GROOMING AND ITS EFFECT ON THE PREVALENCE OF TICK BORNE DISEASES: A CASE STUDY OF WILD YELLOW BABOONS (*Papio cynocephalus cynocephalus*)

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DECLARATION

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

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DEDICATION

To my mother and brother, thank you for your love and support and to the late Professor E.O. Wango for introducing me to the world of research.

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ABSTRACT

Behavioral studies on grooming in nonhuman primates have been carried out by several primatologists over the years. Nonhuman primates spend a lot of time grooming for various reasons which include health and social functions. These nonhuman primates are exposed to ectoparasites in the wild which include ticks that act as vectors in the transmission of several diseases including haemoparasitic infections in animals. This study aimed at determining whether there is a relationship between the frequency of grooming received by a baboon and its ectoparasite load. It also aimed to find out whether reduced ectoparasite load results in lower prevalence of haemoparasitic infections transmitted by ticks. The study focused on a population of wild yellow baboons (*papio cynopcephalus cynocephalus),* and examined the relationship, in each study subject, between grooming behavior, tick load, and haemoprotozoan infection status. The methodology included ad libitum and focal sampling methods of measuring grooming behavior; it also included darting and physical examination of animals for disease indicators, haematological laboratory examination of samples such as blood smears, packed cell volume (PCV) determination, and molecular diagnosis of haemoparasites using polymerase chain reaction (PCR). Data management and analysis was carried out using STATA 10 statistical software. The results showed that the frequency of grooming received was influenced by the age, rank, sex and the social group to which an individual animal belonged. The ectoparasite load was influenced mainly by the age of the animal such that older animals were more likely to have a higher tick load compared to the younger ones ($p = 0.025$, Odds Ratio = 1.118, $n = 59$), and to a lesser extent it was also influenced by the frequency of grooming received ($p = 0.083$, Odds Ratio = 0.968, $n = 59$) and dominance rank ($p = 0.056$, Odds Ratio = 0.870, $n = 59$). The results also showed a low prevalence of Babesia species in this population of animals. The physical examination did not reveal any signs of acute infection by this parasite. Other physiological

indices of the presence of infection such as packed cell volume were affected by the ectoparasite load, age and sex. The data strongly suggested a relationship between grooming and tick load, such that animals that were groomed more had fewer ticks. The effect was significant in a nonparametric bivariate test of total tick count versus grooming ($p = 0.0036$), and showed a strong trend in a multivariate analysis using a categorical variable (ticks present vs ticks absent; $p = 0.086$). However, the results did not reveal any significant relationship between haemoprotozoan infections verses grooming.

Key words: grooming, ticks, haemoparasitic infections, baboons.

1.0 INTRODUCTION

Nonhuman primates live in social groups and exhibit a range of behaviors such as aggression, consorting, mating, grooming, playing and feeding. These social behaviors contribute to either profitable or adverse effects to animals. Primatologists have done a lot of studies on grooming among nonhuman primates over the years. This work has focused mainly on the social functions of grooming. For instance, (Meller et al. 1980; Boccia et al. 1989 and Aureli et al. 1999) correlated the effect of grooming and psychological well being of Macaca species in semi-captivity. Few studies have been carried out on the health benefits of grooming such as the reduction of cortisol, a stress hormone, in primates giving grooming (Shutt et al. 2007), and production of endorphins also providing for the wellbeing of the animals (Keverne et al. 1989).

Nonhuman primates in the wild are exposed to various pathogens such as ectoparasites and endoparasites which may cause adverse effects to them. Behavior such as grooming is assumed to play an important role in reducing the ectoparasites on the animals and thus further reducing the indirect haemoparasitic infections transmitted by the same. However, comprehensive data demonstrating an effect of grooming on health related to ectoparasites and on indirect infections by haemoparasites infecting baboons is lacking.

This study examined the relationship between grooming behavior and the prevalence of ticks and of tick borne infections, in baboons. This is of interest as some of these haemoparasitic infections such as Babesiosis are emerging zoonotic diseases. Worldwide, very little is known about the prevalence of *Babesia* in malaria endemic countries where misidentification as Plasmodium occurs, (Minnesota department of Health October 2007). It is also important to note that in some areas like our study location (Amboseli), wild animals, domestic animals and humans live together and share an environment and thus the possibility of emerging zoonoses should be identified, investigated and used to develop control strategies in the study location. In

addition to this the increased use of baboon xenografts in humans makes identification of these haemoparasitic infections prior to transplantation important to avoid possible transmission of zoonotic agents (Brondson et al. 1999). Nonhuman primates are also commonly used as laboratory animal models in biomedical research for various tropical and infectious diseases such as schistosomiasis, leishmaniasis, malaria and trypanosomiasis (Institute of Primate Research, Biennial report, 2008) and it is therefore important to have a basic understanding of disease and disease transmission in these organisms (especially when using wild-caught animals).

2.0 LITERATURE REVIEW

2.1 GROOMING

2.1.1 Definition

Grooming is any activity that encompasses all forms of care and attention to the body surfaces (Saunders 1988). It constitutes a major social activity in nonhuman primates and has been studied extensively by field primatologists over the years. Many nonhuman primates invest at least one fifth of their social time to grooming (Dunbar 1991; Shutt et al. 2007). Most of the grooming is done with the hands, though teeth and lips can also be used to perform this behavior. Grooming solicitation behavior in primates varies with species (Saunders 1988). Several hypotheses have been brought forward on the functions of grooming but none have been exhaustively tested (see section 2.1.3).

2.1.2 Types of grooming

Grooming can be directed to another individual (allogrooming), or to the groomers own body (self grooming.) Self grooming, also known as autogrooming is mainly directed to the legs and lower arms and to a lesser extent directed to the head and back areas (Saunders 1988; Lazaro-Perea et al. 2004). Allogrooming has been described for a number of mammalian species and is directed to the areas of the body which the animal cannot reach by itself. In contrast to self grooming, little allogrooming is directed to the legs, genitals or tail and most is directed to the head and back areas (Saunders 1988; Singh 2006). Compared to self grooming, allogrooming yields a higher quality of grooming and is consequently more beneficial to groomee. Where the removal of ectoparasites and cleaning the pelage is concerned, one can speculate that allogrooming and self grooming tend to complement each other in terms of the areas/sites preferred. This ensures that all parts of the body receive grooming.

2.1.3 Functions of grooming

Grooming may serve a health function, and it may serve a social function. These functions may have both short term and long term benefits.

a) Social functions of grooming

Grooming has been used as the quantitative measure of the strength of dyadic social relationships (Lazaro-Perea et al. 2004). The social functions of grooming include the establishment and maintenance of affiliative relationship and the reduction of tension and aggression between individuals (Saunders 1988; Terry 1970). Grooming and maintaining proximity represent the major components of female baboon social time and are widely considered to provide meaningful measures of social relationships in nonhuman primates (Cords et al. 1997; Silk et al. 2003). Studies carried out in yellow baboons suggest that sociality, specifically social integration, plays a role in the reproductive success of females as females who spend time grooming and being groomed and in proximity to others are more likely to successfully rear their infants (Silk et al. 2003). The mechanisms that underlie the relationship between sociality and infant survival are not clear. In general, sociality may have beneficial effects on health and the well being of females and this could contribute to their ability to rear their infants successfully. Most of the studies that have been carried out have emphasized social functions of grooming as opposed to its health functions.

b) Health functions of grooming

The health functions of grooming involve cleaning of wounds and cleaning the pelage to rid the body of ectoparasites such as ticks and fleas (Saunders. 1988), which maintains the integrity of the skin. Grooming also provides direct benefits to the groomee by increasing its psychological and physiological well-being. Increase in the release of β-endorphins, decreased

heart rate and the eliciting of a hedonistic (gratifying) effect have been reported (Meller et al. 1980; Keverne et al. 1989; Boccia et al. 1989; Aureli et al. 1999). Grooming also reduces the levels of stress. This has been demonstrated in a study done by Shutt et al. (2007) in Barbary macaques where they found that levels of the stress hormone cortisol were decreased in groomers compared to groomees.

2.1.4 Cost of grooming

Grooming others is assumed to be costly because time spent grooming could be invested in other activities, such as foraging and vigilance or self grooming. These costs may be manifested as nutritional deficits or increased stress, both of which may increase an individual's vulnerability to disease (Shutt et al. 2007; Saunders 1988). In addition, close proximity of animals during grooming could increase the chance of transmission of ectoparasites and infectious disease such as haemoparasitic infections due to the mechanical transfer of parasites such as midges, flies, lice and ticks from one animal to the other.

2.1.5 Grooming quality and quantity

Grooming quality involves the way that an animal grooms or how well they groom and depends on how well the groomer plays his/her role. Grooming quantity describes the frequency or duration of grooming (Saunders 1988). Table 2.1.1 shows the criteria for determining a groomer's concentration score, which is a quality of measure of grooming developed by Saunders (1988).

ABLE 2.1.1 Criteria for determining a groomer's concentration score (Adapted from Carol Saunders (1988)).

SIGNS OF POOR CONCENTRATION	SIGNS OF GOOD CONCENTRATION
1. Partial attention on the area being groomed: a) Many (>1 per minute) self induced glances or b) Any occurrence of closed eyes	1. Full attention on the area being groomed: a) Few (< 1 per minute) self induced glances ….usually associated with…. b) Close distance between groomer's muzzle and groomee's body
2. Many (>1 per min.) or long (>1 min .) self induced pauses	2. Few (<1 per min.) self induced pauses
3. Hand movements: a) Slow or no hand movements …or … b) One handed grooming	3. Hand movements: a) Continuous sequences of hand mouth movement …and… b) Two handed grooming
4. Body parts: a) Same areas groomed repeatedly (>2 times per bout)	4. Body parts: a) systematic sequence of areas groomed with few (< 2 repeats per bout

Concentration score	Decision Rule
1	if there are no definite good signs
2	if there are more poor signs than good signs
3	if there are roughly equal no. of good and poor signs
4	if there are more good signs than poor signs
5	if there are no definite poor signs

High concentration scores are associated with high quality grooming which could have a hygienic function. Hygienic grooming appears to be more time consuming and may have more impact with respect to fitness benefits. The higher the quality of grooming the higher the probability that ectoparasites and debris will be removed from the skin. High quality grooming may also serve as an important social role.

Lower concentration scores are associated with low quality of grooming and may not improve hygiene. However, low quality grooming plays a role in certain social functions of grooming such as that which occurs when males solicit for mating from the females during estrus or when infants groom their mothers to gain access to milk (Saunders 1988; Altmann 1980).

