken by cheetahs in the Serengeti being Thomson's gazelles (Cheetah News 2002). Infected Thomson's gazelles pose a high probability of transmission of mange to cheetahs.

The prevalence of dogs (4.66%) was the second highest among the study animals. All the infected dogs were observed in shopping centers of Aitong, Sekenani and Ololoimutiak where they scavenge for food and their population is high. There were no infected dogs observed in the Manyatta's and the grazing field. The prevalence of mange in dogs reported in this study is much lower than the 14% that had been reported by Kathryn and Williamson (1998) in dogs in remote Aboriginal communities in Australia. Kathryn and Williamson (1998) study was conducted across the whole community area whereas this study was based in areas where cheetahs occur and this could be the reason for the difference. It is also important to note that although the prevalence in dogs was high, there was a low risk of transmission to cheetahs since cheetahs have a tendency to avoid settled areas. However, secondary transmission could occur from dogs to cheetahs through other wild and domestic animals.

Among livestock, it's only in sheep that significant prevalence of 0.76% was reported. In cattle and goats only 2 and 1 infected animals were observed respectively hence the very low prevalence (0.09%) in both species. The prevalence of mange in sheep and goats recorded in this study was very low compared to the 11% previously reported in Kenya and 24-33% reported in Nigeria (Kusiluka and Kambarase, 1996). For cattle it was also much lower than the 25.4% reported by Biu and Wakawa (2004) in Nigeria. However, this prevalence especially that of sheep is quite significant since they share resources in the same range with cheetah and other wildlife species, with a possibility of parasite transmission. However, phenotypic characterization of mites from sheep revealed *Psoroptic* as opposed to *Sarcoptic* mites in cheetah but mixed infection cannot be ruled out and more studies are required to investigate this. It is also important to note that no sheep were observed in blocks 1,2 and 3 which are protected areas.

Although, mange was observed in wildebeest accidentally in the course of the study, its presence is of great significance since wildebeest especially calves are alternative prey species for cheetah (Hayward *et al*., 2006). Wildebeest do migrate seasonally (Estes, 1966; Estes, 1992) and could act as vehicles of mange transmission. More studies are required to determine the prevalence and the role that wildebeest can serve in transmission of mange in the Masai Mara ecosystem.

Climatic seasonality has been identified as an extrinsic factor that affects the prevalence of mange in wildlife (Christophersen, 1996). Christophersen (1996) observations agree with the results of this study which shows that there is high prevalence of mange in dry than wet season. During dry season prey populations disperse in search of pasture and water making it quite difficult for cheetahs to hunt, in contrast to wet season when prey populations congregate and breed increasing the chance of food availability in cheetahs. The dry season causes increased stress due to prey unavailability as cheetahs have to move long distances in search of prey (Houser *et al*., 2009) thereby expending a lot of energy. Malan *et al*., (1997) also attributed appearance of clinical signs of sarcoptic mange in wildlife to stress. The same trend of higher prevalence in dry than wet season was also noted in Thomson's gazelles, a difference that was significant (P = 0.0001). More studies will be required to focus deeply into the role of climatic season and its association to the epidemiology of mange and other parasites in wildlife.

This study revealed that there was high prevalence of mange in cheetahs in block 5 (Koiyaki) and blocks 1 and 3 (Masai Mara National Reserve) which share the same boundary with a lot of wildlife-livestock interaction. The fact that prevalence was high in Thomson's gazelle and sheep in block 5 (Koiyaki), where prevalence in cheetah was also high, this interphase might be acting as an important area of mange transmission. However, no statistical analysis could be carried out since some of the blocks had no positive cases

The effect of time of sampling on prevalence of mange was analysed. In all study species there was higher prevalence of mange-like diseases in the year 2007/2008 than the year 2008/2009. In Thomson's gazelle the difference was significant (P = 0.001). By the middle of the year 2008/2009 hardly could any positive case of mange be picked in most animal species. It is worth noting that all positive wildlife cases that were captured and domestic animals that had mange were treated with 0.2 mg/Kg bwt of 1% Ivermectin (Kalamectin 1% w/v, Kela NV, St. Lenaartseweg, Belgium). Bornstein *et al*., (2001) had earlier observed that although treatment of single infected wild animals is usually of little value in wild populations, where mange is having an impact on small, isolated, and threatened population (e.g., arctic fox or ibex), it may be worthwhile to capture, treat and release such animals. This has been done successfully with artic foxes in northern Sweden (Morner *et al*., 1988). This method of control might be hindered by the difficulties of capturing all affected wildlife individuals. This study does not have enough evidence to conclude that the treatment led to the decrease in prevalence and it is important for the role of therapeutic treatment as a method of control of mange to be further investigated.

In conclusion, this study determined prevalence of mange in free-ranging cheetahs and Thomson's gazelle that had not been described earlier in the literature. It also recommends that more studies on the importance of therapeutic treatment as a method of control of mange in free-ranging wildlife be undertaken.

CHAPTER 5: ASSESSMENT OF THE LEVEL OF KNOWLEDGE OF MANGE AMONG PASTROLISTS AND WILDLIFE OFFICERS

5.1 Introduction

Although mange is a well known disease in Masai Mara ecosystem (Ngoru and Mulama, 2002; Mwanzia *et al*., 1995; KWS Veterinary Field Reports), no survey has been conducted to assess the level of the information about the disease that the pastoralists and wildlife officers have. The pastoralists are usually very close to their animals and harbour a lot of information about the challenges that their animals face. Since they live close to wildlife, and the wild and domestic animals mix freely during grazing, they also observe wildlife behaviour closely. The pastoralists use various ways to control diseases in their livestock and it is imperative to gather information on how they control mange in the ecosystem. On the other hand, since wildlife officers are on the frontline in disease reporting it is important to gather how much information they have on the epidemiology of the disease so as to identify areas for improvement in the reporting system.

Most of the reports of mange infected wildlife to KWS veterinary services department come from tour operators and wildlife officers (Veterinary Field Reports). However, reports from areas outside the protected areas are brought to the attention of wildlife officers by the pastoralists. In this case the pastoralists are able to give early reports of infection, which prevents spread of the disease to other non-infected wild and domestic animals.

It is on this background that the study was undertaken to understand how much information the pastoralists have on mange and other skin infections. This information was expected to help in coming up with mange control strategies.

5.2. Materials and methods

5.2.1 Study area

The study area was as discussed in section 3.2.1

5.2.2. Experimental design

Pre-tested questionnaires (Appendix 1 and 2) were administered to collect data on the level of knowledge of the economic importance and transmission of the disease among pastoralists and wildlife officers. The questionnaire focused on what the targeted groups knew about the disease, if they had any knowledge of its transmission and the health impacts on domestic and wild animals. In total 7 questions were posed to the respondents.

Interpretation of the questions using Masai language was done for most pastoralists. The questionnaires administered to pastoralists were filled at the manyatta as the team was doing observation and sampling of domestic animals. Wildlife officers were given the questionnaires to fill and submit. A few wildlife officers who were not very conversant with English were guided by the research team members in filling the questionnaires.

5.2.3. Data management and analysis

The data collected was categorical or discrete with nominal variables. The outcome was binary variables (yes or no). The proportions were calculated using Ms Excel program (Microsoft Operation, USA).

5.3 Results

Fifty six (56) pastoralists and 30 wildlife officers responded to the questionnaires. The proportions of pastoralists and wildlife officers who responded in the affirmative to the specific question are as shown in Table 5.1 and Fig. 5.1. Table 5.2 shows the percentage of pastoralists and wildlife officers who identified various species of animals as being affected by mange.

Table 5.1: Responses of pastoralists and wildlife officers to questions on knowledge, aetiology and control of mange

Responses	Pastoralists (%)	Wildlife officers (%)
Heard of mange	92.9	99.7
Knows aetiology of mange	23.3	62.1
Heard of mites	66.1	96.7
Seen infected domestic and/or wildlife	85.7	93.3
Cross infection between domestic and wildlife occurs	69.6	72.4
Institute treatment or control measures	67.9	90.0
Knows other skin diseases affecting domestic and/or wildlife	55.4	40.0

Table 5.2: Percentages of pastoralists and wildlife officers identifying various species of animals affected by mange

Species of animal	Pastoralists (%)	Wildlife officers (%)
Sheep	77.6	0
Cattle	14.3	0
Goats	57.7	3.3
Dogs	24.4	13.3
Cheetah	12.2	57.1
Lions	19.5	7.0
Thomson's gazelle	2.4	48.2
Wildebeest	0	21.4
Vervet monkeys	0	3.5
Wild dog	0	3.5

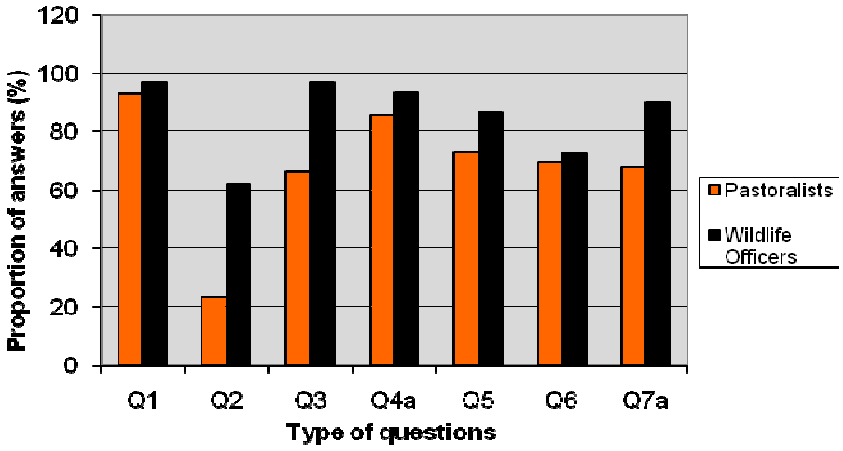


Figure 5.1: A bar graph showing the proportion of positive answers given by wildlife officers and pastoralists

The highest positive responses in both group was in question number 1 where the respondents were asked if they had heard of mange. Ninety two point nine percent (92.9%) of the pastoralists and 99.7% for wildlife managers responded in the affirmative. The lowest positive response was in question number 2 where respondents were asked if they knew the etiology of mange. 62.1% of

wildlife officers and only 23.3% of the pastoralists responded in the affirmative. The respondents were further asked if they had heard about mites and 66.1% of pastoralists and 96.5% of wildlife officers responded to the affirmative respectively.

When respondents were asked if their domestic animals had ever been infected by mange, for pastoralists, and wild animals, for wildlife managers, 85.7% of pastoralists and 93.30% of wildlife managers responded to the affirmative. The pastoralists who answered in the affirmative were asked to specifically state which species of domestic animals had seen infected. 77.6% identified sheep, 57.7% goats, 22.4% dogs and 14.3% cattle. Wildlife officers who responded to the affirmative were asked which species of wildlife they had seen infected. Fifty seven point one percent (57.1%) identified the cheetah, 42.8% identified Thomson's gazelles, 21.4% identified wildebeest, 7.0% identified lion and 3.5% identified vervet monkey and wild dog. The pastoralists and wildlife officers were further asked if they had encountered infected wild animals and domestic animals respectively. Seventy two point seven percent (72.7%) of pastoralists and 86.7% of wildlife officers responded to the affirmative. Twelve point two percent (12.2%) of pastoralists identified cheetah, 19.5% lions, 14.6% Thomson's gazelle, 2.4% identified wildebeest, impala, buffalo and hyena. Wildlife officers identified only dogs and goats at 13.3% and 3.3% respectively among domestic animals they had encountered infected by mange. The respondents were asked if they thought there was cross-infection of mange

between domestic and wild animals. Seventy two percent (72%) of wildlife officers and 69.6% of pastoralists answered in the affirmative.

When the pastoralists and wildlife officers were asked if they instituted any treatment, preventive or control measures once they came across affected domestic or wild animals respectively, about 68% of pastoralists answered in the affirmative compared to 90% of wildlife officers. The two groups were asked to identify the methods they used to treat, prevent and control the disease. Among the pastoralists 29.0% said they spraye or dipped their animals with acaricides, 21.1% reported to veterinary personnel, 21.1% injected terramycin, 13.2% separated the affected individuals from the non-affected ones, 7.8% shaved the affected animals, 5.3% used old engine oil, 2.6% of each visited an agro-vet, injected penicillin or injected ivermectin. Among wildlife officers, 85.2% said they reported to the veterinarian with 52.1% saying that they reported to KWS veterinarian while 7.4% said they reported to park management.

The 2 groups of respondents were asked if they were aware of any other skin diseases that affected animals. Fifty five point four percent (55.4%) of pastoralists and 40% of wildlife officers answered in the affirmative. They were further asked to generally identify the skin diseases that they had come across. Pastoralists identified scabies, fungal disease which they locally referred to as “oloandaban”, sheep/goat pox, papillomatosis and photosensitization Orf, lumpy skin disease and ticks. Wildlife officers

identified lumpy skin disease, fungal infections, giraffe ear disease, scabies, foot and mouth disease and skin rashes.

5.3 Discussion

Mange is a well known disease among pastoralists and wildlife managers in the Masai Mara ecosystem. This has been proved by the fact that over 90% of both groups responded to having heard about the disease. Actually the figure was close to 100% among wildlife officers showing that the disease is a challenge to wildlife conservation in the ecosystem. This agrees very well with previous reports (Ngoru and Mulama, 2002; Mwanzia *et al*., 1995; KWS Veterinary Field Reports). Although the two groups had heard about the disease, quite a large number of them did not know its etiology. This was more so among the pastoralists where only 23% had an idea of what causes the disease compared to 62.1% of wildlife managers. However, when they were asked if they had heard about mites, 66.1% of the pastoralists and up to 96.5% of wildlife managers responded in the affirmative. These results show that majority of pastoralists are aware of the disease and a large number are also aware of the causative parasite but they don't know that mites cause the disease. In contrast, majority of wildlife managers knew about the disease and its causative agent. The most likely explanation to this difference is the literacy level, where wildlife officers have higher education than the pastoralists and have some training on identification of wildlife diseases during their wildlife management course.

The response to the question on possibility of cross-infection of mange between domestic animals and wildlife was quite interesting. Up to 70% of respondents in both groups thought that the disease is transmitted from domestic to wild animals and vice versa. This observation agrees with what has previously been reported by Pence and Uckermann (2002) that various wildlife species are often infected through contact with their domestic counterparts.

Over 85% of respondents in the two groups were able to confirm that, they had seen affected animals in their herds for pastoralists and in wildlife for wildlife officers. They went further and specifically identified the animals that they had encountered having been affected. Pastoralists identified sheep, goats, dogs and cattle in that order. Although these animals have been reported to be affected by mange (Siegmund *et al*., 1973; Mugera, *et al*., 1979; Blood and Radostitis, 1989; Ngoru and Mulama, 2002; Kahn *et al.,* 2005), the results differ with the findings of this study (Chapter 4) where we found insignificant numbers of cattle and goats infected. Wildlife officers identified cheetahs, Thomson's gazelles, wildebeests, lion, vervet monkeys and wild dog in that order. These findings agree with other authors (Pence and Uckermann, 2002; Ngoru and Mulama, 2002; Mwanzia *et al*., 1995) who have reported some of the above animals to be affected by mange. Individuals of all the above species have been diagnosed with mange in various parts of the country (KWS Veterinary Field Reports).