2.1.6 Factors affecting grooming

a) Dominance rank

Individual dominance rank is important in determining grooming relationships. Studies show that females form bonds with other females of the same rank. Thus females of adjacent ranks groom each other (Silk et al. 2006; Seyfarth 1977; Saunders 1988). Previous studies have shown that subordinate primates will direct grooming towards dominants to receive coalitionary support i.e. grooming is directed up the hierarchy (Seyfarth 1977; Lazaro-Perea et al. 2004). In baboons, high ranking animals are more preferred as grooming partners compared to low ranking animals. However, in other primates, grooming is directed down the hierarchy (O' Brien 1993; Di Bitetti 1997; Parr et al. 1997; Lazaro-Perea et al. 2004). Studies in several species have demonstrated that grooming of subordinates by dominants reduces tension by reducing potential aggression (Lazaro-Perea et al. 2004). Studies done on the yellow baboons in Amboseli have shown that low ranking females provide better quality grooming as compared to high ranking females, and adult males receive better quality grooming than females (Saunders 1988).

b) Age and sex

Participation in grooming bouts varies with both age and sex. Baboons are classified by age into infants, juveniles, subadults and adults. Several studies have shown that nonhuman primate females form strong alliances with one another. Grooming amongst other factors has been known to aid in the formation of these social bonds (Kervene 1989). Formation of social bonds amongst females has been hypothesized to have several advantages such as enhancing the probability of receiving coalitionary support during within group contests and provision of a benign environment for raising and socializing of offspring (Silk 2003). New mothers are more frequently groomed than females of other reproductive statuses, but the majority of this grooming received is of poor quality (Saunders 1988). Studies by Saunders (1988) showed that adult females initiated more than half of the grooming bouts with juvenile males, related juvenile females, and infants and received more than half the initiations from adult, subadult males and unrelated female juveniles. Infants mainly groom their mothers when soliciting for breast milk during weaning; thus, this grooming is not high quality (Saunders 1988). Adult females groom adult males for longer duration in exchange for protection during aggression. This may also come with reproductive benefits such as conception and protection against infanticidal attacks (Smuts 1985; Saunders 1988; Silk et al. 2003). However, formation of social bonds amongst males appears constrained and the bonds formed are temporary (Saunders 1988).

c) Kinship

Kin tend to groom each other and grooming is frequently directed to the closest relatives of the groomer. Silk et al. (2006) demonstrated that females form strong bonds with close maternal and paternal kin. The development of social bonds is mediated to some extent by grooming. Adult female baboons tended to choose groomers from the closest relatedness class

available and focused their grooming efforts on either one or several members within that class (Saunders 1988).

d) Reproductive state

Changes in reproductive state also influence the grooming behavior of females. Studies by Lindburg (1973) and Rowell (1964) showed increased amount of grooming received by an adult female following the birth of an infant. Other female baboons groomed the new mother so as to get a closer proximity to the infant. In these cases, grooming quality is expected to be low (Saunders 1988). In some nonhuman primate species such as capuchin monkeys, estrous females engage in more grooming bouts with adult males than with non estrous females (Di Bitteti 1997).

e) Ectoparasite host relationship

Ectoparasite loads and especially ticks on the host do not impose a significant constraint in baboon grooming behavior as showed by studies carried out by Saunders (1988). Field tick densities are normally high following a period of rainfall thus there is a corresponding increase in the number of ticks on the baboons. However, the overall grooming patterns of the baboon groups are not altered by the tick densities probably due to seasonal differences in activity budgets, with grooming allocated less time than feeding after rains (when food is plentiful).

2.2 TICK BORNE DISEASES

2.2.1 Ectoparasites of baboons in Amboseli

a) Definition of ectoparasites

A parasite is an organism that cannot live independently and depends on the host for nourishment. Ectoparasites are parasites that live on or in the skin but not within the body of an animal. They are thus semi- dependent organisms because they live on the surface of their hosts but possess the ability to live free from their hosts or move from one host to another (Saunders 1988). These parasites normally have particular predilection/preferred sites where they attach to the host's body and remain attached for a significant amount of time. The time the parasite remains attached depends on several factors such as grooming, hair type, temperature, and acquired resistance (Marshall 1981). Ectoparasites found in baboons include mites, ticks, midges, and fleas.

b) Detrimental effects of baboon ectoparasites

Ectoparasites may cause significant harm to the host either directly or indirectly. The direct effects include irritation to the baboon, and sucking of blood thus predisposing the baboon to anemia depending of the severity of the infestation. Indirect harm constitutes the transmission of other haemoparasites. They act as vectors of several diseases to animals (see next section).

2.2.2 Ticks and tick borne diseases

a) Definition and classification of ticks.

Ticks are small arachnids in the order Acarina. They are obligate ectoparasites in that they need to ingest a blood meal to transform to their next stage of development. They feed on the blood of mammals, birds and sometimes reptiles and amphibians. They are mainly found in

tall grass and shrubs where they wait to attach to hosts (Swanson 2006; Iqbal et al. 2006; Saunders 1988; ILRI handbook 2004). There are two major families of ticks, the *Ixodidae* or hard ticks, which have thick outer shells made of chitin, and *Argasidae* or soft ticks, which have a membranous outer surface. Soft ticks live in crevices and emerge briefly to feed, while hard ticks will attach themselves to the skin of a host for long periods of time. Approximately 865 species of ticks exist worldwide of which approximately 650 species are classified in the family *Ixodidae* (Swanson 2006). The common genera of hard ticks (*Ioxididae*) include *Amblyomma, Anocentor, Boophilus, Dermacentor, Ixodes, Haemaphysalis, Hyalomma and Rhipicephalus* where as those of soft ticks include *Ornithodorinae* and *Argasine*. Over 60 different species are found in East Africa (ILRI handbook 2004). The tick family of greatest veterinary and medical importance is *Ixodidae*.

b) Tick species found in Amboseli

A study done by Carol Saunders (1988) showed that the Amboseli baboon population had several species of ticks (Table 2.2.1). Most of the ticks found in the baboons in Amboseli are also found in the domestic animals in Amboseli, e.g., cattle, sheep and goats, and also in the wild ungulates, e.g. wildebeests, zebras, gazelle.

TABLE 2.2.1

Tick species found in baboons in Kenya (Compiled from Myers and Kuntz 1965 and Saunders 1988 with modifications)

TICK	LIFE CYCLE
Amblyomma cohaerens	3 host
A. varigetum	3 host
Hyalomma truncatum	3 host
Ixodes cavipalus	3 host
I. rasus	3host
Rhipicephalus appendicultus	3 host
R. evertsi	2 host
R. pulchellus	3 host
R. pravus	3 host
R. sanguineus sanguineus	3 host
R. simus simus	3 host

c) Distribution and Life Cycle

The life cycle of ticks depends on their host type. *Ixodidae* ticks can be classified as one, two or three host ticks. One host ticks remain on the same host from the time they attach themselves to the host until the tick's death. They thus undergo all the stages of development on the same host (i.e. from larvae to adult). The one host ticks include *Boophilus* species. In the two

host ticks, larvae attach to the host, feed, and then moult into nymphs. The nymphs drop to the ground and moult into adults. The adults find a new host (ILRI handbook 2004). The two host ticks include some species of *Rhipicephalus.* The three host ticks drop off and reattach to a new host during each life stage until the adult females lay their batch of eggs. The three-host ticks include *Hyalomma* and *Rhipicephalus* species.

The general life cycle of a typical *Ixodidae* tick starts from a fully adult female who drops off her host after a few days and lays a single large batch of several thousand eggs on the ground, at the bottom of a tuft of grass and other vegetation. The female adult then dies. After a few weeks or months, depending on the species of tick, the temperature and the humidity of the environment, six legged larvae (pepper ticks) hatch out of the eggs. The larvae climb up the grass stems or other plants and may congregate in small clusters to wait for a host to attach on. A period of quiescence accompanied by structural changes on the skin of each larvae takes place after feeding. The larvae then feed and moult to nymphs, which in turn feed and moult into adults. The adults mate while on the host and the females drop off to start the cycle all over again. The males remain on the host for months before dying (ILRI handbook 2004). Under unsuitable conditions ticks can live for long periods without feeding at all.

d) Economic Consequences of Ticks

Ticks are of medical and economic importance because of their ability to transmit disease to both humans and animals (Iqbal et al. 2006). They are excellent vectors for disease transmission second only to mosquitoes as vectors of human disease, both infectious and toxic (Edlow 1999). Several factors make ticks potential vectors of disease; these include, their resistance to environmental stress, freedom from predators, accessibility to a wide range of hosts, fairly long life cycles and a high reproductive potential (ILRI handbook 2004). The direct

detrimental effects to the host include inflammation from the bites and itches, allergic reactions to protein in tick saliva, secondary anemia leading to death, paralysis and myiasis (infestation of tissue with fly larvae due to mechanical transmission of flies by ticks). A remarkable array of pathogens such as bacteria, protozoa, viruses, nematodes, and toxins can be transmitted by ticks and can cause disease. Infection by these pathogens leads to presentation of the various tick borne diseases. These diseases have been studied and documented in wild animals, domestic animals and man. They include major diseases such as Babesiosis which is seen in animals and man, and Anaplasmosis, Heart-water and East Coast Fever (Theileriosis) which affect domestic animals. However, most of the reservoirs of the pathogens responsible for these diseases are wild animals (ILRI handbook 2004; Iqbal et al. 2006; Saunders 1988).

The common tick species of baboons are the *Rhipicephalus* ticks. These include *Rhipicephalus simus, Rhipicephalus pulchellus* and *Rhipicephalus getrudae*. Little is known about tickborne diseases transmitted to baboons by these tick species. *R.simus* has been known to have effects in humans. It transmits *Coxiella brunette* which causes Q fever in man, causes tick paralysis because of the neurotoxin it produces, and also transmits a rickettsia organism causing tick typhus (Saunders 1988). In baboons tick infestations, skin lesions and infections are rare. However, when the infestations occur they may be fatal. A study carried out by Brain and Borhmann (1992) demonstrated that tick infestation is a contributing factor to baboon infant mortality in the *Papio ursinus* baboons found in the Namib Desert.

e) Tick borne haemoparasitic infections in baboons

Tick borne diseases generally affect the blood or the lymphatic system (Iqbal et al. 2006). *Ixodidae* ticks transmit both haemoparasitic and lymphatic infections. The ticks become infested with the causative organisms of these diseases while they are feeding on infected animals. These parasites include *Babesia* species which are some of the most wide spread blood parasites in the

world. They are intraerythrocytic parasites, and are commonly referred to as piroplasms due to their pear-shaped forms found within infected red blood cells. Babesiosis has been recorded in a wide variety of mammalian hosts including man, where it is gaining increasing interest as an emerging zoonosis. The species of greatest interest is *Babesia microti* (Homer et al. 2000). The major tickborne disease recorded in nonhuman primates is enzootic (constantly present in an animal community but in a small number of cases) Babesiosis. This disease is characterized by fatal haemolytic crisis in immune-compromised baboons (Brondson et al. 1999). Babesial parasites require both a competent vertebrate (mammal e.g. man, domestic or wild animals) and invertebrate (tick) host to maintain transmission cycles. The life cycle of *Babesia* occurs in three stages; the first stage includes the formation and fusion of gametes inside the tick gut, the second involves asexual reproduction in the salivary glands of the ticks and the last stage involves asexual reproduction in the vertebral host. As ticks feed on the vertebral host, the sporozoites formed in the second stage are inoculated in the host where they multiply and may cause disease.

Entopolypoides macaci is a *Babesia*-related haemoparasite that has been described in a wide variety of nonhuman primates (including rhesus macaques, chimpanzees, vervets and baboons) (Jeneby et al. 2008) and rarely in humans. However these parasites do not appear to affect the health of baboons although there may be substantial alteration in haemoglobin and red blood cell counts (Cogswell 2000). It has a similar life cycle as *Babesia* species. Ehrlichiosis, which is also transmitted by ticks, has been seen in some nonhuman primates. Yabsley et al. (2004) carried out studies in lemurs which demonstrated molecular and serologic evidence of tick borne *Ehrlichia* but no clinical signs of disease. Studies done in hamsters and white foot mice have shown that the length of time that a tick is attached to the vertebrate host directly affects the efficacy of sporozoite transmission from the tick to the host. This means that the longer the tick is attached the higher the probability of sporozoite transmission (Homer et al.

2000). Grooming in nonhuman primates may thus reduce the time the tick is attached to the baboon thus reducing transmission. However, this is a hypothesis that has not yet been tested.

3.0 HYPOTHESIS

This study examined the relationships between grooming and ectoparasite load, etoparasite load and prevalence of tick borne disease, and grooming and prevalence of tick borne diseases in yellow baboons. This study took advantage of three different data sets to examine the relationship between grooming, tick loads, and tick-borne disease in wild baboons of the Amboseli basin in southern Kenya. One data set included behavioral data on grooming, mined from the long-term database (Babase) of the Amboseli Baboon Research Project. The second data set included tick species and tick counts from baboons darted in Amboseli. The third data set focused on haemoparasite screening data which included physical examination of darted baboons, blood smear screening, haematocrit analysis and PCR screening data. These three data sets were used to test the hypothesis.

3.1 NULL HYPOTHESIS

There is no relationship between the frequency of grooming received, the ectoparasite load and the prevalence of tick borne diseases in wild yellow baboons (*Papio cynocephalus cynocephalus).*

3.2 ALTERNATE HYPOTHESIS

There is a relationship between the frequency of grooming received, the ectoparasite load and the prevalence of tick borne diseases in wild yellow baboon *(Papio cynocephalus cynocephalus).*

4.0 OBJECTIVES

1. To determine the relationship between the frequency of grooming received in wild yellow baboons and ectoparasite (tick) load.
2. To determine the relationship between tick load on wild yellow baboons and haemoparasitic infection.
3. To determine the presence of haemoparasitic infection in wild yellow baboons and its relationship with grooming received.

5.0 MATERIALS AND METHODS

5.1 STUDY AREA AND SITES

5.1.1 Amboseli

The baboons were from the Amboseli region of East Africa immediately north and west of Mount Kilimanjaro. The Amboseli Baboon Research Project (ABRP) team has been involved in monitoring the Amboseli baboon population and its ecology since 1971. The baboons found in this area are yellow baboons (*Papio cynocephalus*) that experience some admixture with neighboring populations of Anubis baboons *(Papio anubis*) (Alberts & Altmann 2001; Tung et al. 2008). The ABRP has carried out detailed behavioral studies throughout the lifetime of individual animals. Other parameters that have been studied include life history data, genetics, hormones, nutrition and hybridization. (Amboseli Baboon Project Bibliography, http://www.princeton.edu/~baboon). The total population of the baboons being monitored by the Amboseli Baboon Project during the study period was approximately 310. The total number of animals used in this study was 65. This number was limited by the cost of darting each baboon and the logistics of capture and release of each baboon to its group. The darting was also done under the strict regulations of the Kenya Wildlife Service and the Amboseli Baboon Research Project team such that infants and pregnant animals and previously darted animals did not qualify for darting.

Blood samples used in this project were obtained from 65 individuals anesthetized by a dart delivered from a hand-held blowgun, darted in June-July 2007 and June-July 2008 (Altmann et al. 1996). The behavioral data used in this study were extracted from a larger data set (housed

in BABASE, the Baboon Project Database) that covers many years. Here I focused on the behavior data in the 6 months prior to the date each baboon was darted.

5.1.2 University of Nairobi and International Livestock Research Institute (ILRI)

Part of the work was done in the Department of Medical Physiology laboratory. This analysis of blood smears for parasites, differential white blood cell counts and mining of data from the Amboseli baboon project database (Babase). The tick work (identification and counts) was done in collaboration with the tick unit based at the International Livestock Research Institute (ILRI).

5.1.3 Institute of Primate Research

This is a biomedical research institute situated in Karen, Nairobi. It is a branch of the National Museums of Kenya and is a World Health Organization collaborating centre. It is responsible for the coordination of the field primate field studies in Kenya. IPR offers affiliations to scientists including those from the Amboseli Baboon Research Project and carries out research projects in ecology, nutrition, reproductive or human disease related aspects of various primate species. The work that was done in IPR was mainly the PCR based parasite screening. This was carried out at the Tropical and Infectious Diseases Department laboratories.

5.2 CONSTRUCTION OF DATA SETS FOR HYPOTHESIS TESTING

5.2.1 Determining grooming quantity and factors affecting grooming in wild yellow baboons

a) Grooming data

Grooming data have been collected for approximately the last 30 years by the Amboseli Baboon Research Project team and are stored in a dedicated database, Babase. The observational methods used for sampling grooming include the ad libitum and focal animal sampling methods. Ad libitum sampling is used in most observational studies. As described by Altmann (1974), in ad libitum sampling the observer records as much as he can of whatever is readily observed of the social behavior of a group; behaviors, individuals and often the time for behavior sessions are chosen on an ad libitum basis. Focal sampling involved collecting data on a particular animal using a Psion workabout computer. The sampling is normally 10 minutes long and the points are collected once each minute, when the timer beeps. In the case of Babase grooming data, it includes records of every observed instance of grooming between any two animals, and the direction, i.e. who groomed who. Grooming data in this project were collected by mining the existing data base (BABASE) for the specific animals being studied in this project. The data of interest were the counts of grooming received by each of the study animals in the six months prior to their date of darting (referred to as the frequency of grooming received). The darted animals belonged to six different groups. However, one of the groups was a non study group (Group 6) thus there were no grooming data on the three individuals in this group, (see table 5.1.1).

Table 5.1.1: Distribution per Group of Darted Animals.

Group Code	Group Name	No. of Animals darted	No of males darted	No of females darted	Age ranges in years	Percentage of Total Sample size
1.1	Nyayo	11	4	7	8 – 24	17
1.21	Omo	8	4	4	4 – 10	12
1.22	Viola	13	5	8	6 – 15	20
2.1	Linda	12	3	9	6 – 26	18
2.2	Weaver	18	8	10	6 – 23	28
6	Non study	3	3	0	-	5
TOTAL		**65**	**27**	**38**		**100**

b) Dominance rank

The dominance rank for each individual was determined by field observations of dyadic agonistic interactions and these were then used to compute social dominance rank data for each animal. The dominance ranks used here were extracted from the database, and represent the dominance rank of each animal in the month that it was darted or, for females darted in June-July 2008, in December 2007 (because females ranks for 2008 were not yet calculated). Dominance rank varies little over the lifetime of females, so the Dec 2007 ranks are a good approximation to the ranks at the time of darting for females darted in 2008. Dominance rank in males is very dynamic and changes frequently unlike females.

c) Demographic data

This involved mining of age, sex, and kin data from the existing ABRP database.

d) Environmental and Climate data

The vegetation in the Amboseli national park has changed drastically over the past century. It comprises mainly of Suaeda/Salvadora scrub and grassland with open bush lands. The other kind of vegetation found is woodlands and bushlands which unfortunately have thinned or contracted (Western 2006). The climate data was also mined from the existing (Amboseli Baboon Research Project) ABRP database and this included the temperature and the rainfall recorded 5 to 6 months prior to darting as illustrated in tables 5.1.2 and 5.1.3

Table 5.1.2 Rainfall Data in Amboseli (2006, 2007 and 2008)

Month	**Rainfall in 2006/2007 in (mm)**	**Rainfall in 2007/2008 in (mm)**
December	243.8	45.4
January	64.4	32.2
February	11	10.6
March	2.8	73.5
April	2.5	4.4
May	8	4.0
June	0	0
July	0	0
TOTAL	**332.5**	**170.1**

The temperature was recorded with using a WeatherHawk thermometer which records mean temperature (+/- standard deviation) hourly, and a min and maximum thermometer. The data from both types of thermometers are shown in table 5.1.3.

Table 5.1.3 Temperature Data in Amboseli (2007 and 2008)

Month	Year	Weather hawk temperature (°C)	Minimum temperature (°C)	Maximum temperature (°C)
June	2007	20.73 +/- 5.03	8-17	30-36
July	2007	20.03 +/- 4.69	9-14	26-36
June	2008	19.93 +/- 4.60	9-15.5	23-36
July	2008	19.36 +/- 4.36	8-15	25-34

5.2.2 Determining ectoparasite populations on yellow baboons

a) Tick collection

Adult ticks were collected from each darted baboon, plucked from the animal using forceps, and and placed in a well-labeled container and fixed with 70% alcohol. Pepper ticks (immature ticks) were not plucked from the animal but were counted as observed on the baboon's body.

b) Tick identification and distribution

Ticks were identified by morphological characterization under a microscope and by the help of an atlas of pictures of the different types of tick species recorded in the past. The basic characteristic features used for tick identification included presence or absence of eyes, shape and location of eyes, shape of their mouth parts, leg or body markings, presence or absence of festoons and anal plates, and the number size and shape of anal plates.

c) Tick counts/loads

This was done by manual counting of the already collected ticks and recording them under the following categories; adult females, adults females engorged, adult males and immature ticks.