Around 67.9% of pastoralists provide certain treatment, prevention or control initiatives when they observe suspected mange within their herds. Although, majority of them use acceptable interventions such as spraying or dipping their animals using acaricides, others report to veterinary personnel or separate the affected from un-affected animals. Others use methods such as administration of antibiotics which have no effect on mange and can lead to antibiotic build-up in animal tissues and increased antibiotic resistance. On the other hand, some pastoralist use traditional methods such as use of old engine oils, which have unknown effect on mange control. Interestingly a few pastoralists use Ivermectin, the drug of choice for treatment of mange (Pence and Uckermann, 2002; Kahn *et al*., *2005*; Blood and Radostitis, 1989). The wildlife managers report cases of mange in wildlife to the KWS veterinarians or management for intervention. This is expected since for any treatment to be instituted the animals have to be immobilized and it's only KWS veterinary personnel who have that capacity.

Several other skin diseases were identified by both pastoralists and wildlife officers to affect domestic and wild animals. A number of pastoralists identified scabies as a different skin infection from mange. This shows that some pastoralists did not understand that mange and scabies are one and the same disease. However, quite a large number of pastoralists identified fungal diseases, sheep and goat pox, papillomatosis, photosensitization, lumpy skin disease and Orf. Fungal diseases, sheep and goat pox, papillomatosis and photosensitization have been reported as differential diagnosis of mange in

domestic animals (Kusiluka and Kambarase, 1996; Craig 2009). Wildlife officers identified lumpy skin disease, fungal infections and giraffe ear disease. Fungal diseases, specifically dermatomycosis have been reported to be a differential diagnosis of mange in wildlife (Frederick, 2001). Interestingly, a few wildlife officers like pastoralists still identified scabies as a different infection from mange. This was unexpected due to their higher literacy level.

In general, wildlife officers have more knowledge of the disease dynamics than the pastoralists. However, it's important to note that with their low literacy levels, pastoralists have a lot of information about the disease.

In conclusion, this study shows that there is a lot of information about mange among pastoralist and wildlife officers in the Masai Mara ecosystem. However, the results of this study are not conclusive enough to determine if the disease they identified is really mange. A Participatory Rural Appraisal (PRA) approach combined with Participatory Disease Search (PDS) is required to gather enough data and determine if the disease is mange. It will also help in determining if the two groups can positively identify the other skin diseases they mentioned.

CHAPTER 6: PHENOTYPIC CHARACTERIZATION OF ISOLATED MANGE MITES

6.1 Introduction

Although field observations of clinical symptoms of mange-like skin conditions are important in coming up with tentative diagnosis and extent of infection, it is only after sampling and laboratory phenotypic characterization of the causative agent that a confirmatory diagnosis is made. This is often difficult due to the submacroscopic size of *S. scabiei* and the plethora of associated clinical lesions they can cause, especially in hypersensitised hosts where the mites have largely disappeared (Pence and Uckermann, 2002). Sample collection in wildlife is quite challenging since each has to be captured individually either chemically or physically. It is much easier in domestic animals since only physical restraint is required. However, a sample from wildlife that is positive confirms presence of infection in the environment.

Sarcoptes scabiei has already been confirmed in the laboratory to be the causative agent of mange in cheetahs in the Masai Mara ecosystem (Mwanzia *et al*., 1995). However, no reports of confirmation in other wild and domestic animals are available from this ecosystem. Although most host species are affected by a single species of mites (Fain, 1978), reports of mixed infections by different species of mites exist, such as *sarcoptes and notoedres* in cheetahs (Pence and Uckermann, 2002) and *psoroptes* and *sarcoptes* in sheep (Radostitis *et al*., 1999). It is important to phenotypically characterize the causative agent

to determine the species of mites that infest the affected animal and also reveal if an animal is affected by one species of mites or a mixture of species.

This study was designed to confirm the presence of mange mites in wild and domestic animals observed to have mange-like disease conditions and phenotypically characterize the isolated mites.

6.2. Materials and methods

6.2.1 Study area

The study area was as discussed in section 3.2.1.

6.2.2 Sampled animals

Animals showing at least 3 of the 5 symptoms (pruritus, alopecia, crust formation, skin roughening and poor body condition) describing mange-like skin disease were sampled. In domestic animals, since they are easier to restrain, all animals that fitted the description were sampled. This was with the exception of dogs which were stray in shopping centers and could not be manually restrained. The dogs that we could closely approach were darted and sampled. In Thomson's gazelles, due to their challenges of capture, it is only those that could be captured that were sampled among the ones that fitted the case description mentioned above. In cheetahs only those that were heavily infested and required treatment were sampled. Cheetahs that looked mildly infested were not sampled since cheetahs are highly sensitive to immobilization

drugs (personal observation). Any other wildlife species that was observed to be mange infested was conveniently sampled.

6.2.3 Sampling method

A purposive random sampling method was used to get the sampling units. The same method of stratification as in prevalence determination was used. Since sampling was being done to detect disease the following formula was used to calculate sample sizes (Pfeiffer, 2002)

$$n = [1-(1-ß)^{1/d}] [(N-d/2) + 1/2]$$

Where, n = Sample size

ß = Confidence level (as proportion) – the probability of observing at least one Diseased, if prevalence is d/N

N = Population size

d = number of diseased animals – can be calculated from prevalence and total population, assuming expected prevalence is 10% (P = d/N)

As per the above formula, the sample sizes of the various animal species expected to be sampled were; cheetahs (22), Thomson's gazelles (29), livestock (cattle - 29, goats - 29, sheep - 29) and dogs (25)

6.2.4 Capture of wildlife and dogs

Cheetahs, lions and wild dogs were captured via chemical immobilization using a combination of 100 mg/ml Ketamine (Agraket^{R}, Agrar Holland Bv) and 100mg/ml Xylazine (500mg Xylazine^{R}, Kyron Laboratories (Pty) Ltd) at 8mg/kg body weight and 1mg/kg body weight respectively propelled via a Daniject (Daniject^{R} Denmark) remote projectile system. The sedative effect of Xylazine was reversed after 45 minutes using 5mg/ml Atipemazole hydrochloride (Antisedan^{R}, Pfizer Laboratories (Pty) Ltd) at 1/10 total dose of Xylazine used. The reversal in cheetah was done after 45 minutes to allow Ketamine to wear out of the body so as to avoid muscle tremors associated with it. For lions and wild dog the reversal was done immediately after sampling since they don't show tremors as is the case with cheetahs. The reversal of Xylazine was to make sure that animals were alert prior to release to avoid being predated upon.

Thomson's gazelles, wildebeest and impala were captured via chemical immobilization using a combination of 9.8 mg/ml Etorphine hydrochloride (M99 ^{R}, Norvatis South Africa (Pty Ltd/(Edms) Bpk) and Xylazine at 50µg/Kg body weight and 0.2mg/Kg body weight respectively propelled via a Daniject remote projectile system. The lepto-analgesic effect of Etorphine was reversed using 12 mg/ml Diprenorphine hydrochloride (M5050^{R}, Novartis South Africa (Pty Ltd/(Edms) Bpk) at 3X total dose of Etorphine used while that of Xylazine was reversed using Atipemazole at 1/10 total dose of Xylazine used. The reversal was done immediately after sampling.

Domestic dogs were also captured via chemical immobilization using a combination of 100 mg/ml Ketamine and 100mg/ml Xylazine at 5 mg/kg body weight and 2 mg/kg body weight respectively propelled via a Daniject remote projectile system. The dogs were observed until they recovered from anaesthesia.

6.2.5 Sample collection

Affected area of skin was scrapped with a scalpel blade until it bled to obtain hairs and crusts for parasitological examination. The scrapings were placed in universal labeled bottles containing 70% ethanol and transported to the laboratory.

In the laboratory, 100 to 200 milligrams of scrapings were placed in a beaker with 30ml of 10% potassium hydroxide and heated, without boiling in a stirrer plate until all hairs were dissolved. The suspended material was poured into a centrifuge tube and centrifuged for 5 minutes at 700 revolutions per minute, and the supernatant discarded. Pellets were suspended in saturated sucrose solution, specific gravity 1.22 and centrifuged again for 5 minutes at 700 revolutions per minute. Each tube was filled with sucrose solution to form a slight meniscus at the top, and a cover slip was placed in contact with the sucrose for 5-10 minutes. Each cover slip was thereafter carefully removed, placed on a glass slide and microscopically examined for the presence of mites and eggs. The types of mites and their stages of development were identified

using various identification marks as described by Margaret and Russell (1978).

6.3 Results

Different species of animals showed similar symptoms in affected areas which included alopecia, encrustation and skin roughening (Fig. 6.1, 6.2, 6.3, 6.4 and 6.5). The symptoms differed depending on severity of infestation. Figures 6.1 and 6.2 also show characteristic tissue necrosis in long standing cases.

Figure 6.1: A photo of mange infested cheetah head showing necrotised areas

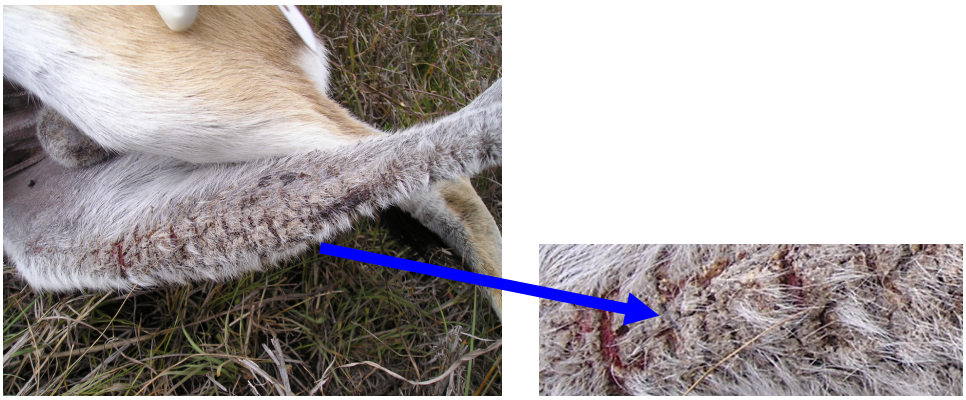


Figure 6.2: A photo of mange infested Thomson's gazelle hind limb showing necrotised areas

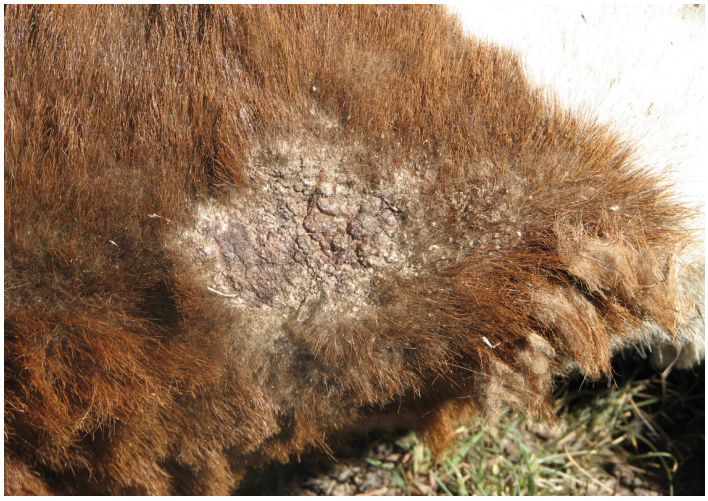


Figure 6.3: A photo of a mange infested sheep rump showing alopecia and encrustation

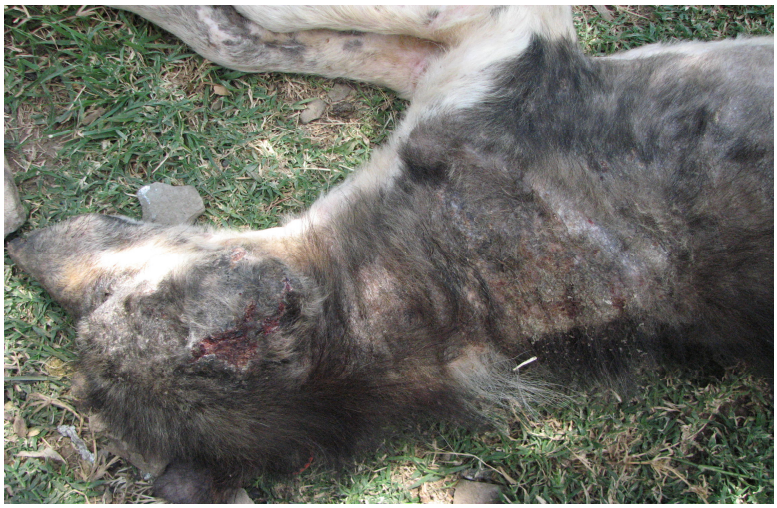


Figure 6.4: A photo of a mange infested head and neck region of a dog showing alopecia, encrustation and necrosis

Figure 6.5: A photo of a mange infested wildebeest calf showing alopecia and encrustation on the hind limb

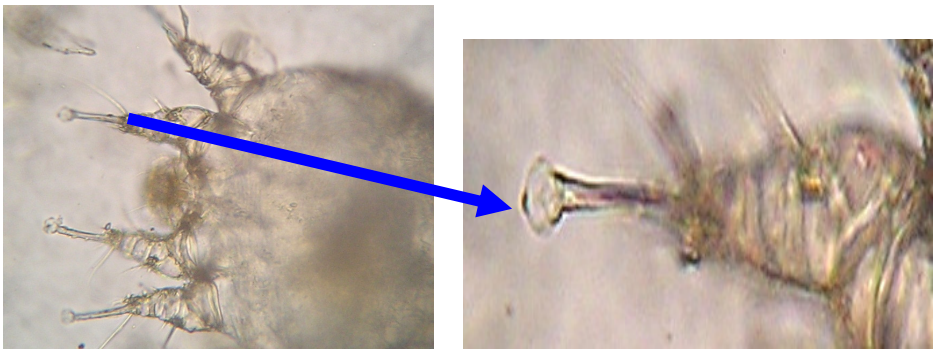
Seventy eight samples were collected from different species of wild and domestic animals (Table 6.1). Out of these, 8 were from cheetahs, 10 from Thomson's gazelles, 51 from sheep, 9 from dogs, 5 from wildebeest, 2 from cattle, 2 from lion, 1 each from goat, wild dog and impala. All (100%) of

cheetahs within the study were positive, 8 (80%) of Thomson's gazelles were positive, 27 (52.9%) of sheep were positive, 1 (11.1%) of dogs were positive, 5 (100%) of wildebeest were positive, 2(100%) of lions were positive, the wild dog sample was positive while cattle, goat and impala samples were negative.