5.2.3 Determining haemoparasite infection in wild yellow baboons

a) Packed Cell Volume (PCV)

Packed cell volume (PCV) also known as haematocrit was used to determine whether or not the animals had signs of anemia, presumably due to tick infestation or infection by haemoparasites. PCV was measured by using the microhaematocrit. Heparinized capillary tubes were filled with a sample of venous blood obtained from each animal. The tube was about three quarters full and one end of the tube was sealed using critoseal. The tubes were then placed in a centrifuge with the sealed end facing the outer circumference. The blood sample was spun at 1500 revolutions per minute at ambient temperature for 5 minutes. Results were read using a microhaematocrit reader. For each animal, at least two readings were taken, and the PCV value assigned to the animal was the mean of these two readings. In most cases, the two readings were identical.

However due to lack of a portable haemoglobin analyzer and a microscope in the field set up, the red blood cell counts, haemoglobin levels and other red blood cell indices of anaemia were not collected in this study.

b) Lymph nodes traits

Examination of each lymph node was done by palpation of the anaesthetized baboon. Lymph nodes were assessed by examining the following lymph nodes; right and left submandibular, inguinal and axillary. The traits that were examined were the size of the node (in

relation to the size of the animal), the consistency (hard, soft or granulated), the symmetry between the right and left nodes, and lobulations.

c) Laboratory tests

i) Blood smears

Blood smears screening was done to identify acute or primary infections of the various haemoparasites. The blood smears were taken from the anaesthetized baboon by pricking the tip of the ear and placing a drop of blood on a clean non greased glass slide. Each slide was labeled with the date, month year and name of the animal using an alcohol resistant marker pen before making the smear. Another slide was used to spread the drop and the slide was air dried. The blood smear was then fixed using 70% methanol for one minute. Staining of the smear was done using 10% Giemsa stain after which it was observed under a microscope. The Giemsa stained smears were examined for the presence of haemoparasites under the light microscope first under 40x and then at 100x objectives. At least 3 blood smears were made for each baboon. Examination of the blood smear involved the screening for the presence of the following piroplasms (*Babesia, Entopolypoides*) in the red blood cells and *Theileria* in the lymphocytes.

ii) Differential White Blood Cell counts

Differential counts involved the estimation of the relative proportion of the different kinds of leucocytes. This was done by making blood smears and staining them with the Giemsa stain as previously described. The count was done towards the tail of the film where the cells lie evenly under the oil immersion objective. A total of 100 cells were counted following a zig zag path and categorized into the different kinds of leucocytes. This was useful for identifying any cellular reaction to the parasitic infections being studied.

iii) Genomic DNA preparation and PCR amplification

PCR screening was carried out to detect haemoparasitic infections in animals with low parasitemia that could not be detected microscopically. This technique is thus a highly sensitive and specific diagnostic tool. The study focused on determining whether the animal was infected or not by the parasites as indicated in the primer sets and not on the quantification of the parasites present. Whole-blood DNA from each baboon was extracted with the DNeasy kit (Qiagen) and was kept at -20 °C. Piroplasm-specific DNA was amplified using four different primer sets specific for the following parasites; *Babesia microti, Entopolypoides macaci*, and 2 primer sets derived from the b-tubulin gene of 2 *Theileria* species and 7 *Babesia* species (*Theileria sergenti, Theileria annulata, Babesia bigemina, Babesia bovis, Babesia major, Babesia equi , Babesia caballi, Babesia divergens,* and *Babesia microti.*), as recorded and carried out by Caccio et al. (2000). The amplifications were done on a PTC-200 Peltier Thermal cycler model and the PCR products were visualized on a 2% agarose gel stained with ethidium bromide and photographed. PCR amplification was then carried out for the target species (Table 5.2.1). The master mix for the different amplification runs and the specific conditions (temperature and time) the runs were carried out are summarized in the tables 5.2.2 and 5.2.3.

Table 5.2.1: Summary of Targeted Parasites.

Target species and gene	Primer name	Primer sequence (5' to 3'end)	Expected base pair size
Babesia microti (18S rRNA)	Bmic F1 Bmic F2	CCTGCGGCTTAATTTGACTC GGATCACTCGATCGGTAGGA	505
Entopolypoides macaci	Emac F1 Emac R1	ATACAGCGAAACTGCGAATG GAAGGGTTTAGATCCCCATCA	437
Theileria and *Babesia*	F34 R323	TGTGGTAACCAGAT(t/c)GG(a/t)GCCA TCnGT(a/g)TA(a/g)TGnCC(t/c)TT(a/g)GCCCA	310 – 460 bp
Theileria and *Babesia*	F79 R206	GA(a/g)CA(t/c)GGnATnGA(t/c)CCnGTAA AC(a/t/g)GA(a/g)TCCATGGT(a/t/g)CCnGG(t/c)T	169 -319 bp

Table 5.2.2: PCR Master Mix Constituents per Reaction

All reagent amounts in µl	*Babesia microti*	*Entopolypoides macaci*	*Theileria* and *Babesia* (primary PCR)	*Theileria* and *Babesia* (nested PCR)
PCR water	17.35	16.85	27.7	18.1
10x buffer	2.5	2.5	5	2.5
Mgcl2 (25mM)	1.5	2.0	3	1.5
dNTPs (20mM)	0.5	0.5	3	0.25
Forward primer	0.25	0.25	0.5	0.25
Reverse primer	0.25	0.25	0.5	0.25
Taq polymerase	0.15	0.15	0.3	0.15
DNA	2.5	2.5	10	5.0
TOTAL	**25**	**25**	**50**	**25**

Table 5.2.3: Specific Primer Conditions

PCR stage	*Babesia microti*	*Entopolypoides macacai*	*Theileria* and *Babesia* (primary PCR)	*Theileria* and *Babesia* (nested PCR)
	°C time	°C time	°C time	°C time
Initial Denaturation	95 - 5 min	94 – 5 min	95 - 5min	94 - 5 min
2nd	95 – 30 min	94 – 30 sec	95 – 30sec	94 – 30 sec
Annealing	59 – 30sec	62 – 30 sec	59 – 30sec	62 – 30sec
Extension	72 – I min	72 – 1 min	72 – 1 min	72 – I min
Further extension	72 – 9 min	72 – 5 min	72 – 9 min	72 - 5 min
Additional conditions			20 – 1 min	
No Of cycles	30	34	30	30

5.3 STATISTICAL ANALYSIS OF RESULTS

These results focus on the three main response variables (grooming received, ectoparasite load and packed cell volume) and how different predictor variables contribute to these responses among the Amboseli baboons. The statistical analyses involved both initial, exploratory bivariate analyses, and multivariate analyses to control for the effects of confounders on the predictor variables. The results presented below are of the multivariate statistical analysis except for a few cases where bivariate analyses are presented for the purposes of illustrating relationships. The statistical software used for these analyses was STATA 10. Some of these results are expressed as the mean +/- SEM or mean +/- SD depending on the type of data.

6. 0 RESULTS

6.1 DETERMINATION OF GROOMING RECEIVED AND FACTORS AFFECTING GROOMING IN WILD YELLOW BABOONS

The frequency of grooming received was defined as the total number of grooming bouts received by an animal over the six month period prior to darting. The average frequency of grooming received by individual animals over the six months prior to their darting was 41+/- 3 (SEM) counts, n = 60. Because the frequency of grooming received is a count variable, the selected statistical test was poisson regression analysis. The predictors that were included in the multivariate analysis were sex, age, dominance rank and group, (Table 6.1.1).

Table 6.1.1: Results of Multivariate Analysis Model on Grooming Recieved

Response Variable: Frequency of grooming received over 6 months

Predictor Variable	Pseudo R2(%)	n	P	B	SE	Direction
Sex		60	<0.0001	-5.587	0.048	Females receive more grooming than males
Age		60	<0.0001	-0.0129	0.0047	Grooming received declines with age
Dominance Rank		60	<0.0001	-0.0300	0.0040	Grooming received reduces down the hierarchy
Group		60	<0.0001	-	-	Grooming received differs with groups
Combined model	41	60	<0.0001	-	-	

β - regression co-efficient, ***Pseudo R2(%)*** *- percentage explained by the model -,* ***n*** *– number of animals sampled,* ***p*** *– probability* ***SE*** *– standard error,* ***Direction*** *– effect by the predictor,* ***Combined model*** *– summarizes the entire effect of the four different predictor variables on grooming received.*

6.1.1 Grooming received and sex

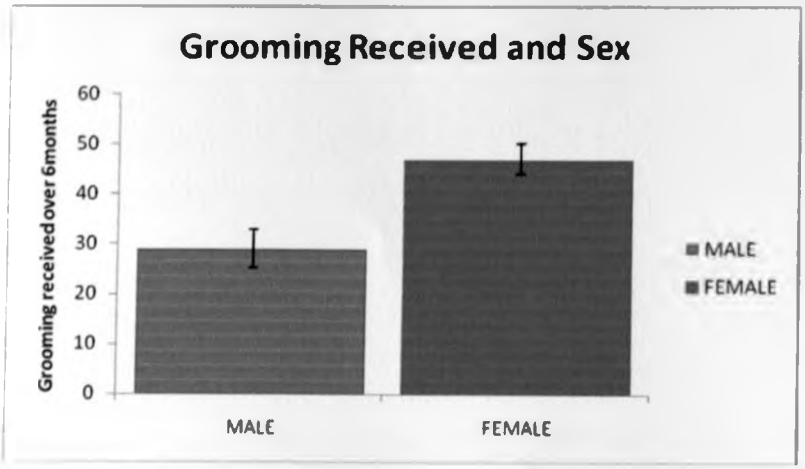
The average frequency of grooming received by females was 47 +/- 4.075, (n = 38), whereas males received an average of 29 +/- 3.90, (n = 22) counts of grooming (Table 6.1.2). The multivariate poisson regression analysis revealed that when age, group and rank have been taken into account, grooming received differs significantly by sex (β = -5.59, p = < 0.001, and n = 60) with females receiving more grooming than males (Figure 6.1.1, table 6.1.1).