Table 6.1: A summary of number of samples collected and type of mite isolated

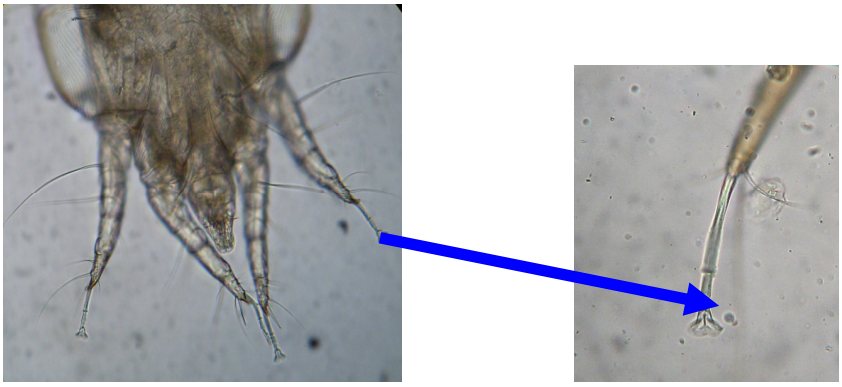
Species	**No. Sampled**	**Positive**	**Negative**	**Mite species isolated**
Cheetah	8	8	0	*Sarcoptes scabiei*
Thomson's gazelle	10	8	2	*Sarcoptes scabiei*
Dogs	9	1	8	*Sarcoptes scabiei*
Sheep	41	22	19	*Psoroptes communis*
Wildebeest	5	5	0	*Sarcoptes scabiei*
Lion	2	2	0	*Sarcoptes scabiei*
Wild dog	1	1	0	*Sarcoptes scabiei*
Cattle	2	0	2	Negative
Goats	1	0	1	Negative
Impala	1	0	1`	Negative
Total	78	47	31	Negative

Sarcoptes scabiei (Fig. 6.6) was isolated from cheetahs, Thomson's gazelles, dogs, wildebeest, lion and wild dog while, *Psoroptes communis* (Fig. 6.7) was isolated in sheep. The developmental stages of *Sarcoptes scabiei* were also observed (Fig. 6.8).



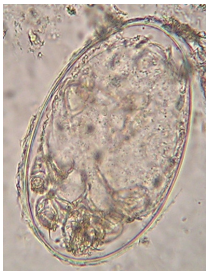
X 400

Figure 6.6: Isolated *S. scabiei*: the protruding stalk at the end of the legs is the pedicle.

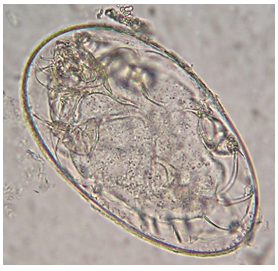


X 400

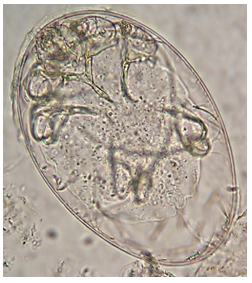
Figure 6.7: An isolated *Psoroptes spp.* showing characteristic suckers on long jointed stalks



x400



x400



x400

Eggs at different stages of development

x400 x400

Six-legged larval stage Adult with eight legs

Figure 6.8: Developmental stages of the *S. scabiei* observed in a cheetah sample

6.4 Discussion

The clinical picture observed in the study animals is that of alopecia, pruritus, acute dermatitis, suppurative encrustation, skin roughening and poor body condition. The clinical symptoms observed in this study are similar to those reported by other authors in wild and domestic animals affected by sarcoptic mange (Siegmund *et al*., 1973; Fain, 1978; Pence *et al*., 1983; Blood and Radostitis, 1989; Morner, 1992; Ippen, *et al*., 1995; Rossi *et al*., 1995; Yeruham *et al*., 1996; Fernandez *et al*., 1997; Scott *et al.,* 2001; Pence and Uckermann, 2002; Fitzgerald *et al*., 2004; Ljunggren *et al*., 2003; Kahn *et al.,* 2005; Ljunggren *et al*., 2006; Williams *et al*., 2008). Similar symptoms which included papules, crusts, excoriation, and lichenfication were described by Kahn *et al.,* 2005 in psoroptic mange infested sheep. Due to the pruritus, animals spend most of their time scratching. The dermatitic areas erode leaving

necrotic wounds that attract flies. The scratching and disturbance by flies leaves very little time for the affected animals to feed which leads to loss of body condition. Wild carnivores that are severely affected cannot hunt especially if they are solitary, they succumb to malnutrition.

The number of sampled animals in this study differed with the calculated sample size. With the exception of sheep all the number of other animals sampled were fewer than the calculated sample size. For goats and cattle where the calculated sample size was 29, only 2 and 1 animal were sampled respectively. This was due to lack of active cases of skin infections. In cheetahs and dogs with a calculated sample size of 22 each we only sampled 6 and 9 respectively. However it's important to note that all cheetahs observed in Chapter 4 to have mange-like skin condition were sampled. This was not the case in dogs, where only 9 out of the 13 dogs observed to have mange-like disease were sampled. The reason for this was due to the difficulty in capturing some of the stray dogs which ran into bushes once they realised that we were pursuing them.

The calculated sample size of Thomson's gazelles was 29. However, only 10 out of the 81 Thomson's gazelles observed with mange-like skin disease were captured. Although, the sampled figure looks relatively small compared to the number observed, this was not unexpected. Thomson's gazelles are very difficult to capture by darting due to their flight distance and small body size. Aiming the target during darting is very challenging due to the long distance to

and small size of the target. Further, once they realised they were being hunted, they became unapproachable. In case the first dart missed, the animal became almost impossible to get it again. Mass capture using nets is the method of choice, but it is also very challenging where you are targeting a specific individual. It only works well when you are targeting a herd or a group of individuals as you have no assurance that the targeted animal will be pushed into the net. We did attempt the method twice without success. In sheep the number of animal sampled were beyond the calculated sample size and all animals observed with mange-like skin disease were sampled. It's important to note here that the epidemiological formula used to calculate sample sizes might not be practical in wildlife due the myriad of challenges in the wild. This observation is supported by Lindberg and Walker (2007) who reported that logistical challenges of working with some wildlife species can limit sample size. Wildlife veterinarians should come up with epidemiological formulae that take into consideration all challenges of wildlife sampling.

The results of this study show that all the cheetahs sampled were positive for mange mites. Six of the cheetahs were sampled during the study period while 2 had been sampled before commencement of the study and the samples stored. These results show that it's highly likely that most cheetahs depicting symptoms of skin disease are affected by mange. This observation agrees with that of Weber and Rabinowitz (1996) that cheetahs are highly vulnerable to mange. Although there was impression that the study targeted only those cheetahs that were severally affected, this was not the case on the ground at the

time the study was done, there were no cheetahs that were mildly affected. The same trend was noted in lions where the 2 opportunistic sampling were positive for mange mites. From this result, we can conclude that wild cats are highly vulnerable to mange. In all other sampled animals the percentages of positive samples ranged from 11.1% in dogs to 80% in Thomson's gazelle with exception wildebeest which had 100%. This shows that some of the skin conditions in these animals could be non-mange. During the course of the study it was realised that certain Thomson's gazelles had discoloration that resembled mite infestation. In such cases, the individuals were observed until signs of pruritis were seen before they were immobilized. The results of 100% positive cases of wildebeest were biased since it was targeted sampling of affected ones.

Sarcoptes scabiei was isolated in all positive animals except in sheep where *Psoroptes communis* was isolated. This shows that *S. scabiei* is the commonest species of mites affecting wild and domestic animals. This observation agrees with that of Bornestein *et al*., (2002) who described sarcoptic mange as the commonest mange infection in mammals and Pence and Uckermann, (2002) who described it as commonest in wildlife. It's important to also note that the infections in most animals were active since all stages of the life cycle were observed. *Psoroptic* mange also referred to as "sheep scab" caused by *P. communis ovis* has been reported to be commonest in sheep (Mugera, *et al*., 1979; Blood and Radostitis, 1989; Kusiluka and Kambarase, 1996). Similar results were observed in this study. However, a mixed infection of *S. scabiei*

and *P. communis* in sheep cannot be ruled out. According to Radostitis *et al.*, (1999), the occurrence of sarcoptic mange in sheep is quite uncommon, but does occur. It is possible that *S. scabiei* could be present in sheep in this area at very low concentration and are missed out during isolation since *P. communis* are the majority. More investigations are required to verify if mixed infections do occur in this species in the study area

In conclusion this study shows that *S. scabiei* is the commonest cause of skin condition in wildlife in Masai Mara ecosystem while *P.* communis is commonest in sheep. Further, it can also be concluded that epidemiological formula developed for calculation of sample sizes of domestic animals will not always work in wildlife.

CHAPTER SEVEN: GENOTYPIC CHARACTERISATION OF ISOLATED MITES

7.1 Introduction

Although *S. scabiei* has previously been confirmed phenotypically in the laboratory as the causative agent of mange in Thomson's gazelle and cheetahs in Masai Mara ecosystem (Mwanzia *et al*., 1995; *KWS Veterinary reports*), no attempts have been made to determine if the isolated mites are host specific or mites affecting the same species are different.

Although *Sarcoptes* mites have been described as a single species with variable sub-species that are predominantly host specific, morphological and experimental studies have failed to identify any significant differences between mite populations (Fain, 1978 Arlian *et al*., 1984; Arlian *et al*., 1988a; Arlian, 1989). Due to these difficulties, scientists have resulted to molecular studies to genotypically characterise and identify differences in mites affecting same or different host species and geographical locations (Walton *et al*., 1999; Walton *et al*., 2004b; Alasaad *et al*., 2008b).

Microsatellites have previously been shown to provide differentiation of *S. scabiei* in to geographically discret populations, population level and even at skin-scale level of individual host (Bowcock *et al*., 1994; Walton *et al*., 2004a; Alasaad *et al*., 2008a). Evidence of gene flow between *Sarcoptes* mite populations between different hosts though rare has been proven using multi-locus genotyping by applying microsatellite markers (Walton *et al*., 1999). The

same molecular marker was used to describe differences in host-taxon- derived (carnivore- herbivore-, and omnivore- host-derived) *Sarcoptes* mite population (Rasero et al., 2010). This molecular marker was found to be suitable for differentiation of mites in these study populations.

It is on the above background that this study was designed to determine if mites isolated from different wild and domestic animal hosts were genetically different. The study also aimed at determining if there was any genetic difference between mites isolated from the same host. The understanding of the host specificity or non-specificity is important in the design and institution of mange control strategies.

7.2 Material and Methods

7.2.1. Study area

The study area was as discussed in section 3.2.1.

7.2.2 Samples

From isolates recovered in chapter 6, mites isolated from 35 skin scrapings of cheetah (8), lion (2), wildebeest (2), Thomson's gazelle (8) and sheep (15) were used for genotypic characterisation. Mites from domestic dog and wild dog were not characterised genotypically because the concentration of mites was quite low and the few that were recovered were exhausted during the initial genotypic trials.

7.2.3 Isolation of gDNA

DNA of individual mites was extracted using the NucleoSpin Tissue kit procedure (Macherey-Nagel, Düren, Germany), with some modifications proposed by Soglia *et al*., (2009), and the HotSHOT Plus ThermalSHOCK technique (Alasaad *et al*., 2008b).

Each individual mite was placed in a 1.5ml Eppendorf tube containing 180 microlitres (µl) of T1 buffer with the help of a needle under a dissecting microscope. The sample was then subjected to a heat shock, consisting of -80ºC step for 2 minutes followed by 70 ºC step for 2 minutes, repeated 3 times. To pre-lyse the sample 25 µl of Proteinase K was added to the sample and incubated at 56 ºC overnight.

After overnight incubation the sample was vortexed and complete lysis was achieved by adding 200 µl of Buffer B3. The sample was then vortexed vigorously and incubated at 70ºC for 10 minutes. After the incubation, the gDNA binding conditions were adjusted by adding 210 µl of absolute ethanol to the sample and vortexing vigorously. To bind the gDNA, the resultant solution was placed in a 2-ml Collection Tube containing a NucleoSpin® Tissue Column and centrifuged at 11,000 x g for 1 minute. The flow-through on the column was discarded and the column placed back into the collection tube. To wash the silica membrane of the column, 500 µl of Buffer BW was added and centrifuged at 11,000 x g for 1 minute. The flow-through was discarded and the column placed back in the collection tube. The silica

membrane was washed again by adding 600 µl of Buffer B5 to the column and centrifuging 11,000 x g for 1 minute. The flow-through was again discarded and the column placed back into the collection tube. In order to dry the silica column and remove residual ethanol, the column was centrifuged for 1 minute at 11,000 x g.

The gDNA was eluted by placing the NucleoSpin® Tissue Column into a 1.5ml Eppendorf tube and adding 100 µl of Buffer BE that was pre-warmed at 70ºC. The gDNA was incubated at room temperature for 1 minute and then centrifuged at 11,000 x g for 1 minute. The gDNA was ready for use.

7.2.4 Fluorescent-based PCR analysis of microsatellite DNA

From an *S. scabiei* microsatellite panel described by Walton *et al.* (1997), ten microsatellites (Sarms 33-38, 40, 41, 44 and 45) (Table 7.1.) were selected and analysed with one 10× multiplex PCR, with one primer from each set 5' labelled with 6-FAM, VIC, NED or PET® fluorescent dye tag (Applied Biosystems, Foster City, CA, USA). Each 15 µl PCR reaction mixture consisted of 3 µl of the single mite gDNA, together with the PCR mixture containing all primer pairs which ranged from 0.04 to 0.1 µM per primer, 200 µM of each dATP, dCTP, dGTP and dTTP, 1.5 µl of 10× PCR buffer (200 mM KCl and 100 mM Tris-HCl, pH 8.0), 1.5 mM $MgCl_2$ and 0.15 µl (0.5 U/reaction) HotStar Taq (QIAGEN, Milano, Italy). The samples were subjected to the following thermal profile for amplification in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA): 15 min at 95°C (initial

denaturing), followed by 37 cycles of three steps of 30 seconds at 94°C (denaturation), 45 seconds at 55°C (annealing) and 1.5 minutes at 72°C (extension), before a final elongation of 7 min at 72°C.

Table 7.1: Microsatellite primers for *Sarcoptes* mites used in the study

Primer	**Forward**	**Reverse**
Sarms45	5-ATG GTA TGG ATG CGG AAG AG-3	5-GGA TTC TGG TAA GGA TCG AG-3
Sarms44	5-CAA TCA TCT CAT CGG CGA AG-3	5-CGA AGC GCA TCA CAA CAT C -3
Sarms41	5-CTA CGA ATC TGT CGG GAT CC-3	5-CTA TTG CCA TTC AGC AGC ACC-3
Sarms40	5-CGC GCC AAT GAT TTC TGT CTG-3	5-GGA AAT GCG CGT ATT CCG-3
Sarms38	5-CAC CAA AGG GTT ACG GTG AG-3	5-GCG ATC CTT TTG AGC TGT TCG-3
Sarms37	5-CGG TCC TCA TCT TAT CAT CAC CCA CC-3	5-CTG GAA GAC CTC GTG ACC-3
Sarms36	5-CCA GTG GAC TGT GGA TCT TCA ATC G-3	5-CTC GAT GAA AAG TGA GGA GTG-3
Sarms35	5-CTG TCA CTC TCT TTC GCT ATC CG-3	5-GGA GCC TAA GGT CCT AAC-3
Sarms34	5-CAC CTC CAT CAT CCA GTA G-3	5-GCT GCT GCT TTG GAT TCA G-3
Sarms33	5-GGT GTG TGG TTC TGA GTA C-3	5-GAG GTT GAG AAT AGG TTC ACG-3

7.2.5 Genotyping of PCR products

Using 96-well plates, aliquots of 12 μL of formamide with Size Standard 500 Liz (Applied Biosystems, Foster City, CA, USA) and 2 μl PCR product were prepared. Then, the plates were heated for 2 min at 95°C and chilled to 4°C. Fluorescent PCR amplification products were analyzed by ABI PRISM 310 Genetic Analyzer with pop4. Allele coding was performed using the GeneMapper v. 4.0 software (Applied Biosystems, Foster City, CA, USA). To track and minimize the amount of error associated with genotyping, the genetic data were collected twice.

7.2.6 Descriptive statistics and cluster analysis

CONVERT 1.31 software (Glaubitz, 2004) was used to reformat files for the statistical software. Descriptive statistics and diversity analyses were carried out with GenAlEx v. 6.2 (Peakall and Smouse, 2006), Genepop v. 4.0 (Raymond and Rousset, 1995), Fstat v. 2.9.3 (Goudet, 1995) and Arlequin v. 3.1 (Excoffier *et al*., 2005) software to determine the number of private alleles, allele frequencies and unbiased expected (He) and observed (Ho) heterozygosity, and also to test for Hardy-Weinberg (HWE) and linkage equilibriums (LE), and *F* statistics. The analysis of the structure and relationships between host-specific mite populations were studied using two different approaches:

(i) The multilocus genetic Distance based on Proportion of Shared alleles (Dps) was computed between all possible pairs of individual mites using Microsat software (Minch, 1997), ignoring any preliminary information regarding the

origins of the parasites. One thousand datasets were generated by resampling the input data (bootstrapping); the Neighbor-Joining algorithm was implemented by the Phylip v. 3.6 packages (Felsenstein 1989) to obtain a consensus dendrogram. The dendrogram was visualized using the Dendroscope v. 2.2.2 software (Huson *et al*., 2007).

(ii) The analysis of relationships between mites was then improved by a Bayesian assignment test using the Structure v. 2.2 software (Pritchard *et al*., 2000). I performed 10,000 MCMC (Markov chain Monte Carlo) replicates following a burn-in period of 10,000 steps. This parameter set was run 10 times for each different number (K) of the genetic clusters of the multilocus genotypes; all values of K from 1 to 20 were tested. The probability of the multilocus genotype of any individual mite occurring in each of the K clusters was computed. I used the admixture model (each mite drew some fraction of its multilocus genotype from each of the K clusters), thereby allowing the allele frequencies to be correlated between clusters. This configuration has been described as the best in cases with subtle population structures (Falush *et al*., 2003). The height of the modal value of the distribution of ΔK was used to estimate the uppermost number of clusters capturing the overall mite sample structure, as suggested by Evanno *et al*., (2005). I then associated all individual mites with the cluster that corresponded to its greatest membership (q), that is, the fraction of its multilocus genotype; a threshold value $q \geq 0.9$ was used. Finally, each of the inferred clusters was associated with the component populations of its mites.

7.5 Results

Variability and similarities were noted in *Sarcoptes* mites affecting same host species and different host species (Fig. 7.1 and Fig. 7.2). *Sarcoptes* mites isolated from wildebeest had the highest genetic variability while those of lions had the least genetic variability. There were 4 main clusters with wildebeest mites appearing in 3 and lion mites appearing in 1 of the 4 clusters. Cheetah and Thomson's gazelle mites appeared in 3 and 1 clusters of the 4 clusters respectively. There was higher genetic diversity in cheetah than Thomson's gazelle. The high genetic diversity of wildebeest and cheetah mites was also exemplified by having some mites from the 2 species out of the major clusters. Both lion and Thomson's gazelle mites appeared in one cluster each but the Thomson's gazelle mites appeared in more sub-clusters within the major cluster hence had more diversity.

Microsat Software

Ghe=Cheetah
Leone=Lion
Gazz=Gazelle
Gnu=Wildebeest

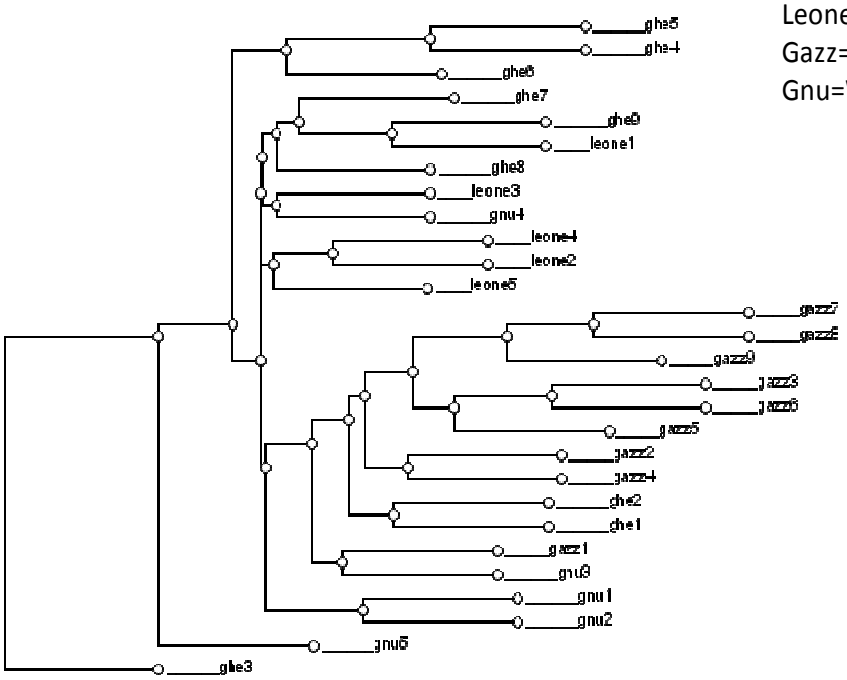


Figure 7.1: Unrooted Dps consensus dendrogram for individual *Sarcoptes* mites from four sympatric host-derived mite populations in Masai Mara, Kenya. Numbers at the nodes are the percentage values of 100000 bootstraps supporting the same branching structure

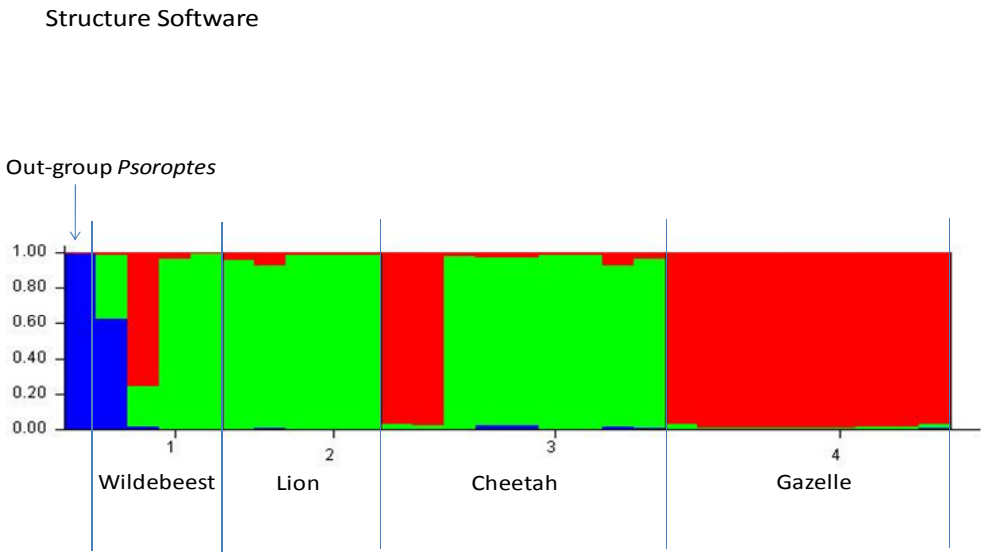


Figure 7.2: Cluster structure where the colors bars show separately the proportion of member-ship of each Sarcoptes individual in the genetic clusters for each *Sarcoptes* population from Masai Mara, Kenya

Sarcoptes mites from wildebeest, cheetah and lions were more similar while those of Thomson's gazelle were totally different. Mites from the first 3 species appeared together in one of the major clusters. The similarity between mites from lion and wildebeest was very close as they appeared together in one sub-cluster. The same similarity was also observed in mites from lion and cheetah which appeared in the same sub-cluster. There was some relationship between mites of Thomson's gazelle, cheetah and wildebeest since they appeared together in one major cluster but the similarity appeared to be more in cheetahs and Thomson's gazelle since they appeared in 2 close sub-clusters.

The mites that were isolated and analysed from sheep were *Psoroptes* species and were detected as an out-group as shown in Fig. 7.2.

7.3 Discussion

The results from this study showed genetic diversity of mange from the same host species. Mites from wildebeest had the highest genetic diversity followed by those of cheetah, then Thomson's gazelle while lion had the lowest. Wildebeest are seasonal migratory species (Estes, 1966; Estes, 1992) and the high diversity could be as a result of infection by mites from different geographical locations. Cheetahs have large home ranges (Ngoru and Mulama, 2002; Creel and Creel, 2002; Broomhall *et al*., 2003; Houser *et al*., 2009) and could be infected by mites of different geographical locations just as is the case with wildebeest. This is in contrast to lions that have smaller home ranges that are governed by food availability and energy expenditure during territorial defense (Gittleman and Harvey, 1982; Van Orsdol *et al*., 1985; Lehmann *et al*., 2008) and Thomson's gazelles that are territorial with small home ranges that increase depending on food availability (Stuart and Stuart, 2006). The probability of infection of these two species with mites from different geographical locality is minimal hence the low genetic variability. The observation of genetic diversity of mites from the same host agrees with earlier reports by Walton *et al*., 2004a who described genetic differences of mites isolated from humans living in the same household and also in dogs living in the same locality. The argument that the greater genetic diversity of mites from wildebeest and cheetah could be possibly related to geographical localities is supported by Walton *et al*., 2004a and Rosero *et al*., 2010 who described genetic differences between mites of same species from different geographical localities. However, mites used by the above authors to prove genetic diversity

were isolated from same host in different countries. This study is therefore not conclusive and analysis of mites from the same host in different geographical areas needs to be done in Kenya and the region to prove the hypothesis that mites of these 2 species differ due to geographical factors.

The mites isolated from cheetah, wildebeest and lions were genetically similar while those of Thomson's gazelle were different though a few are slightly similar to those of cheetahs. These results show that there is gene flow between mites of cheetah and wildebeest, cheetah and Thomson's gazelle, cheetah and lion and, lion and wildebeest. There is no gene flow noted between lion and Thompson gazelle and, wildebeest and Thomson's gazelle.

The cheetah is known to prey upon Thomson's gazelles (Ngoru and Mulama, 2002; Hayward et al., 2006) and wildebeest especially calves (Hayward et al., 2006) in contrast to lions which are known to prey on wildebeest and rarely Thomson's gazelle (Viljeon, 2003; Owen-Smith and Mills, 2008; Fryxell *et al*., 2009). There is high likelihood of transmission of mites from the prey to the predator during feeding which can lead to the observed gene flow. This phenomenon explains the existence of gene flow between mites of cheetah and wildebeest, lion and wildebeest and, cheetah and Thomson' gazelle. However, although lion can prey upon Thomson's gazelle, this is quite rare especially in areas where there are other big game species (Owen-Smith and Mills, 2008) like the Masai Mara ecosystem (Ottichilo *et al*., 2000). This explains the lack of detectable gene flow between mites of lion and Thomson's gazelle.

The mites isolated from cheetah and lions are similar while those isolated from Thomson's gazelle and wildebeest are not similar. This shows that there is gene flow between mites of the lions and cheetahs but non between wildebeest and Thomson's gazelle. The cheetah and lion are genetically closely related (Order: Carnivora; Family: Felidae). The genetic similarity of mites from these closely related wild cats could be due to host-taxon-derived effect (Rosero *et al*., 2010). These authors reported that there is lack of gene flow or recent admixure between carnivore-, herbivore-, and omnivore- derived *Sarcoptes* populations. Mite transmission occurs within each carnivore-, herbivore-, and omnivore-derived mite cluster but extremely rare or absent between them. Further, no epidemiological relationship was found to exist in Europe between mange foci affecting wild ruminants, wild boars and carnivores (Berilli *et al*., 2002). It's important to note that despite wildebeest and Thompson gazelle being genetically closely related (Order: Artiodactyla; Family: Bovidae) and mostly sharing the same range, the host-taxon derived effect was not observed. This could be attributed to a predator/prey effect. In the study reported by Rosero *et al*., (2010) there was lack of interaction between carnivore, herbivore and omnivore hosts, while in a predator/prey system there is real interaction, which could lead to alteration in host-taxon phenomenon up to predator/prey degree of contact. This requires further investigation. This is the first report of *Sarcoptes* mite gene flow in predator/prey system, which alternated the host-taxon effect where no clear predator/prey relations exist.

Although, mites isolated from sheep were *Psoroptes* and there genetic relationship with wildlife mites could not be compared, it is not advisable to dismiss sheep as having no role to play in epidemiology of mange in Masai Mara ecosystem. Infected sheep were consistently observed in the ecosystem throughout the study period and we could have missed *Sarcoptes* mites due to high concentration of *Psoroptes*. The same case applies to dogs where, although we isolated *Sarcoptes* mites we could not get enough mites for genetic characterisation study due the low concentration of mites in the infected animals and whatever was there we had used for earlier trials. Further investigation on the role of sheep and dogs in the epidemiology of mange in the ecosystem is important.