Table 6.1.2: Summary Statistics of Grooming Received

	Median	Mean +/- SEM	Number of animals
Females	46	47 +/- 4.075	38
Males	28	29 +/- 3.902	22
Overall	40	41 +/- 3.14	60

Median *– Midpoint in frequency distribution,* ***Mean*** *– average counts,* ***SEM*** *– standard error of the mean,* ***number of animals*** *– number of baboons sampled*

Figure 6.1.1: Sex Differences in Grooming Received

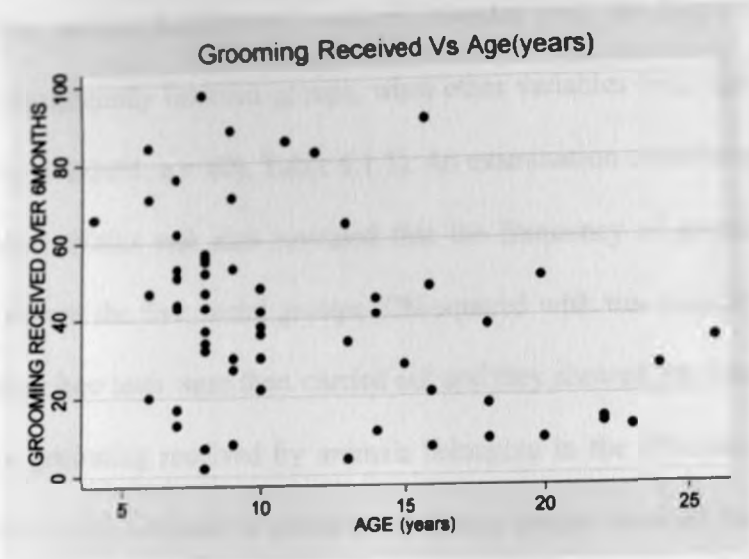


Differences in the amount of grooming received by sex, the error bars are +/-SE.

6.1.2 Grooming Received and Age

The average age of the animals darted was 11 +/- 5years with a range of 4 to 26 years, (n=60). The multivariate poisson regression analysis showed that grooming received by an animal declined significantly with increasing age, when the other variables (group, rank and sex) were taken into account ($\beta = -0.0129$, $p = < 0.001$, $n = 60$), see table 6.1.1. The animals with ages between 5 to 10 years had high counts of grooming received ranging between 20 to 100 counts where as the animals over 15 years had counts ranging between 20 to 50 counts of grooming received as illustrated in the scatter plot, (Figure 6.1.2).

Figure 6.1.2 Grooming Received and Age



Grooming received for each animal compared against its age, each dot represents an animal.

6.1.3 Grooming Received and Group

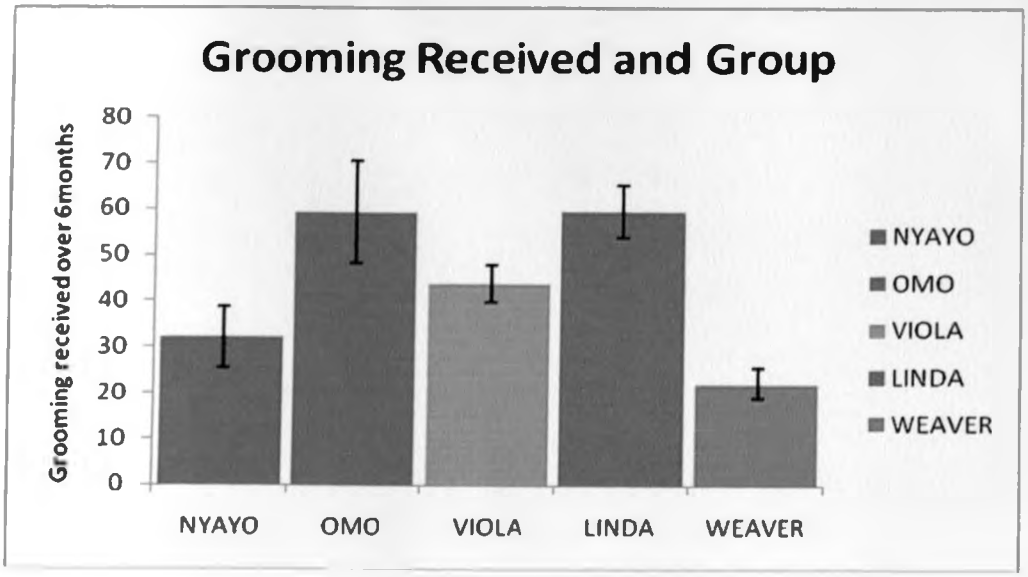
The poisson multivariate analysis revealed that the frequency of grooming received differed significantly between groups, when other variables (sex, age and rank) were taken into account ($p < 0.0001$, $n = 60$), Table 6.1.1). An examination of the bivariate analysis results using the Kruskal Wallis test also revealed that the frequency of grooming received significantly differed across the five social groups (Chi-squared with ties = 23.557 with 4 d.f., $p = 0.0001$). Further post hoc tests were then carried out and they showed the absolute differences in average frequency grooming received by animals belonging in the different social groups, (Table 6.1.3 and figure 6.1.3). Animals in Linda's and Omo's groups received the highest average frequency of grooming, (59 counts for both males and females). Viola's group received an average of 42 counts, Nyayo's group received 32 counts and Weaver's group had the lowest frequency of grooming, 22 counts, (Figure 6.1.3).

Table 6.1.3 Post Hoc Bivariate Analysis Results of Grooming Received vs Group

Group Name	N	Average Frequency of Grooming received	Groups that showed Significant differences ($p<0.05$)
(1) LINDA	12	59	2 and 5
(2) NYAYO	11	32	1 and 3
(3) OMO	8	59	2 and 5
(4) VIOLA	12	42	5
(5) WEAVER	17	22	1, 3, and 4

Group name *– baboon social group,* ***n-*** *number of animals in each group, the 4th column shows the groups (indicated by a number) with significant differences in the amount of grooming received.*

Figure 6.1.3: Multiple Comparisons Graphs of Grooming Received vs Group

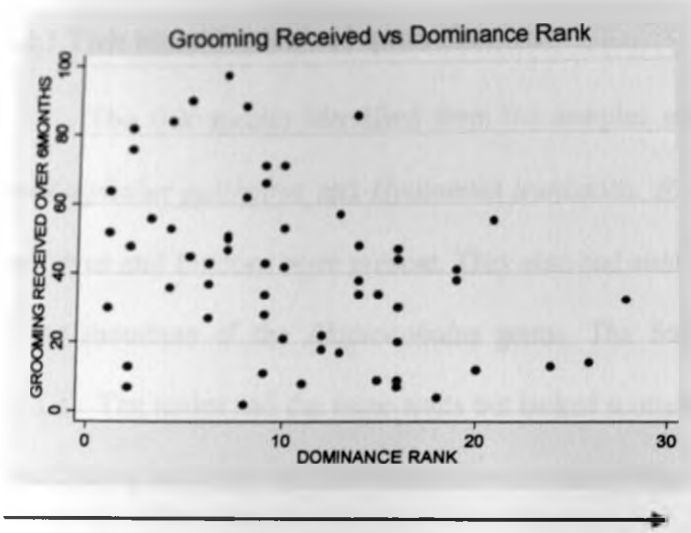


This figure illustrates the differences of grooming received by the five baboon social groups. The error bars are +/-SE.

6.1.4 Grooming received and dominance ranks

Multivariate poisson regression analysis results also identified a significant relationship between grooming and dominance rank, when other variables were taken into account (β =-0.0300, p = < 0.0001, n = 60). Figure 6.1.4 showed that the frequency of grooming received declined down the hierarchy. Specifically, higher ranking individuals received more grooming than low ranking individuals.

Figure 6.1.4: Grooming Received Vs Dominance Rank



High rank Low Rank

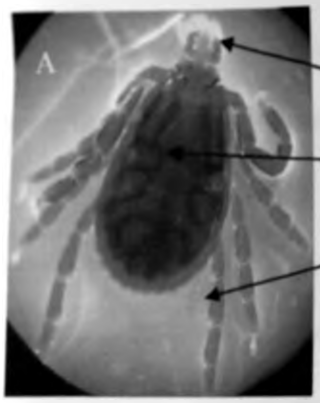
Grooming received for each animal compared against its dominance rank, each dot represents an animal.

6.2 DETERMINATION OF TICK LOAD ON WILD YELLOW BABOONS

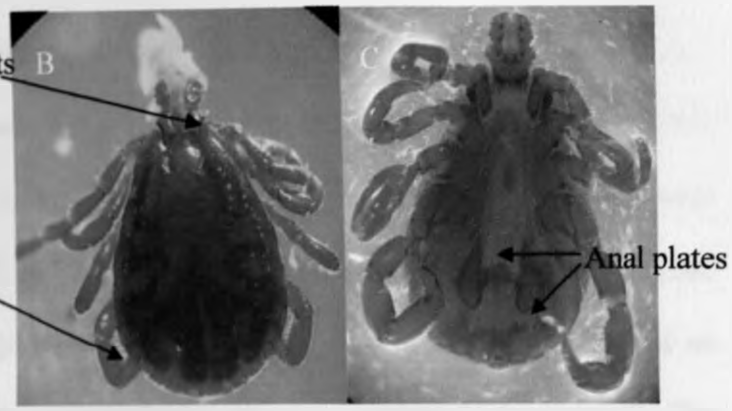
6.2.1 Tick identification and quantification

The tick species identified from the samples collected included *Rhipicephallus simus, Rhipicephalus pulchellus* and *Hyalomma truncatum*. *R. simus* ticks were brown in colour, and their eyes and festoons were present. They also had anal plates and brown marked legs like most of the members of the *Rhipicephalus* genus. The females had scutums at the back (figure 6.2.1.a). The males had the same traits but lacked scutums (figure 6.2.1b). The engorged females were brown in colour and the festoons were not visible (figure6.2.1c). The eyes however were visible. The *R. pulchelus* ticks had brown legs, presence of eyes, anal plates, festoons and white and brown patterns on the back of both the males and females. The females had white scutums (figure 6.2.1.d). The *Hyalomma* species were also brown in colour but had slightly bright leg markings and longer mouthparts as compared to the *Rhipicephalus* species. The females also had scutums (figures 6.2.1e and f). The dominant tick species collected was *Rhipicephallus simus. R. pulchellus* and *Hyalomma* were relatively rare. Table 6.2.1 shows the number of ticks of each species found on the 64 baboons sampled. These results include the immature ticks that were found on the body of the animal.