In conclusion this study shows that there is genetic diversity of *Sarcoptes* mites isolated from the same host species. There is also genetic similarities and differences of *Sarcoptes* mites isolated from different host species. There is gene flow between *Sarcoptes* mites of different hosts mostly due to transmission of mites between genetically related hosts as a result of host-taxon derived effect and predator-prey relationship. This is important in understanding the epidemiology of mange in wildlife and therefore will help in coming up with prevention and control strategies. However, the effect of geographical locality to genetic similarities and/or differences of *Sarcoptes* mange in same host, lack of host-taxon derived effect in Thomson's gazelle and wildebeest, and role of sheep in epidemiology of mange in the Masai Mara ecosystem require further investigation.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Discussion

This study was conducted in Masai Mara ecosystem and aimed at describing the epidemiology of mange among wild and domestic animals sharing the same range within the ecosystem. This was done by determination of the spatial and temporal distribution and, prevalence of mange, the risk factors for infection and characterization of the causative mites by phenotypic and genotypic approaches. In an effort to achieve these objectives, a number of studies were conducted.

Cross-sectional observation survey of mange-like skin disease revealed that the disease was distributed in most parts of the study area with higher concentration being noted along the boundary of the protected area and community ranches. It is in these areas that there is significant interaction between wild and domestic animals with increased potential for disease transmission. This phenomenon of increased disease observations in wildlife at the wildlife livestock interphase has previously been described (Bengis *et al*., 2002; Jones *et al*., 2008) and has been associated with expansion of human and livestock into wildlife areas. The study further revealed that affected cheetahs were found close to affected Thomson's gazelle showing that there could be parasite transmission from Thomson's gazelle to cheetah during feeding as Thomson's gazelle is favourite prey for cheetah (Ngoru and Mulama, 2002; Hayward *et al*., 2006). There was also observation of infected sheep close to

infected cheetahs and Thomson's gazelles which could point to the likelihood of parasite transmission among the 3 species. Interestingly, the study revealed that infection of dogs was concentrated in shopping centers among stray dogs but none in grazing fields and Manyattas. These points to the low likelihood of transmission to wild animals. The study further revealed the high possibility of getting infected animals in dry than in wet season. This is likely to be as a result of increased stress brought about by reduced food availability leading to manifestation of clinical signs in formerly sub-clinical animals as described by Malan *et al*., (1997).

The prevalence study of mange-like skin disease revealed that cheetah had the highest prevalence (12.77%) among the study animals. This is first prevalence report of mange in free-ranging cheetahs in the literature and this figure was close to that reported in other wild carnivores (Todd *et al*., 1981). The study also found a prevalence of mange of 0.81% in Thomson's gazelle. This prevalence just like that of cheetah is the first reported prevalence of mange in free-ranging Thomson's gazelles in the literature. Since Thomson's gazelle is the favourite prey of cheetah (Ngoru and Mulama, 2002; Hayward *et al*., 2006) it poses high probability of transmission of mange to cheetah. The study further revealed that, although dogs had the second highest (4.66%) prevalence, the affected dogs were observed only in shopping centres with a low likelihood of transmission to cheetah and other wild animals. Among domestic animals sheep had highest period prevalence of 0.76%. This however was quite low compared to previous studies (Kusiluka and Kambarase, 1996; Biu and

Wakawa 2004) in sheep. Nevertheless, the prevalence is still significant with a possibility of transmission to cheetah and other wildlife species. Sheep share the same range with cheetahs and other wildlife species and can act as source of infection. However, phenotypic characterization studies revealed a different species of mange from that isolated from cheetah but mixed infections cannot be ruled out. The study also found mange infection in wildebeest which is an alternative prey of cheetah (Howard *et al*., 2006). Wildebeest can therefore act as a potential source of mange infection for cheetah.

The study revealed that geographical location (study blocks), climatic season and time of sampling have effect on prevalence of mange. The prevalence was higher in study blocks with high wildlife/livestock interaction, in dry than wet season and in the year 2007/2008 than 2008/2009. However, more studies focusing on the association between the 3 factors and prevalence of mange requires to be undertaken. It is worth noting that the prevalence of mange decreased as sampling continued and by the middle of the year 2008/2009 hardly any positive case could be picked. All captured wild animals and all domestic animals were treated with Ivermectin before they were released. Although this study did not gather enough evidence to conclude that the treatment led to the decrease in prevalence, it is important that the role of therapeutic treatment as a method of control of mange be further investigated.

The study on assessment of level of knowledge of disease dynamics of mange through pre-tested questionnaires revealed that most of the pastoralists and

wildlife officers were aware of the disease. However, a large number of pastoralists and a significant number of wildlife officers were unaware of its etiology, higher numbers have heard about mites but could not connect them to the causative agent of mange. The study showed that most pastoralists were also aware of the disease but not the causative agent as opposed to wildlife officers who were aware of both the disease and causative agent. This could be due to the higher literacy level of wildlife officers and the fact that they are trained to identify wildlife diseases compared to pastoralists. The study also revealed that majority of the respondents believed that there was cross-transmission of the disease between wild and domestic animals, an observation that agrees with Pence and Uckermann, 2002 who reported that various wildlife species are often infected through contact with their domestic counterparts. The respondents also identified sheep, dog, cattle and goat as the domestic animals affected and cheetahs, Thomson's gazelle, wildebeest, lion, vervet monkey and wild dogs as the wild animals affected. Most of the above animals have been reported to be infected elsewhere (Pence and Uckermann, 2002; Kahn *et al*., 2005). The study also revealed that most pastoralists administered certain treatment or control interventions when their animals were affected. The commonly used interventions were spraying with acaricides, reporting to a veterinarian, separating infected animals and administration of antibiotics. Wildlife officers reported cases of affected wild animals to KWS veterinary personnel. The study also revealed that both pastoralists and wildlife officers were aware of other skin diseases. Pastoralists identified fungal infections, sheep and goat pox, papillomatosis and photosensitization as the

commonest, all of which have been identified as differential diagnosis of mange (Kusiluka and Kambarase, 1996; Craig 2009). On the other hand, wildlife officers identified lumpy skin disease, fungal infections and giraffe ear disease as the commonest. Among them, only fungal diseases and specifically dermatomycosis had been previously reported to be a differential diagnosis of mange in wildlife (Frederick, 2001). However, the results of this study are not conclusive enough to determine if the disease they identified was really mange. A PRA approach combined with PDS is required to gather enough data and determine if the disease is mange. It will also help in determining if the two groups can positively identify the other skin diseases they mentioned.

The study identifies the following clinical symptoms in affected animals; alopecia, pruritus, acute dermatitis, suppurative encrustation, skin roughening and poor body condition. The symptoms occurred together or in a combination of several of them. The same symptoms have been reported in affected wild and domestic animals by other authors (Siegmund *et al*., 1973; Fain, 1978; Pence *et al*., 1983; Blood and Radostitis, 1989; Morner, 1992; Ippen, *et al*., 1995; Rossi *et al*., 1995; Yeruham *et al*., 1996; Fernandez *et al*., 1997; Scott *et al.,* 2001; Pence and Uckermann, 2002; Fitzgerald, 2004; Ljunggren *et al*., 2003; Kahn *et al.,* 2005; Ljunggren *et al*., 2006; Williams *et al*., 2008). The study also found that it is difficult to achieve the calculated sample sizes in wild animals due to challenges of capture. It is also important to note that all the cheetahs observed with mange-like skin disease in this study were captured and all were positive for mange on laboratory analysis. This is in contrast to

other animals where the positives ranged from 11% to 80% in captured animals with mange-like skin condition. This observation reveals that cheetahs are highly vulnerable to mange as reported by Weber and Rabinowitz, (1996). *Sarcoptes scabiei* was isolated in all positive animals except sheep where *Psoroptes communis* was isolated. These observations agree with the reports by other authors; Pence and Uckermann, (2002) identified sarcoptic mange as the commonest in wildlife; Mugera, *et al*., 1979, Blood and Radostitis, 1989 and Kusiluka and Kambarase, 1996 reported psoroptic mange as the commonest in sheep.

Genetic characterization of isolated *Sarcoptes* mites revealed genetic diversity within the same host especially in wildebeest and cheetah but very low diversity in Thomson's gazelle and lion. This genetic diversity could be as a result of infection of mites from different geographical locations due to seasonal migration of wildebeests (Estes, 1966; Estes, 1992) and larger home ranges of cheetahs (Ngoru and Mulama, 2002; Creel and Creel, 2002; Broomhall *et al*., 2003; Houser *et al*., 2009). Genetic similarities were observed between mites isolated from different hosts. The mites isolated from cheetah, lion and wildebeest were very similar while those from Thomson's gazelle were different though slightly similar to those of cheetahs. This shows that there is some gene flow between cheetah and wildebeest, cheetah and Thomson's gazelle, cheetah and lion and, lion and wildebeest. The gene flow potential between cheetah and wildebeest, cheetah and Thomson's gazelle and, lion and wildebeest is due to predator-prey relationship which creates a rich

platform of mite transmission hence genetic exchange. Thomson's gazelle and wildebeest are common prey of cheetah (Ngoru and Mulama, 2002; Hayward et al., 2006) while wildebeest is the common prey of lion (Viljeon, 2003; Owen-Smith and Mills, 2008; Fryxell *et al*., 2009). The gene flow between cheetah and lion is due to host-taxon derived effect where mites from genetically closely related species have been found to be similar as described by Rosero *et al*., 2010. The two belong to the same order and family and therefore they can share a carnivore-derived mite population. This is in contrast to wildebeest and Thomson's gazelle that share same order and family but did not show herbivore-derived host-taxon derived effect. Rosero *et al*., (2010) reported lack of interaction between carnivore, herbivore and omnivore hosts, while in a predator/prey system there is real interaction, which could lead to alteration in host-taxon phenomenon up to predator/prey degree of contact.

The study also revealed that although infected sheep were consistently observed in the ecosystem throughout the study period, only *Psoroptes* mites were observed to affect them. There could have been a possibility of a mixed *Psoroptes*/*Sarcoptes* infection where *Sarcoptes* could have been missed due to high concentration of *Psoroptes*. On the other hand, low concentration of *Sarcoptes* mites were isolated from affected dogs indicating that dogs may not be important reservoirs for transmission of mange to cheetahs.

8.2 Conclusions

The following conclusions were made from this study;

- The spatial distribution of mange infected species of animals is related to areas with close interaction between wild and domestic animals and climatic seasonal.
- The prevalence of mange in free-ranging cheetah and Thomson's gazelle was determined to be 12.77% and 0.81% respectively
- There is a lot of information about mange among pastoralists and wildlife officers in Masai Mara ecosystem, which can be used when developing prevention and control strategies of mange but more studies using PRA approach requires to be undertaken.
- *Sarcoptes scabiei* is the commonest cause of mange in wild animals while *P. communis* is the commonest in sheep
- There is genetic diversity of *Sarcoptes* mites isolated from the same host species within Masai Mara ecosystem
- There are genetic similarities and differences of *Sarcoptes* mites isolated from different host species within the Masai Mara ecosystem
- There is a possibility of gene flow between *Sarcoptes* mites of different host species probably due to transmission of mites between genetically related hosts as a result of host-taxon derived effect and predator-prey relationship

7.3 Recommendations

- The importance of therapeutic treatment as a method of control of mange in free-ranging wildlife require to be further investigated
- Since most infections were observed in areas with high wildlife/livestock interaction, control measures targeting mange should be focused more in these areas
- More studies require to be undertaken with a view of further understanding the association between geographical location , climatic season and epidemiology of mange and other parasites in wildlife in Masai Mara ecosystem
- A PRA study is required to gather enough data and determine if the disease the pastoralists and wildlife officers identified as mange through questionnaires is really mange. The study will also help in determining if the two groups can positively identify the other skin diseases they mentioned.
- The lack of herbivore-derived host-taxon effect of mites isolated from wildebeest and Thomson's gazelle needs to be investigated
- The role of sheep and dogs in the epidemiology of mange within the Masai Mara ecosystem needs further investigation

REFERENCES

Alasaad S., Soglia D., Sarasa M., Soriguer R.C., Perez J.M., Granados, J.E., Rasero R., Zhu X.Q and Rossi L. (2008a). Skin-scale genetic structure of *Sarcoptes scabiei* populations from individual hosts: empirical evidence from Iberian Ibex-derived mites. Parasitology Research **104**: 101-105

Alasaad S., Rossi L., Maione S., Sartore S., Soriguer R.C., Pére, J.M., Rasero R., Zhu X.Q., Soglia D., (2008b). HotSHOT Plus ThermalSHOCK, a new and efficient technique for preparation of PCR-quality *Sarcopte*s mite genomic DNA. Parasitology Research **103**: 1455–1457

Arlian L.G., Runyan R.A. and Estes, S.A. (1984). Cross infectivity of *Sarcoptes scabiei*. Journal of American Academy of Dermatology **10**: 979-986.

Arlian L.G., Runyan R.A and Vyszenski-Moher D.L. (1988a). Water balance and nutrient procurement of *Sarcoptes scabiei var canis* (Acari: Sarcoptidae). Journal of Medical Entomology **25**: 64-68.

Arlian L.G., Vyszenski-Moher D.L. and Cordova, D. (1988b). Host specificity of *Sarcoptes scabiei var canis* (Acari: Sarcoptidae) and the role of host odour. Journal of Medical Entomology **25 (1)**: 52-56.

Arlian L.G., Ahmed M., Vyszenski-Moher D.L., Estes S.A. and Achar S. (1988c). Energetic relationship of *Sarcoptes scabiei var. canis* (Acari: Sarcoptidae) with laboratory rabbit. Journal of Medical Entomology **25**: 64-68.

Arlian L.G. and Vyszenski-Moher D.L. (1988). Life cycle of *sarcoptes scabiei* v*ar. canis*. Journal of Parasitology **74**: 427-430.

Arlian L.G. (1989). Biology, host relations, and epidemiology of *Sarcoptes scabiei*. Annual Review of Entomology **34**: 139-161.

Arlian L.G., Vyszenski-Moher D.L. and Pole M.J. (1989). Survival of adults and development stages of *Sarcoptes scabiei var. canis* when off the host.Experimental and Applied Acarology **6**: 181-187

Bates P. (2003). Sarcoptic mange (*Sarcoptes scabiei var vulpes*) in a red fox (*Vulpes vulpes*) in a population in north-west Surrey. Veterinary Record **152(4)**:112-114

Bengis R.G., Kock R.A. and Fisher J. (2002). Infectious animal diseases: the wildlife/livestock interface. Review Science Technology **21**:53-65

Berilli F., D'Amelio S. and Rossi, L. (2002). Ribosomal and mitochondrial DNA sequence variation in *Sarcoptes* mites from different hosts and geographical locations. Parasitology Research **88**: 772-777.

Biu, A.A. and Wakawa M.M. (2004). Chorioptic mange infestation in cattle in Borno state, Nigeria. Pakistan Veterinary Journal **24(3)**: 155-156

Blood D.C and Radostitis O.M. (1989). In: Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 7th Edition. Printed by the University printing House Oxford. Pages 1094-1099.

Bornstein S. (1995). *Sarcoptes scabiei* infections of the domestic dog, red fox and pig. Dissertation PhD, Department of Veterinary Microbiology, section at the Swedish University of Agricultural Sciences and National Veterinary Institute, Uppsala, Sweden. ISBN 91-576-4951-0

Bornstein S., Zakrisson G. and Thebo P. (1995). Clinical picture and Antibody response to experimental *Sarcoptes scabiei var. vulpes* infection in red foxes(*Vulpes vulpes*). Acta Verinaria Scandinavica **36**: 509-519

Bornstein S., Thembo P. and Zakrisson G. (1996). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of canine sarcoptic mange. Veterinary Dermatology **7**: 21-28

Bornstein S. and Wallgren P. (1997). Serodiagnosis of sarcoptic mange in pigs.Veterinary Record **141**:8-12

Bornstein S., Roken B. and Lindberg R. (1997). An experimental infection of a lynx (*Felis lynx*) with *Sarcoptes scabiei vulpes*. 16th International Conference of the World Association for Advancement of Veterinary Parasitology, 10-15 August, Sun City, South Africa, page 11

Bornstein S., Morner T. and Samuel W.M. (2001). *Sarcoptes scabiei* and sarcoptic mange. In: parasitic Disease of wild Animals, 2nd edition (W.M. Samuel, M.S Pybus and A.A Kocan eds). Iowa State University Press Ames, pages 107-119.

Bourn D and Blench R (Eds) (1999). Can Livestock and Wildlife Co-exist? An Interdisciplinary approach. Overseas Development Institute (ODI) and The Environmental Research Group Oxford (ERGO). Publishers, London, UK; Page 58.

Bowcock A.M., Ruiz-Linares A., Tomfohrde J., Minch E., Kidd J. and Cavalli-Sforza L.L (1994). High resolution of human evolutionary trees with polymorphic microsatellites. Nature **368**: 455-457

Bowman D.D. (1999). Georgis' Parasitology for Veterinarians, 7th edition. W.B. Saunders Company Philadelphia, Pennsylvania. Pages 63-66.

Broomhall L.S.S., Mills M.G.L. and du Toit J.T. (2003). Home range and habitat use by cheetahs (*Acinonyx jubatus*) in Kruger National Park. Journal of Ecology **261 (2)**: 119-128

Burney D.A. (1980). The effects of human activities on Cheetah in the Maasai Mara region of Kenya. Msc. Thesis, University of Nairobi, Kenya.

Burgess I. (1994). *Sarcoptic scabiei* and scabies. Advances in Parasitology **33:** 235-292.

Caterino M.S.C. and Sperling F.A.H. (2000). The current state of insect molecular systematics: a thriving tower of Babel. Annual review of Entomology. **45**: 1-54.

Cheetah News (2002). The Cheetah *Acinonynx jubatus* Schreber: (newsletter) Zoological Society of San Diego, Centre for Reproduction of Endangered Species, San Diego Zoo, Box 551, San Diego CA, 92112, USA.

Christophersen J. (1996). Epidemiology of scabies. Parasitology Today. **2**:247-248

Craig M. 2009. Sarcoptic mange (*Sarcoptic acariosis*, scabies) in dogs. Companion Animal, The journal for the Veterinary Surgeon in Practice 14(2) 61-66

Creel S. and Creel M.N. (2002). Home ranges and habitat selection. In: The African wild dog behaviour, ecology and conservation (Creel S. and Creel M.N. eds). Princeton University Press, 41 street, Princeton, New Jersey 08540: pages 36-65.

Cruickshank R.H., Johnson K.P., Smith V.S., Adams R..J., Clayton D.H. and Page R.D.M. (2001). Phylogenetic analysis of partial sequences of elongation factor 1α identifying major groups of lice (Insecta: Phthiraptera). Molecular Phylogenetics and Evolution **19**: 202-215.

Cruickshank R.H. (2002). Molecular markers for the pylogenetics of mites And ticks. Systematic and Applied Acarology **7**: 3-14.

Dagleish M.P., Ali Q., Powell R.K., Butz D and Woodford M.H. (2007). Fatal *Sarcoptes scabiei* of blue sheep (*Pseudois nayaur*) in Pakistan. Journal of Wildlife Diseases **43**: 512-517

Daszak P., Cunningham A.A.and Hyatt A.D. (2000). Emerging infectious diseases of wildlife: Global threats to biodiversity and human health. Science **287**: 443-449

Davis D.P. and Moon R.D. (1990). Dynamics of swine management. A critical review of the literature. Journal of Medical Entomology **27**: 727-737.

Deem S.L., Noss A.J., Cuellar R.L., Villarroel R., Linn M.J and Forrester J. (2002). Sarcoptic mange in free-ranging pampas foxes in the Gran Chaco, Bolivia. Journal of Wildlife Diseases **38(3)**: 625-628.

East R. (1999). African Antelope Database 1999. IUCN, Gland, Switzerland And Cambridge UK.

Estes R.D. (1966). Behaviour and life history of the wildebeest (*Connochaetes taurinus* Burchelli). Nature **212**: 999-1000.

Estes R.D. (1992). Hartebeest, Topi, Blesbok and Wildebeest: Tribe Alcelaphine. In:The Behavioural Guide to African Mammals including Hoofed Mammals, Carnivores, Primates. Richard Despard Estes Eds. University of California Press, Berkeley and Los Angeles, California. Pages 133-167

Ellis S. (2001). Global cheetah conservation action plan workshop briefing book.IUCN/SSC. Conservation breeding specialist group. Apple Valley MN.

Evanno G., Regnaut S., Goudet J., (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**: 2611–2620.

Excoffier L., Laval, G., Schneider, S., (2005). ARLEQUIN ver 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**: 47–50.

Fain A. (1978). Epidemiological problems of scabies. International Journal of Dermatology **17**: 20-30.

Fain A. (1991). Origin, variability and adaptability of *Sarcoptes scabiei*. In: **Dusbabek F, Bukva V**, editors. Modern Acarology 1. The Hague: SPB Academy Publication: 261-265.

Falush D., Stephens M., Pritchard J.K., (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164**: 1567–1587.

Felsenstein J. (1989). PHYLIP - Phylogeny inference package (Version 3.2). Cladistics **5**: 164–166.

Fernandez M.J., Gomez S., Ballesteros F., Quiros P., Benito J.L., Feliu C. And Nieto J.M. (1997). Epizootiology of sarcoptic mange in a population of cantabrian chamois (*Rupicapra pyrenaica parva*) in northwestern Spain. Veterinary Parasitology **73**: 163-171.

Fischer K., Holt C.D., Harumal, P., Currie, B.J., Walton F.S. and Kemp D.J.(2003). Generation and characterization of cDNA clones from *Sarcoptes scabiei* Var. *hominis* for an expressed sequence tag library: identification of homologues of house dust mite allergens. American Journal of Tropical Medicine and Hygiene **68 (1)**: 61-64

Fitzgerald S.D., Cooley,T..M., Muphy A., Cosgrove M..K. and King B.A. (2004). Sarcoptic mange in raccoons in Michigan. Journal of Wildlife Diseases **40**: 347-350.

Frederick A.L. (2001). Dermatomycosis. Bacterial and Mycotic diseases. In: Infectious Diseases of Wild Mammals. (Elizabeth S.W. and Ian B.K. eds). 3rd Edition. Blackwell Publishing 2121 State Avenue, Ames, Iowa 50014. Pages 489-491.

Fryxell J.M., Mosser A., Sinclair A.R.E and Packer C. (2009). Group formation stabilizes predator-prey dynamics. Nature **449**:1041-1043.

Fthenakis G.C., Papadopolous E., Himonas C., Leontides L. and Kritas S.P. (2000). Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Veterinary Parasitology **87**:207-216.

Gittleman JL. And Harvey PH (1982). Carnivore home-range size, metabolic needs and ecology. Behavioural Ecology and Sociobiology **10**:57-63

Glaubitz J., (2004). CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes **4**: 309–310.

Gortazar C., Villafuerte R., Blanco J.C. and Fernandez-de-Luko D. (1998). Enzootic sarcoptin mange in red foxes in Spain. Z Jagdwiss **44**: 251-256.

Goudet J., (1995). FSTAT (vers. 1.2): a computer program to calculate F-statistics. Journal of Heredity **86**: 485–486.

Gregory M.W. (1981). Mites of hedgehogs *Erinaceus albiventris* Wagner in Kenya: Observations on the prevalence and pathogenicity of *Notoedres oudemansi* Fain, *Caparinia erinacei* Fain and *Rodentopus sciuri* Fain. Journal of Parasitology **82**: 149-157.

Gros P. (1998). Status of the cheetahs (*Acinonyx jubatus*) in Kenya. A field interview assessment by Wildlife, Fish and Conservation Biology Department, University of California, One shields Avenue, Davis CA95616. U.S.A. Biological Conservation **85**: 137-149.

Goldstein D.B. and Clark A.G. (1995). Microsattellite variation in North American populations of *Drosophila melanogaster*. Nucleic Acid Research **18 (14)**: 4123-4130.

Gu X.B. and Yang G.Y. (2008). A study on the genetic relationship of mites in the genus Sarcoptes (Acari: Sarcoptidae) in China. International Journal of Acarology **32**: 183-190.

Hayward M.W., Hofmeyer M., O'Brien J.O. and Kerley G.I.H. (2006). Prey preferences of cheetah (*Acinonyx jubatus*) (Felidae: Carnivora): Morphological limitations or need to capture rapidly consumable prey before kleptoparasites arrive. Journal of Zoology **270**: 615-627.

Hill P.B. and Steinberg H. (1993). Difficult dermatological diagnosis. Journal of American Veterinary Medical Association **202**: 873-874

Houser A., Somers M.J. and Boast L.K. (2009). Home range use of free-ranging cheetah on farm and conservation land in Botswana. South African Journal of Wildlife Research **39 (1)**: 11-22.

Hoyet H.M.D. and Manson R.S. (1961). Sarcoptes scabiei in the dingo (*Canis antarcticus*). Australian Veterinary Journal **37**: 53-54.

Huson D.H., Dezulian T., Franz M., Rausch C., Richter D.C. and Rupp R. (2007). Dendroscope - an interactive tree drawer. BMCB **8**: 460.

Ippen R.S., Nickel S. and Schroder H.D. (1995). Krankheiten des Jadgbaren Wildes. Berlin. Deutscher Land-wirtschaftverlag, pp. 189-195.

Jones K.E., Patel N.G., Levy M.A., Storeygard A., Balk D., Gittleman J.L. and Daszak P. (2008). Global trends in emerging infectious diseases. Nature **451**: 990-993

Kahn C.M., Line S., Allen D.G., Anderson D.P., Jeffcoh L.B., Quesenberry K.E., Radostitis O.M. (2005). Acariasis mite infestation. In: The Merck Veterinary Manual. 9th Edition. Published by Merck and Co Inc. Whitehouse Station, New Jersey U.S.A. Pages 742-749.

Kalema G., Koch R.A.and Macfie E. (1998). An outbreak of sarcoptic mange In free-ranging mountain gorillas (*Gorilla gorilla berengei*) in Bwindi Impenetrable National Park, South Western, Uganda. Proceedings AAZV and AAWV Joint Conference, Omaha, Nebraska, USA page 438.

Kathryn W. and Williamson P. (1998). The dog health program in Aboriginal communities – a method for dog management in remote Aboriginal communities.Urban Animal Management Proceedings – Text Copyright @ AVA Ltd Pages 1-11.

Kemp D.J., Walton S.F., Harumal P. and Currie B.J. (2002). The scourge of scabies. Biologist (London England) **49**: 19-24.

Kock RA, Wambua JM, Mwanzia J, Wamwayi H, Ndungu EK, Barett T, Kock ND and Rossiter PB. (1999) Rinderpest epidemic in wild ruminants in Kenya 1993 – 1997. Veterinary Record **145**:275-283.

Kurtdede A., Aktas M.S., Cingi C.C., Ural K. and Kar S. (2007). Sarcoptic Mange in a gazelle (*Gazella gazelle*) in Ankara, Turkey. Journal of Applied Biological Sciences **1 (3**): 111-112.

Kusiluka L. and Kambarase D. (1996). Diseases caused by arthropods and fungi. In: A Handbook. Communicable diseases of Small Ruminants in Sub-saharan Africa. Edited and published by VETAID, Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian EH259RG, Scotland.

Laurenson M.K. (1995a). Implications of high offspring mortality for cheetah population dynamics. In: Serengeti II: Dynamics, management and conservation of an ecosystem (A.R.E. Sinclair and P. Archese Eds.). The University of Chicago Press, Chicago. Pages 385-399.

Laurenson M.K. (1995b). Early maternal behaviour of wild cheetahs. Implications for captive husbandry. In: Zoo Conservation Biology **12**: 31-43.

Lehmann MB., Fuston P.J., Owen C.R. and Slotow R. (2008). Home range utilization and territorial behaviours of lions (Panthera leo) on Korongwe Game reserve, South Africa. PLoS ONE **3(12)**. Doi:10.1371/journal.pone.0003998.

Lindberg M.S. and Walker J. (2007). Satellite Telemetry in Avian Research and Management: Sample size considerations. Wildlife Society **71**: 1002-1009.

Lindstrom E. and Morner T. (1985). The spreading of sarcoptic mange among Swedish red foxes (*Vulpes vulpes L*) in relation to fox population. Review Ecologie (Terre View) **40**: 211-216.

Little S.E., Davidson W.R., Howerth E.W. and Nettles V.F. (1998). Diseases diagnosed in red foxes from Southeastern United States. Journal of Wildlife Diseases **34**: 600-624.

Ljunggren E.L., Bergstrom K., Morrison D.A. and Mattson J.G. (2006). Characterization of a typical antigen from *Sarcoptes scabiei* containing an MADF domain. Parasitology **132**: 1-10.

Ljunggren E.L, Nilson, D. and Mattson J.G. (2003). Expressed sequence tag analysis of *Sarcoptes scabiei*. Parasitology **127**: 193-145.

MaCarthy P.H. (1960). The presence of sarcoptic mange in wild fox (*Vulpes vulpes*) in Central Queensland. Australian Veterinary Journal **36**:359-360.

Malan F.S., Horak I.G., de Vos V. and van Wyk J.A. (1997). Wildlife parasites: Lessons for parasite control in livestock. Veterinary Parasitology **71**: 137- 153.

Margaret W.S. and Rusell L.P. (1978). Mites and ticks (Acarina) of the skin and internal organs. In: Veterinary Clinical Parasitology. 5th edition. Published by IOWA State University Press, IOWA. Pages 146-199.

Mattsson J.G., Ljunggren E.L. and Bergstrom K. (2001). Paramyosin from the parasitic mite *Sarcoptes scabiei*: CDNA cloning and heterologous expression. Parasitology **122**: 55-562.

Michigan Wildlife Disease Manual. (2001-2006). Mange (Sarcoptic and notoedric). Department of Natural Resources. Copyright @ 2001-2006 State of Michigan. File 11A;// Mange htm.

Minch E. (1997). http://hpgl.stanford.edu/projects/microstat/

Morner T., Bornestein S. and Eriksson G. (1988). Successful treatment of wild Arctic Foxes (Alopex lagopus) infested with *Sarcoptes scabiei* var. vulpes. Abstract, 37th WDA Annual Conference, Athens, GA.