Figures 6.2.1 a – f: Tick Identification Pictures

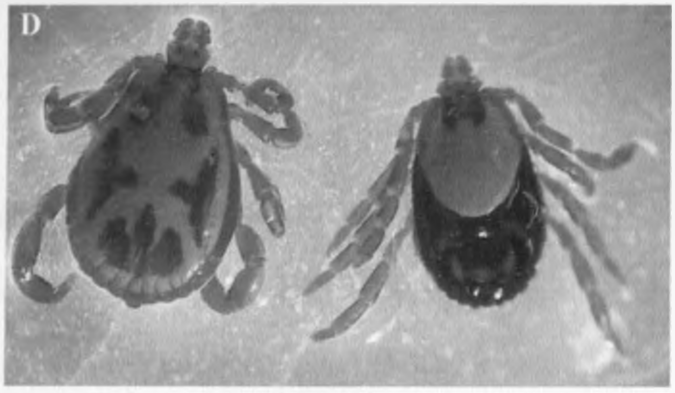


Rhipicephallus simus
Female adult tick

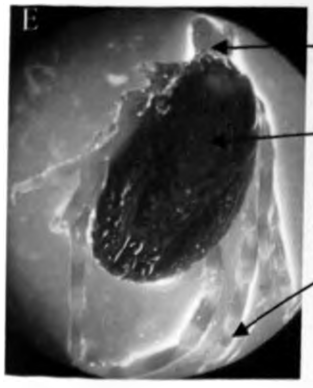


Rhipicephallus simus Male adult tick anterior and posterior views

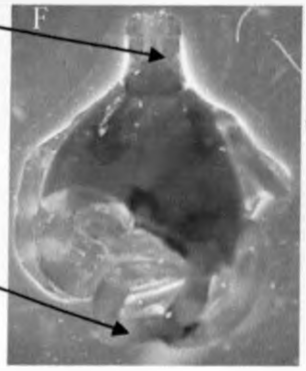
Rhipicephalus simus
Engorged Female adult tick



Rhipicephalus pulchelus
male and female adult ticks



Hyalomma truncatum
Female adult tick



Hyalomma truncatum

6.2.2 Determination of factors affecting tick load

The total tick count on animals included all the three species of ticks and was not normally distributed across baboons; consequently, it was not possible to perform a multivariate analysis on this variable. For this reason, a series of simple nonparametric analyses were performed, which are presented below, and then a second measure of tick load for multivariate analysis was devised, namely, a categorical variable of "Ticks present" and "no ticks". If an animal had any ticks at all (of whatever number) they were categorized as "ticks present". The multivariate analysis on the categorical variable is presented below, after the presentation of the bivariate, nonparametric analyses on total tick count.

a) Bivariate analysis using total tick counts

i) Tick load and sex

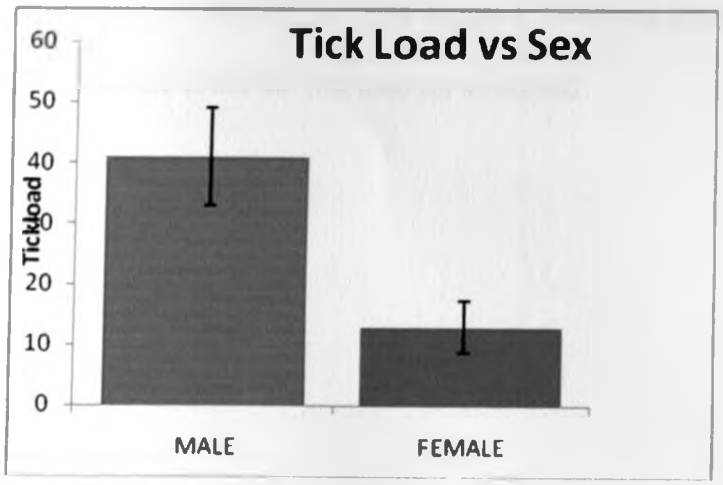
The average tick count in the entire set of animals was 24 +/- 4.46 SEM, n= 64. This number included the larvae and adult ticks and all the tick species counted in each animal. A Mann-Whitney test was carried out to find out if there was a difference in tick load by sex. The results were significant ($p = <0.00015$), and indicated a higher tick load for males than for females. The mean tick load in males was 41+/- 41, (n = 26) and in females 12 +/- 26, (n=38) (Figure 6.2.2).

Table 6.2.1: Total Numbers of Each Tick Species; Pooling Across All 64 Animals

Tick species	N	Mean +/- SEM	Median
R. simus	951	24 +/- 4.3	6
R. pulchellus	17	0 +/- 0	0
H.truncatum	2	0 +/- 0.	0

Tick species *– specific tick species identified,* ***n*** *- Total number of ticks collected,* ***Mean*** *– average number of tick species collected in all animals,* ***Median*** *–50% distribution of ticks*

Figure 6.2.2: Bivariate Analysis on Sex Differences in Mean Tick Load



Differences in tickload by sex, the error bars are +/-SE

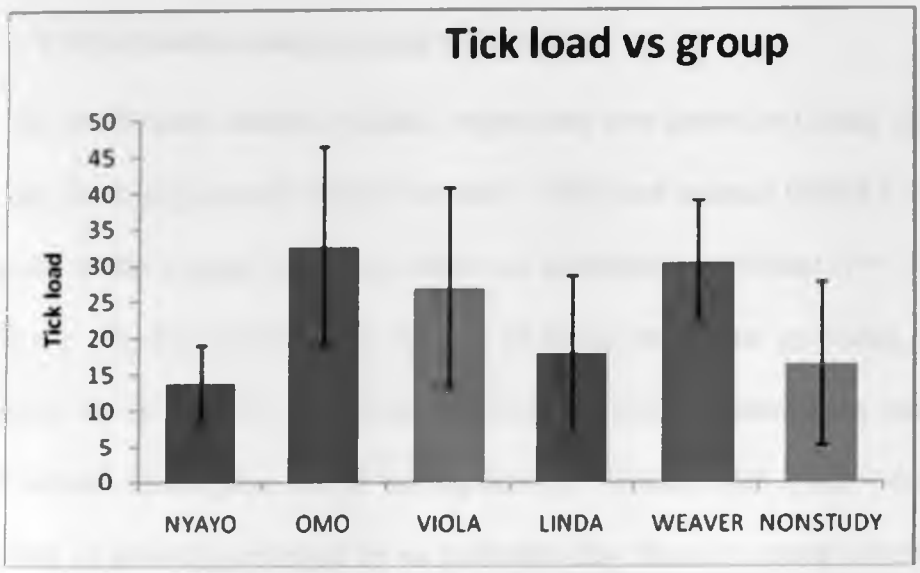
ii) Tick load by group

A nonparametric analysis using the Kruskal Wallis test carried out to check for tick load differences by group did not reveal any significant differences between groups (d.f = 5, p = 0.7005). The highest amount of ticks collected was from Omo's group which had an average of 33 ticks per animal (n = 8 animals). Weaver's group had an average of 30 ticks per animal, (n = 18 animals), Viola's had 27 ticks per animal, (n = 13 animals), Linda's group 18 ticks per animal, (n = 11 animals) and Nyayo's group 13 ticks per animal, (n = 11 animals). The non study group had an average of 16 ticks per animal, (n = 3 animals) (Figure 6.2.3).

iii) Tick load and grooming

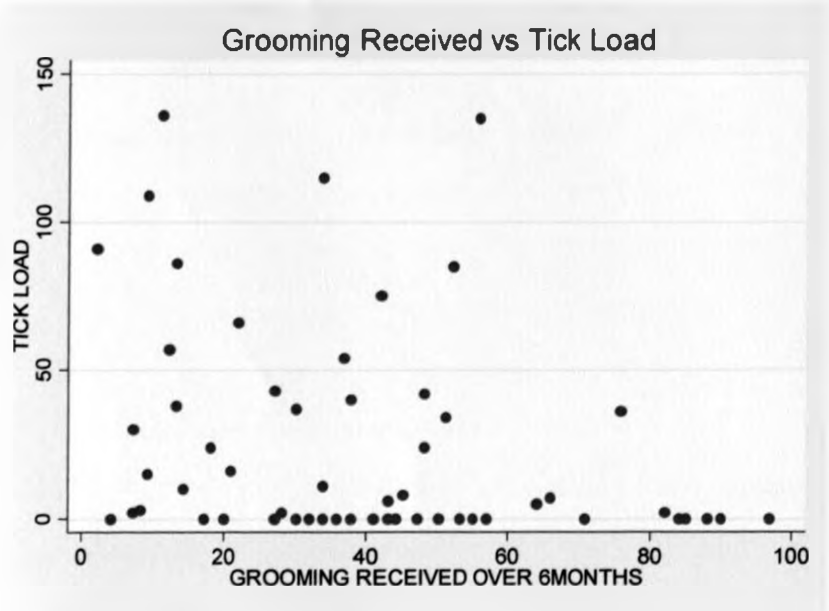
To determine whether there was a relationship between grooming and tick load a Spearman rank correlation was carried out. The Spearman's Rho value was - 0.37 and the p value was significant (p = <0.0036, n = 59). Figure 6.2.4 shows that the higher the grooming received by an individual the lower the tick load on an animal.

Figure 6.2.3: Mean Tick Load per Group.



Bar charts illustrating the differences in tickload by the baboon social groups. The error bars are +/-SE

Figure 6.2.4: Grooming Received vs Tick Load



Tickload on each animal compared against the amount of grooming it received, each dot represent an animal.

b) Multivariate analysis using tick category

The multivariate analysis (logistic regression) was carried out using the categorization of tick load as "ticks present" versus "no ticks". The older animals tended to have more ticks as compared to the younger ones, this effect was statistically significant (P = 0.025, Odds Ratio = 1.118, n = 59). Sex did not affect the risk of having ticks after grooming, rank and age were controlled for (p = 0.157, Odds Ratio = 0.283, n = 59), and dominance rank showed a strong trend towards altering the risk of having ticks (p = 0.056, Odds Ratio = 0.870, n = 59). The frequency of grooming received by an individual also showed a trend towards affecting the risk of having ticks such that animals that were groomed more had fewer ticks (p = 0.083, Odds Ratio = 0.968, n = 59) (Table 6.2.2).