Morner T. (1992). Sarcoptic mange in Swedish wildlife. In: Health and Management of free-ranging mammals, Part One (M. Artois, ed). Revue Scientifique et Technique Office International des Epizooties **11 (4)**: 1115-1121.

Mugera G.M., Bwangamoi O. and Wandera J.G. (1979). Diseases caused by Ectoparasites I. In: Disease of cattle in Tropical Africa. Kenya Literature Bureau Nairobi. Pages 296-304.

Mulheisen M. and Knibbe N. (2001). "*Acinonyx jubatus*" (on –line), Animal diversity web. Accessed June 15,2004 at http://animaldiversity . ummz edu/site/account/information/Acinonyx Jubatus. Html.

Mwanzia J.M., Kock R., Wambua J., Kock, N and Jarret, O. (1995). An outbreak of Sarcoptic mange in the free-living cheetah (*Acinonyx jubatus*) in the Mara region of Kenya. In: Proceedings of American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians Joint Conference, Omaha. Pages 105-112.

Najavas M. and Fenton B. (2002). The application of molecular markers in the study of diversity. Acarology **24**: 751-774.

Ngoru B. and Mulama M. (2002). Cheetah (*Acinonyx jubatus*) population status, problem and possible mitigation measures in Maasai Mara National Reserve and adjacent Group Ranches. Cheetah Conservation Project in Mara. A report to Kenya Wildlife Service, Nairobi, Kenya.

OIE (Office International d'Epizooties), 2007. Health Standards. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.10.4.

Ottichilo W.K., Leeuw J.D., Skidmore A.K., Prims H.H.T. and Said M.Y. (2000). Population trends of large non-migratory wild herbivores and livestock in the Masai Mara ecosystem, Kenya , between 1977 and 1997. African Journal of Ecology **38 (3)** 202-216.

Owen-Smith N. and Mills M.G. (2008). Shifting prey selection generates contrasting herbivore dynamics within a large-mammal predator-prey web.Ecology **89 (4)**: 1120-1133.

Peakall R. and Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**: 288–295.

Pence D.B., Castro S.D. and Samuel W.M. (1975). Variation in the chaetotaxy and denticulation of *Sarcoptes scabiei* (Acarina: Sarcoptidae) from wild canids. Acarologia **17**:160-165.

Pence D.B., Custer J.W. and Curley C.J. (1981). Ectoparasites of wild canids From the Gulf Coastal Prairies of Texas and Lousiana. Journal of Medical Entomology **18**: 409-412.

Pence D.B., Windberg, L.A., Pence B.C and Sprowls, R. (1983). The epizootiology and pathology of sarcoptic mange in coyotes, *Canis latrans*, from South Texas. Journal of Parasitology **69**: 1100-1115.

Pence D.B. and Windberg L.A. (1994). Impact of sarcoptic mange epizootic on a coyote population. The Journal of Wildlife Management **58**: 624-63.

Pence D.B. and Uckermann E. (2002). Sarcoptic mange in wildlife. Review of Scientific Technical committee. Office International Epizootics (OIE).

Pritchard J.K., Stephens M., Donnelly P. (2000). Inference of population structure using multilocus genotype data. Genetics **155**: 945–959.

Pfeiffer D.U. (2002). Sampling of Animal Population. In; Veterinary Epidemiology – An Introduction. Epidemiolgy Division, Department of Clinical Sciences, The Royal Veterinary College, University of London. Pages 28-34

Radostitis O.M., Gay C.C., Blood D.C. and Hinchcliff K.W. (1999). Diseases caused by Arthropod Parasites. In: Veterinary Medicine, A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. (Radostitis O.M., Gay C.C., Blood D.C. and Hinchcliff K.W. eds.). 9th Edition. W.B. Saunders, Elsevier Limited. Pages 1412-1415.

Rasero R., Rossi L., Soglia D., Maione S., Sacchi P., Rambozzi L., Sartore S., Soriguer R.C. Spalenza, V. and Alasaad, S. (2010). Host taxon-derived *Sarcoptes* mite in wild animals. Biological Conservation **143:**1269-1277

Raymond M. and Rousset F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenism. Journal of Heredity **86**: 248–249.

Rossi L., Meneguz P., Martin P. and Rodolfi M. (1995). The epizootiology of sarcoptic mange in chamois, *Rupicara rupicara*, from the Italian Eastern Alps. Parasitologia (Roma) **37**: 233-240

Ryser-Degiorgis M., Ryser A., Bacciarini L.N., Angst C., Gottstein B., Janovsky M. and Breitenmoser U. (2002). Notoedric and Sarcoptic mange in free-ranging Lynx in Switzerland. Journal of Wildlife Diseases **38(1)**: 228-232.

Scott D.W. Miller, W.H and Griffin, C.E. (2001). Parasitic disease. In: Muller and Kirk's small animal dermatology. 6th edition. Saunders company Philadelphia, Pennsylvania. Pages 476-484.

Scott D.F., Cooley A..M., Cosgrove, M.K. and King, B.A. (2004). Sarcopric mange in racoons in Michigan. Journal of Wildlife Diseases. **40**: 347-350.

Siegmund O.H., Fraser C.M., Archilbald J., Blood D.C., Handerson J.A., Howell D.G. and Kitchell R.L. (1973). Mange. In: The Merck Veterinary Manual. 4th Edition. Published by Merck and Company Inc., Rahway, New Jersey., U.S.A. Pages 904-912.

Skerratt L.F., Martin, R.W., and Handasyde K.A. (1998). Sarcoptic mange in wombats. Australian Veterinary Journal **76**: 408-410

Soglia D., Rambozzi L., Maione S., Spalenza V., Sartore S., Alasaad S., Sacchi P. and Rossi L. (2009). Two simple techniques for the safe *Sarcoptes* collection and individual mite DNA extraction. Parasitology Research **105**: 1465–1468.

Stuart C. and Stuart T. (2006). T. gazelle and dwarf Antelope. In: Field Guide to the large mammals of Africa. 3rd Edition. Published by Struik Nature, 80 McKenzie Street, Cape Town 8001 pages 164-182.

Tautz D. and Renz M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research. **12 (10)**: 4127-4138.

Tautz D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Research **17**: 6463-6471.

Todd A.W., Gunson J.R. and Samuel W.M. (1981). Sarcoptic mange: An important disease of coyotes and wolves of Alberta, Canada. In: Worldwide Furbearer Conference Proceedings, 3-11 August 1980, Frostburg, MD, Ed. J.A. Chapman D. and Pursley, Pages 706-729.

Trainer D.O. and Hale J.B. (1969). Sarcoptic mange in red foxes and coyotes inWisconsin. Bulletin of Wildlife disease Association **5**: 387-391

Van Neste D.J. (1988). Human scabies in perspective. International Journal of Dermatology **27**: 10-15

Van Orsdol K.G., Handy T.P. and Bygott M. (1985). Ecological correlates of lion social organization (*Panthera leo*). Journal of Zoology London **206**: 97-112.

Viljeon P. (2003). African Lion Working Group: Dedicated to Africa Lion Conservation, African Working Group. <http://www.african-lion.org/lions e.htm>

Walton S.F., Currie B.J., and Kemp, D.J. (1997). A DNA fingerprinting system for the ectoparasite *Sarcoptes scabiei*. Molecular and Biochemical Parasitology **85**: 187-196.

Walton S.F., Choy J.L., Bonson, A., Valle A., McBroom J., Taplin., D., Arlian L., Matthews, J.D., Currie B. and Kemp J.D., (1999). Genetically distinct dog-derived and human –derived *Sarcoptes scabies* in scabies-endemic communities in Northern Australia. American Journal of Tropical Medicine and Hygiene **61**: 542-547.

Walton S.F., Dougall, A., Pizzutto S., Holt D., Arlian L.G. Morgan M., Curie B.J. and Kemp D.J. (2004a). Genetic epidemiology of *Sarcoptes scabiei* (Acari: Sarcoptidae) in northern Australia. International Journal of Parasitology **34**: 839-849

Walton S.F., Holt D.C., Currie B.J. and Kemp D.J. (2004b). Scabies: new Future for a neglected disease. Advances in Parasitology **57**: 309-376

Weber W. and Rabinowitz A. (1996). A global perspective on large carnivore conservation. Conservation Biology **10 (4)**: 1046-1054

Weber J.L. and May P.E. (1989). Abundant class of human DNA polymorphism which can be typed using polymerase chain reaction. American Journal of Human Genetics **44**: 388-388

Williams J.M., Lonsdorf E.V., Wilson M.L., Scumacher-Stankey J., Goodall J. and Pusey A.E. (2008). Causes of death in the Kasekela Chimpanzees of Gombe National Park, Tanzania. American Journal of Primatology **70**: 766-777.

Yeruham I., Rosen S., Hadani A., and Nyska A. (1996). Searcoptic mange in wild ruminants in zoological gardens in Israel. Journal of Wildlife Diseases **32**: 57-61.

Young E. (1975). Some important parasitic and other diseases of lions, *Panthera leo*, in Kruger National Park. Journal of South African Veterinary Association 46:181-183

Zahler M., Essig, A., Gothe, R. and Rinder H. (1999). Molecular analyses suggest monospecificity of the genus Sarcoptes (Acari: Sarcoptidae). International Journal of Parasitology 29, 759-766

Zeh J.B. (1974). Infestation of sarcoptic mange on red fox in New York. New York Fish and Game Journal 21: 182-183

Zumpt F. and Ledger J.A. (1973). Present epidemiological problems of Sarcoptic mange in wild and domestic animals. Journal of the Southern African Wildlife Management Association **3**: 119-120.

APPENDICES

Appendix I: Questionnaire on knowledge of mange (pastoralists)

Study Area ----------

Question 1: Have you ever heard of mange? Yes------- , No…….

Question 2: Do you know what is the etiology? Yes------- , No…….

Question 3: Have you heard about mites? Yes------- , No…….

Question 4: (a) Have your animals been affected by this disease? Yes------- , No…….

(b) If yes which particular ones?…….

Question 5: Are you aware if wild animals are affected by this condition? Yes---------- , No…….

Question 6: Do you think there is cross-infection between domestic and wild animals? Yes------- , No…….

Question 7: (a) Do you institute any control/preventive measures? Yes-------, No……..,

(b) If yes which methods do you use? …….

Question 8: Which other skin diseases do you know that affect domestic and/or wild animals? ……..

Appendix II: Questionnaire on knowledge of mange (wildlife officers)

Study Area ----------

Question 1: Have you ever heard of mange? Yes------- , No…….

Question 2: Do you know what is the etiology? Yes------- , No…….

Question 3: Have you heard about mites? Yes------- , No…….

Question 4: (a) Have you seen any wild animals with the disease? Yes------- , No……..

(b) If yes which animal species in particular ? ……

Question 5: Are you aware if domestic animals are affected by this condition? Yes-------, No…….

Question 6: Do you think there is cross-infection between domestic and wild animals? Yes------- , No…….

Question 7: (a) When you come across the disease in wildlife, do you take any intervention measures? Yes------- , No……..,

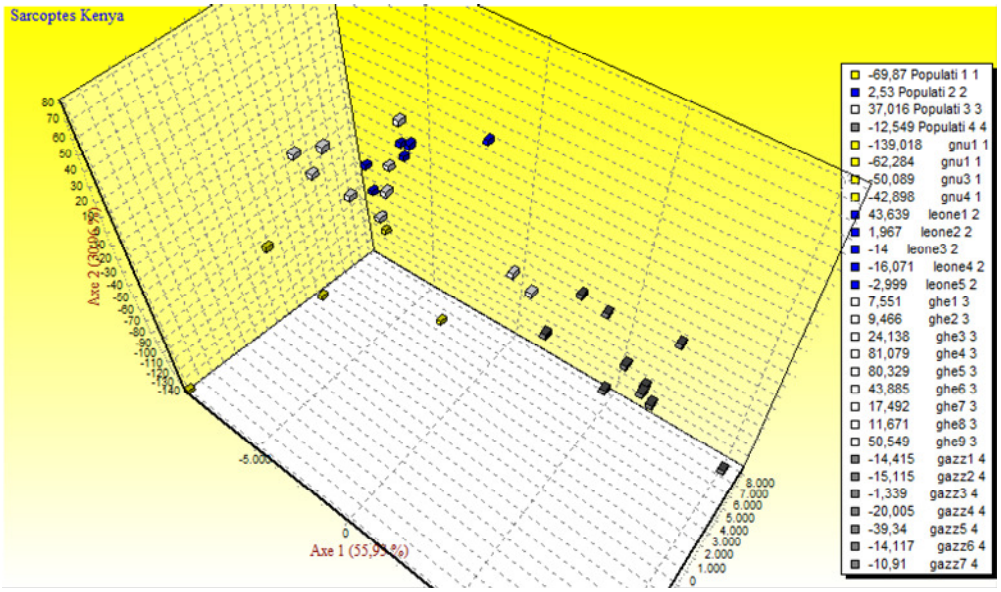
(b) If yes which ones? ……

Question 8: Which other skin diseases do you know that affect domestic and/or wild animals? ……..

Appendix III: Gene flow structures of individual Sacroptes mites from four Sympatric hosts derived mite populations in Masai Mara, Kenya

Genetix Software

Ghe=Cheetah
Leone=Lion
Gazz=Gazelle
Gnu=Wildebeest



Appendix IV: FisTest multi-locus clustering analyses results

05/01/2011 16:03:20
Calcul des paramètres F selon la méthode de Weir & Cockerham 1984
Pour la signification des paramètres consultez l'aide !

FICHIER TRAITE : C:\Users\samer\Documents\Escritorio Samer\Kenia\Analisis\Genetics\test.txt
Nombre de locus : 10

N°	Nom de la population
1 ->	1 4
2 ->	2 5
3 ->	3 9
4 ->	4 9

****
* ESTIMATIONS MULTILOCUS (les details sont en fin de fichier)
*
****

Weir & Cockerham FIS= 0.44541 FIT= 0.57903 FST= 0.24094
Robertson & Hill RH = 0.13499
Corrigé Raufaste RH'= 0.27147

****
Estimation de l'intervalle de confiance à 95% par 1000 BOOTSTRAPS SUR LES LOCUS
FIS (0.31594 - 0.56710)
FIT (0.46180 - 0.68622)
FST (0.15613 - 0.33626)
RH (0.02654 - 0.11496)

****
JACKKNIFE SUR LES LOCUS

Sans loc	1	FIS= 0.39485	FIT= 0.52943	FST= 0.22238
Sans loc	3	FIS= 0.50003	FIT= 0.62924	FST= 0.25844
Sans loc	5	FIS= 0.47816	FIT= 0.57926	FST= 0.19373
Sans loc	9	FIS= 0.44252	FIT= 0.59664	FST= 0.27645
Sans loc	15	FIS= 0.46928	FIT= 0.60644	FST= 0.25844
Sans loc	17	FIS= 0.38448	FIT= 0.52967	FST= 0.23589
Moyenne :		0.44803	0.58197	0.24120
Ecart-type		0.09531	0.08405	0.06105

```
##########################################
Estimation par Jackknife sur les populations
##########################################
=========================================================
========================
1
Sans pop  1     Fis=  0.70325  Fit=  0.77225  Fst=  0.23252 A=   0.1361 B=
0.3160 C=   0.1333 N= 5.0000 NC=4.9333 C2=0.0400

Sans pop  2     Fis=  0.71640  Fit=  0.82358  Fst=  0.37792 A=   0.2856 B=
0.3368 C=   0.1333 N= 5.0000 NC=4.9333 C2=0.0400

Sans pop  3     Fis=  0.76581  Fit=  0.80981  Fst=  0.18787 A=   0.1520 B=
0.5031 C=   0.1538 N= 4.3333 NC=4.3077 C2=0.0178

Sans pop  4     Fis=  0.48705  Fit=  0.71559  Fst=  0.44555 A=   0.3357 B=
0.2035 C=   0.2143 N= 4.6667 NC=4.5714 C2=0.0612

---------------------------------------------------
Moyenne :     FIS=  0.71555 FIT=  0.78429 FST=  0.33354
Ecart-type          0.18553          0.07243          0.18146

=========================================================
========================
3
Sans pop  1     Fis=  0.24771  Fit=  0.39730  Fst=  0.19885 A=   0.1434 B=
0.1432 C=   0.4348 N= 7.6667 NC=7.4348 C2=0.0907

Sans pop  2     Fis=  0.34633  Fit=  0.47199  Fst=  0.19224 A=   0.1560 B=
0.2271 C=   0.4286 N= 7.0000 NC=6.4286 C2=0.2449

Sans pop  3     Fis=  0.12244  Fit=  0.23793  Fst=  0.13161 A=   0.1016 B=
0.0821 C=   0.5882 N= 5.6667 NC=5.1176 C2=0.2907

Sans pop  4     Fis=  0.28773  Fit=  0.38153  Fst=  0.13169 A=   0.0877 B=
0.1663 C=   0.4118 N= 5.6667 NC=5.1176 C2=0.2907

---------------------------------------------------
Moyenne :     FIS=  0.26509 FIT=  0.41671 FST=  0.20012
Ecart-type          0.14224          0.14672          0.05548

=========================================================
========================
5
```

Sans pop 1 Fis= 0.10522 Fit= 0.55654 Fst= 0.50440 A= 0.2967 B= 0.0307 C= 0.2609 N= 7.6667 NC=7.4348 C2=0.0907

Sans pop 2 Fis= 0.10860 Fit= 0.54464 Fst= 0.48916 A= 0.2930 B= 0.0332 C= 0.2727 N= 7.3333 NC=6.9545 C2=0.1550

Sans pop 3 Fis= 0.43396 Fit= 0.83072 Fst= 0.70093 A= 0.4601 B= 0.0852 C= 0.1111 N= 6.0000 NC=5.6111 C2=0.1944

Sans pop 4 Fis= -0.20000 Fit= -0.05026 Fst= 0.12478 A= 0.0264 B= -0.0370 C= 0.2222 N= 6.0000 NC=5.6111 C2=0.1944

Moyenne : FIS= 0.12570 FIT= 0.89955 FST= 0.72599
Ecart-type 0.38832 0.55712 0.36035

==========

9
Sans pop 1 Fis= 0.42274 Fit= 0.47533 Fst= 0.09110 A= 0.0552 B= 0.2330 C= 0.3182 N= 7.3333 NC=7.1364 C2=0.0806

Sans pop 2 Fis= 0.53018 Fit= 0.54118 Fst= 0.02342 A= 0.0146 B= 0.3224 C= 0.2857 N= 7.0000 NC=6.6667 C2=0.1429

Sans pop 3 Fis= 0.66204 Fit= 0.71278 Fst= 0.15012 A= 0.0922 B= 0.3457 C= 0.1765 N= 5.6667 NC=5.4118 C2=0.1349

Sans pop 4 Fis= 0.23003 Fit= 0.21226 Fst= -0.02308 A= -0.0130 B= 0.1328 C= 0.4444 N= 6.0000 NC=5.6111 C2=0.1944

Moyenne : FIS= 0.44308 FIT= 0.50217 FST= 0.06088
Ecart-type 0.27388 0.31167 0.11400

==========

15
Sans pop 1 Fis= 0.25329 Fit= 0.31185 Fst= 0.07843 A= 0.0311 B= 0.0925 C= 0.2727 N= 7.3333 NC=7.1364 C2=0.0806

Sans pop 2 Fis= 0.61351 Fit= 0.64259 Fst= 0.07523 A= 0.0210 B= 0.1587 C= 0.1000 N= 6.6667 NC=6.1500 C2=0.2325

Sans pop 3 Fis= -0.24283 Fit= -0.02053 Fst= 0.17887 A= 0.0548 B= -0.0611 C= 0.3125 N= 5.3333 NC=4.9375 C2=0.2227

Sans pop 4 Fis= 0.31027 Fit= 0.31956 Fst= 0.01346 A= 0.0058 B= 0.1323 C= 0.2941 N= 5.6667 NC=5.1176 C2=0.2907

Moyenne : FIS= 0.36423 FIT= 0.35226 FST= 0.05048
Ecart-type 0.53216 0.40612 0.10268

==========
17
Sans pop 1 Fis= 0.63790 Fit= 0.76135 Fst= 0.34094 A= 0.2485 B= 0.3064 C= 0.1739 N= 7.6667 NC=7.4348 C2=0.0907

Sans pop 2 Fis= 0.89412 Fit= 0.93070 Fst= 0.34554 A= 0.2374 B= 0.4021 C= 0.0476 N= 7.0000 NC=6.4286 C2=0.2449

Sans pop 3 Fis= 0.54545 Fit= 0.73288 Fst= 0.41234 A= 0.2724 B= 0.2118 C= 0.1765 N= 5.6667 NC=5.1176 C2=0.2907

Sans pop 4 Fis= 0.71390 Fit= 0.67353 Fst= -0.14111 A= -0.1017 B= 0.5871 C= 0.2353 N= 5.6667 NC=5.1176 C2=0.2907

Moyenne : FIS= 0.73522 FIT= 0.81164 FST= 0.32930
Ecart-type 0.22181 0.16545 0.38367

****
* DETAIL DU CALCUL LOCUS PAR LOCUS *
****
1(4 populations)

Allele 242 Fis= 1.00000 Fit= 1.00000 Fst= 0.06294 A= 0.0034 B= 0.0500 C= 0.0000 P= 0.0526 S2= 0.0139 H= 0.0000

Allele 248 Fis= -0.02564 Fit= 0.00686 Fst= 0.03168 A= 0.0008 B= -0.0007 C= 0.0263 P= 0.0263 S2= 0.0035 H= 0.0526

Allele 250 Fis= 0.55390 Fit= 0.76789 Fst= 0.47968 A= 0.1088 B= 0.0654 C= 0.0526 P= 0.2632 S2= 0.1270 H= 0.1053

Allele 252 Fis= 0.68301 Fit= 0.81732 Fst= 0.42370 A= 0.1221 B= 0.1134 C= 0.0526 P= 0.5263 S2= 0.1502 H= 0.1053

Allele 284 Fis= 0.67261 Fit= 0.64828 Fst= -0.07431 A= -0.0056 B= 0.0541 C= 0.0263 P= 0.0789 S2= 0.0086 H= 0.0526

Allele 288 Fis= 1.00000 Fit= 1.00000 Fst= -0.01708 A= -0.0009 B= 0.0533 C= 0.0000 P= 0.0526 S2= 0.0103 H= 0.0000

Tous Fis= 0.67998 Fit= 0.78130 Fst= 0.31661 A= 0.2286 B= 0.3355 C= 0.1579 N= 4.7500 NC=4.7018 C2=0.0406
RH = 0.11200
RH'= 0.11200

3(4 populations)

Allele 200 Fis= 0.61553 Fit= 0.67029 Fst= 0.14242 A= 0.0083 B= 0.0308 C= 0.0192 P= 0.0577 S2= 0.0141 H= 0.0385

Allele 204 Fis= -0.11864 Fit= 0.03620 Fst= 0.13842 A= 0.0028 B= -0.0020 C= 0.0192 P= 0.0192 S2= 0.0038 H= 0.0385

Allele 206 Fis= 0.76058 Fit= 0.79416 Fst= 0.14027 A= 0.0131 B= 0.0611 C= 0.0192 P= 0.0962 S2= 0.0233 H= 0.0385

Allele 208 Fis= 0.04027 Fit= 0.21000 Fst= 0.17685 A= 0.0474 B= 0.0089 C= 0.2115 P= 0.4808 S2= 0.0625 H= 0.4231

Allele 210 Fis= 0.31317 Fit= 0.35630 Fst= 0.06280 A= 0.0094 B= 0.0438 C= 0.0962 P= 0.1731 S2= 0.0230 H= 0.1923

Allele 212 Fis= 0.13537 Fit= 0.39743 Fst= 0.30308 A= 0.0484 B= 0.0151 C= 0.0962 P= 0.1731 S2= 0.0555 H= 0.1923

Tous Fis= 0.25456 Fit= 0.38332 Fst= 0.17273 A= 0.1293 B= 0.1576 C= 0.4615 N= 6.5000 NC=6.1538 C2=0.2130
RH = 0.15823
RH'= 0.62995

5(4 populations)

Allele 134 Fis= 0.11538 Fit= 0.57769 Fst= 0.52261 A= 0.1375 B= 0.0145 C= 0.1111 P= 0.6667 S2= 0.1427 H= 0.2222

Allele 136 Fis= 0.11538 Fit= 0.57769 Fst= 0.52261 A= 0.1375 B= 0.0145 C= 0.1111 P= 0.3333 S2= 0.1427 H= 0.2222

Tous Fis= 0.11538 Fit= 0.57769 Fst= 0.52261 A= 0.2750 B= 0.0290 C= 0.2222 N= 6.7500 NC=6.4938 C2=0.1518
RH = 0.52261
RH'= 0.52261

9(4 populations)

Allele 172 Fis= 0.54926 Fit= 0.54964 Fst= 0.00086 A= 0.0002 B= 0.1406 C= 0.1154 P= 0.5385 S2= 0.0307 H= 0.2308

Allele 178 Fis= 0.26298 Fit= 0.26527 Fst= 0.00310 A= 0.0003 B= 0.0274 C= 0.0769 P= 0.1154 S2= 0.0105 H= 0.1538

Allele 180 Fis= 0.43985 Fit= 0.52305 Fst= 0.14854 A= 0.0359 B= 0.0906 C= 0.1154 P= 0.3462 S2= 0.0575 H= 0.2308

Tous Fis= 0.45671 Fit= 0.48958 Fst= 0.06051 A= 0.0365 B= 0.2587 C= 0.3077 N= 6.5000 NC=6.2821 C2=0.1341
RH = 0.05013
RH'= 0.05290

15(4 populations)

Allele 240 Fis= -0.16667 Fit= 0.02086 Fst= 0.16073 A= 0.0066 B= -0.0057 C= 0.0400 P= 0.0400 S2= 0.0085 H= 0.0800

Allele 246 Fis= 0.02041 Fit= -0.00725 Fst= -0.02823 A= -0.0006 B= 0.0004 C= 0.0200 P= 0.0200 S2= 0.0011 H= 0.0400

Allele 248 Fis= 0.22085 Fit= 0.28432 Fst= 0.08145 A= 0.0137 B= 0.0340 C= 0.1200 P= 0.8000 S2= 0.0280 H= 0.2400

Allele 250 Fis= 0.49307 Fit= 0.52446 Fst= 0.06192 A= 0.0078 B= 0.0584 C= 0.0600 P= 0.1400 S2= 0.0216 H= 0.1200

Tous Fis= 0.26623 Fit= 0.32309 Fst= 0.07749 A= 0.0275 B= 0.0871 C= 0.2400 N= 6.2500 NC=5.9467 C2=0.1941
RH = 0.06539
RH'= 0.14171

17(4 populations)

Allele 272 Fis= 0.89153 Fit= 0.90421 Fst= 0.11685 A= 0.0235 B= 0.1581 C= 0.0192 P= 0.2500 S2= 0.0480 H= 0.0385

Allele 274 Fis= 0.59794 Fit= 0.80717 Fst= 0.52038 A= 0.1557 B= 0.0858 C= 0.0577 P= 0.5192 S2= 0.1650 H= 0.1154

Allele 278 Fis= 0.83519 Fit= 0.84265 Fst= 0.04524 A= 0.0055 B= 0.0975 C= 0.0192 P= 0.1346 S2= 0.0217 H= 0.0385

Allele 280 Fis= 0.34394 Fit= 0.35675 Fst= 0.01953 A= 0.0018 B= 0.0302 C= 0.0577 P= 0.0962 S2= 0.0107 H= 0.1154

Tous Fis= 0.70719 Fit= 0.78387 Fst= 0.26190 A= 0.1864 B= 0.3716 C= 0.1538 N= 6.5000 NC=6.1538 C2=0.2130
RH = 0.13154
RH'= 0.13154

Appendix V: NeiTest Results Genetic Analysis

05/01/2011 15:58:55
Calcul des distances de Nei 1972
Fichier traité : C:\Users\samer\Documents\Escritorio Samer\Kenia\Analisis\Genetics\test.txt
Nombre de populations : 4
Nombre de locus : 10

	1	2	3	4
1 (4)	0.000	0.072	0.162	0.251
2 (5)	0.072	0.000	0.087	0.270
3 (9)	0.162	0.087	0.000	0.163
4 (9)	0.251	0.270	0.163	0.000

Appendix VI: Variability test results for allelic polymorphism

05/01/2011 16:07:24
Fichier traité : C:\Users\samer\Documents\Escritorio Samer\Kenia\Analisis\Genetics\test.txt
Nombre de pop. : 4
Nombre de loc. : 10

H exp. = H calculée avec biais
H n.b. = H calculée sans biais (Nei 1978)
H obs. = H obsérvée
P(0.95) = Polymorphisme au seuil 95%
P(0.99) = Polymorphisme au seuil 99%
Seuil 95%, 99% => que le locus est considéré comme polymorphe
si l'allèle le plus fréquent ne dépasse pas 95% (respectivement 99%)
Fis W&C = Fis selon Weir & Cockerham(1984)
R&H = Fis selon Robertson & Hill(1984)

N°	Nom de la population
1	1
2	2
3	3
4	4

FREQUENCES ALLELIQUES POUR CHAQUE POPULATION

LOCUS	POPULATION			
	1	2	3	4
1				
(N)	4	4	6	5
242	0.2500	0.0000	0.0000	0.0000
248	0.1250	0.0000	0.0000	0.0000
250	0.6250	0.6250	0.0000	0.0000