Table 6.2.2: Summary of Multivariate Analysis on Tick Load

Response variable - tick load **("ticks present"** and **"No ticks")**

Predictor variable	Pseudo R2(%)	n	P	Odds ratio	SE	Direction
Combined model	**30**	**59**	**0.0001**			
Sex	-		0.157	**0.2835**	**0.2523**	**NS**
Age	-		0.025	**1.1183**	**0.0887**	Older animals are more likely to have ticks
Dominance rank	-		0.056	**0.8700**	**0.0634**	Higher ranking animals had fewer ticks
Grooming received	-		0.083	**0.9683**	**0.0179**	Animal that received more grooming had fewer ticks

Pseudo R2(%) *- percentage explained by the model -* ***, n*** *– number of animals sampled,* ***p*** *– probability,* ***Odds Ratio*** *– Likelihood of exposure,* ***SE*** *– standard error,* ***Direction*** *– effect by the predictor*

6.3 DETERMINATION OF HAEMOPARASITE INFECTION IN WILD YELLOW BABOONS

The presence of haemoparasite infection was assessed in two ways; determination of the presence of haemoparasites and determination of physiological effects due to the presence of ticks and haemoparasitic infections. Determination of the infection status of each animal was done by blood smear screening and polymerase chain reactions which enabled identification of the species of parasites present and their prevalence. Determination of the secondary physiological effects that may have arisen due to the presence of ticks and the presence of haemoparasitic infection such as anaemia, lymph node enlargement and changes in composition of the white blood cell counts was done by analysis of haematocrit (Packed cell volume), physical examination of lymph nodes and analysis of white blood cell counts. However, lymph node data were not analyzed because all the animals had enlarged lymph nodes thus comparisons could not be made.

6.3.1 White blood cell differential cell counts

Differential counts were carried out on blood smears collected from 45 out of 65 animals. Some of the slides could not be read due to poor fixing and field storage. The white blood cells counted from these smears included neutrophils, lymphocytes, monocytes, eosinophils and basophils. The average counts (+/- SD) for neutrophils was 40 +/- 11, lymphocytes, 55+/- 12, eosinophils, 3+/-3, monocytes 1+/- 1 and basophils which were the least 0+/- 0, (Table 6.3.1.).

Differential white blood cell counts and tick load

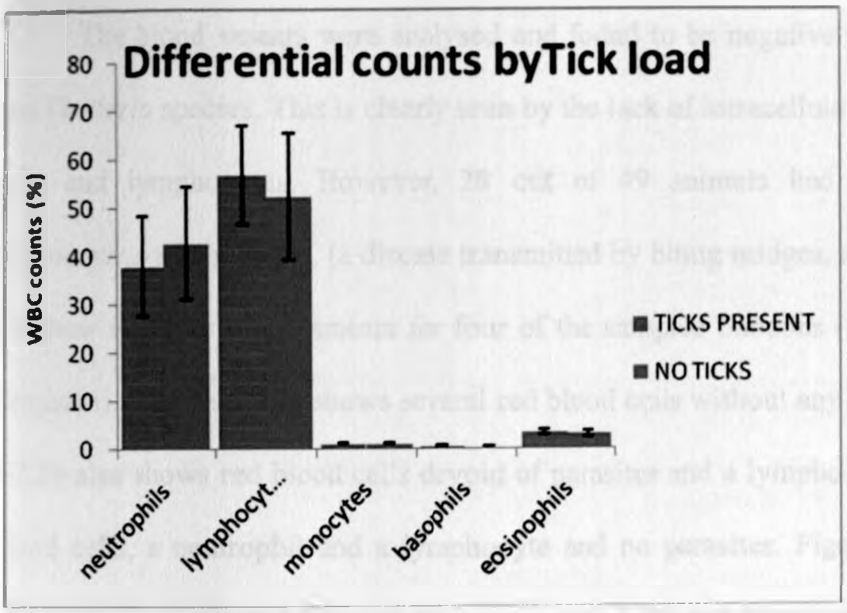
A T-test was carried out to find out if there was a significant difference in the differential white blood cell counts in the animals that had ticks and those that did not have any. The neutrophil counts ($t = 1.4101$, d.f. 43, $p = 0.1657$) and lymphocyte counts were not significantly different ($t = -1.2828$, d.f. 43, $p = 0.2064$) between animals that did have ticks and those that didn't. A Mann-Whitney test was carried out to test for the differences of the various white blood cell counts between the animals that had ticks and those that did not have ticks. These results were also not significant for basophils ($p = 0.7489$, $n = 44$), monocytes ($p = 0.9761$ $n = 43$) and eosinophils ($p = 0.4343$, $n = 44$), (Figure 6.3.1).

Table 6.3.1: WBC Differential Counts

White blood cell type	Mean WBC ranges +/- SD in the sampled wild yellow baboons (%)	Normal WBC ranges in olive baboons (%)
Lymphocytes	55 +/-12	22 – 50
Neutrophils	40 +/- 11	48 – 76
Eosinophils	3 +/- 3	0 – 3
Basophils	0 +/- 0	0 – 1
Monocytes	1 +/- 1	0.5 – 3.5

Mean WBC ranges +/- SD in the sampled wild yellow baboons (%) compared to those in captive olive baboons

Figure 6.3.1: WBC counts Comparisons



Comparison of White Blood Cell (WBC) counts in animals with and without ticks. The error bars are +/-SE

6.3.2 Blood smear screening

The blood smears were analysed and found to be negative for *Entopolypoides, Babesia and Theileria* species. This is clearly seen by the lack of intracellular piroplasms in the red blood cells and lymphocytes. However, 28 out of 49 animals had positive blood smears for *Hepatocystis* trophozoites, (a disease transmitted by biting midges, not by ticks). Pictures 6.3.2 a - d show negative blood smears for four of the sampled baboons (Ganja, Kathryn, Gabriel and Sorghum). Figure 6.3.2a shows several red blood cells without any intracellular parasites, figure 6.3.2b also shows red blood cells devoid of parasites and a lymphocyte, figure 6.3.2c shows red blood cells, a neutrophil and a lymphocyte and no parasites. Figure 6.3.2d was infected with *Hepatocystis kochi* and this can be seen amongst the red blood cells. The parasite was at the gametocyte stage and stained pinkish with brownish granules in the cell with giemsa stain.

Figures 6.3.2 a- d: Blood Smear Screening Pictures

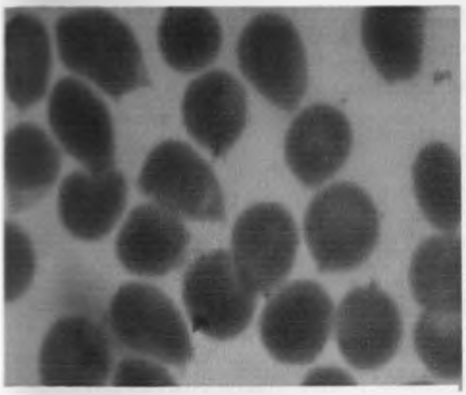


Figure 6.3.2 a, Red blood cells devoid of intracellular parasites

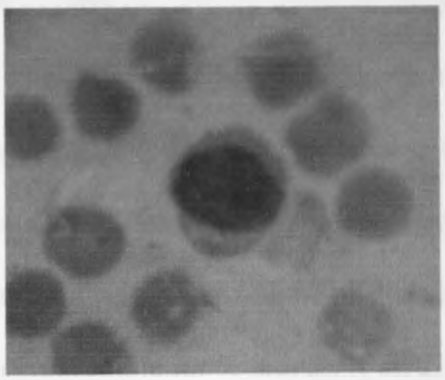


Figure 6.3.2 b, Red blood cells and a lymphocyte devoid of intracellular parasites.

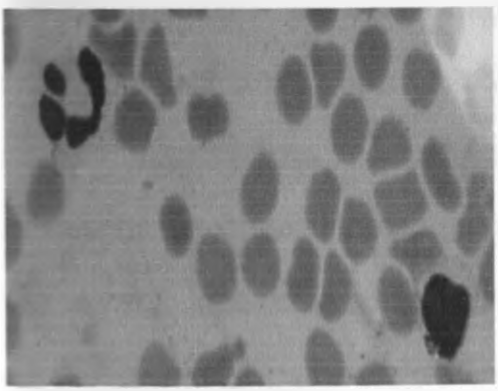


Figure 6.3.2 c, red blood cells and a lymphocyte devoid of intracellular parasites and one neutrophil.

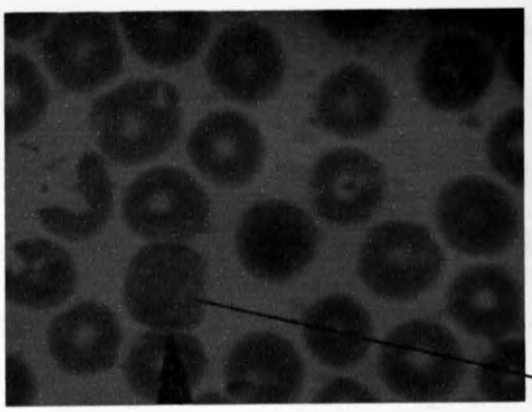
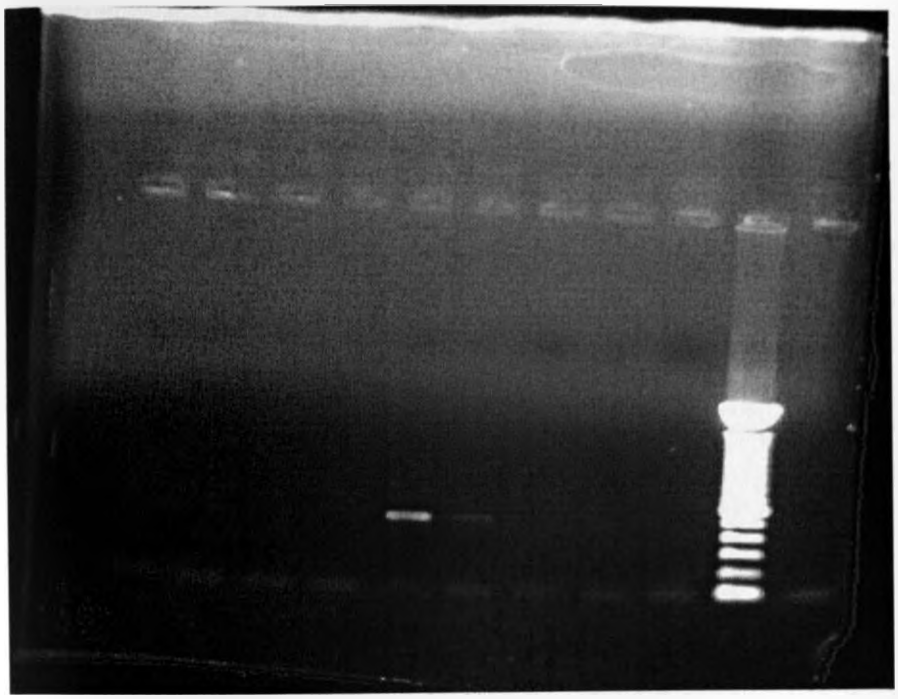


Figure 6.3.2 d, red blood cells devoid of intracellular parasites and *H.kochi* parasite

6.3.4 PCR screening

To confirm the identity of the parasitic species and to carry out a more sensitive analysis than the blood smears results, PCR analysis was used as a diagnostic tool. Samples from 63 animals were screened for various tickborne parasitic infections using the primer sets reported in the methods (Table 5.3.1). Results seen following electrophoretic separation of PCR products (amplicons) are shown in figures 6.3.3 a – d and the marker/ladder used was 100bp. None of the samples tested positive for *Entopolypoides macaci*. A positive control sample for *E. macaci* (Pan 2327) that had been included amplified at approximately 400bp (Figure 6.3.3d). Two samples out of the 63 screened samples were positive for *Babesia microti* and the size of the PCR product was approximately 500bp (Figure 6.3.3a). Fifty-five animals tested positive and 8 tested negative for a test that amplified a non-specific fragment of the b-tubulin gene from *Theileria* and *Babesia* species; hence, these 55 animals were clearly positive for at least one species of either *Theileria* or *Babesia*, (Table 6.3.2). Figure 6.3.3b shows the results of the amplication of this fragment of the b-tubulin gene from two *Theileria* and seven *Babesia* DNAs, as obtained using the primers F34 and R323. After the first amplification, a nested PCR was performed to narrow down on the particular species of *Theileria* and *Babesia* by visualizing the differences in amplicon sizes as was carried out by Caccio et al. (2000). The nested PCR results showed 2 different sizes of amplicons. The 170bp amplicon was *B. microti* and 54 animals tested positive and 9 animals negative. The second amplicon seen after the nested PCR was at 200bp was *B. equi* and two out of 63 animals were positive (Figure 6.3.3c). This figure showed only one of the animals that tested positive number 2 (Lur). Figure 6.3.3d is a summary of all the parasites amplified by the various primer sets.

Figure 6.3.3a: Amplification of *Babesia microti* at 500bp

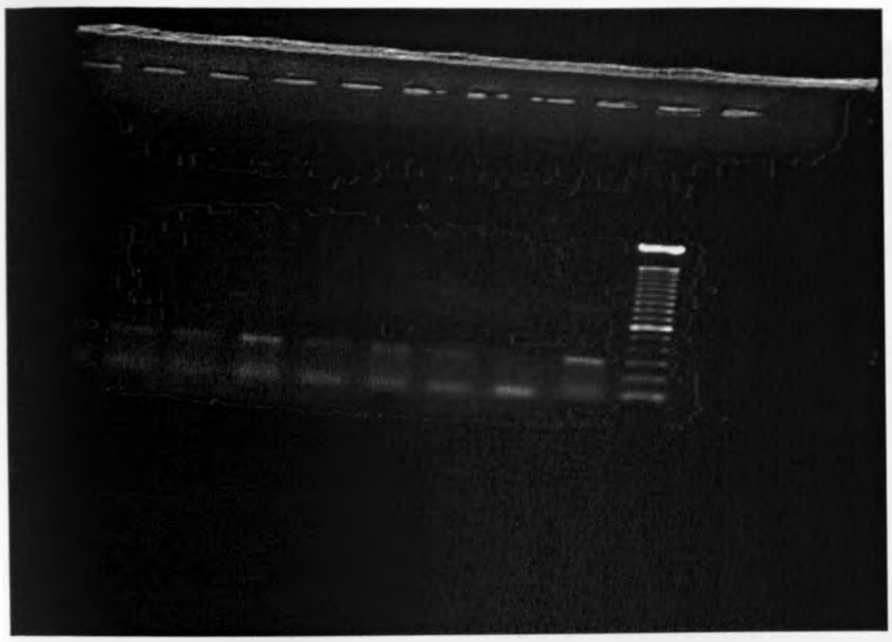


1. Noo
2. Gan
3. Oct
4. Pai
5. **Wab**
6. **Lur**
7. Gab
8. Luna
9. Adr
10. Blank

1 2 3 4 5 6 7 8 9 M 10

Two samples (numbers **5 (Wab) and 6 (Lur))** out of the 63 screened samples were positive for *Babesia microti* and the size of the PCR product was approximately 500bp on 2% agarose gel. **M** is a 100base pair molecular marker.

Figure 6.3.3b: Amplification of *Theileria* and *Babesia* (primary PCR) at 300bp



1 2 3 4 5 6 7 8 9 M

1. Bag
2. Eag
3. Gan
4. Hym
5. Lao
6. Lib
7. Lim
8. Blank
9. Lun

This figure shows the results of the amplication of this fragment of the b-tubulin gene from two *Theileria* and seven *Babesia* DNAs, as obtained using the primers F34 and R323. The positive animals (numbers 1-7, and 9) are those that showed amplicons of approximately 300 bp for *B. microti* on 2% agarose gel. Number 8 was a blank negative and thus showed no amplification. **M** is a 100 bp molecular marker.

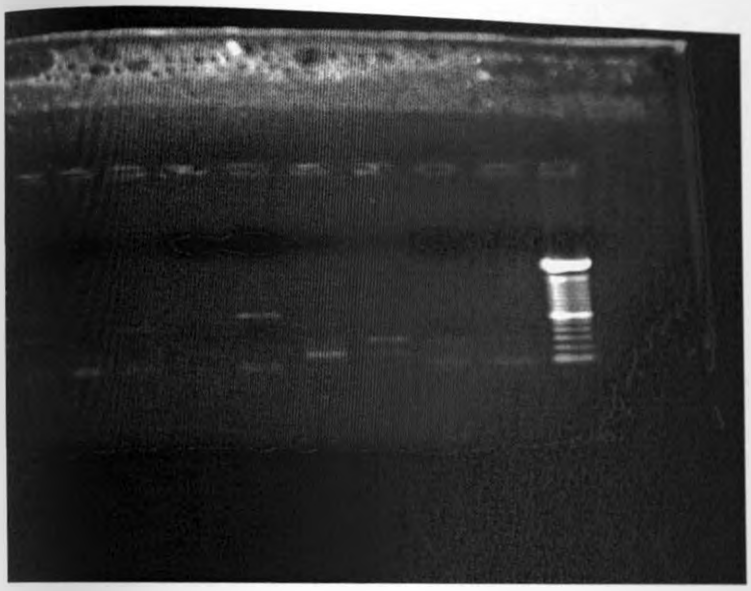
Figure 6.3.3c: Amplification of *Theileria* and *Babesia* (nested PCR)

1. Das	11. Vai
2. Lur	12. Ale
3. Noz	13. Mon
4. Ker	14. Loc
5. Gab	15. Nju
6. Hol	16. Lan
7. Ara	17. Edu
8. Blank	18. Log
9. Cad	19. Rwa
10. Tal	

1 2 3 4 5 6 7 8 9 10 M 11 1213 14 15 16 17 18 19

Figure 6.3.3c shows the results on 2% agarose gel of the nested PCR amplifications done using the F79 and R206 primers. Amplification at the 170bp amplicon indicated the presence of *B. microti.* This is evident for all the animals (numbers 1 to 19) in this picture. The second amplicon seen after the nested PCR was at 200bp was *B. equi.* The animal that was positive for this parasite was number 2 **(Lur).**

Figure 6.3.3d: Summary of All Parasites Amplified



1 2 3 4 5 6 7 8 9 M

1. Pan – *E.macaci*
2. Pai - Negative
3. Wab– *B.microti*
4. Blank
5. Lur - B.*microti*
6. Gan - F79 (nested PCR)
7. Lurch F79 (nested PCR)
8. Lun F34 (primary PCR)
9. Gan F34 (primary PCR)

Figure 6.3.3d is a summary on 2% agarose gel of all the parasites amplified by the various primer sets. 1- A positive control sample for *E. macaci* (Pan 2327) that had been included amplified at approximately 400bp, 2 - was an animal from which no parasite was amplified, 3 – *B.microti*, 4 - was a blank negative sample, 5 - amplicon size 500bp amplification of *Bmicroti*, 6 – *B microti* from nested PCR primers F79, 7 - had 2 amplicons one a 300bp (*B. microti*) and the other at 200bp (*B. equi*). 8 and 9 show faint amplification of *B microti* at 300bp.

Table 6.3.2: Prevalence of Haemoparasitic Infections.

Parasite targeted	Amplicon size in base pairs	NO of animals positive	No of Animals negative
Entopolypoides macaci	437	0	63
Babesia microti	500	2	61
Theileria and *Babesia* (primary PCR)	300	55	8
Theileria and *Babesia* (nested PCR)	170 200	54 2	9 61

Analysis of haemoparasite infection status and Grooming and Tick load.

The analysis of the status of haemoparasitic infection (positive or negative) and tickload and counts of grooming received was carried out using the results of the F34 and F79 primers which as stated before amplified a series of *Babesia* and *Theileria* (see section 5.2.3) parasite DNA in the baboons.

Haemoparasite infection vs tickload

To test the hypothesis that presence of ticks reduces the risk of haemoparasitic infection, a logistic regression analysis was done. For the primary PCR (F34) the results showed that there was no significant difference in the prevalence of infection in animals with or without ticks ($p = 0.304$, Odds ratio = 0.4144, $n = 64$). The analysis using the nested PCR (F79) verses presence of ticks also showed that there was no significant difference in the prevalence of infection ($p = 0.564$, Odds ratio = 0.645, $n = 64$)

Haemoparasite infection vs grooming received

The study hypothesis was that an increase in grooming would reduce the tickload and thus reduce the exposure to haemoparasitic infection. This was analysed by carrying out a logistic regression with the response variable as status of infection and the predictor variable as counts of grooming received. The results showed that the amount of grooming did not influence the presence of infection in the baboons. Primary PCR (F34) results verses counts of grooming received showed no significance, ($p = 0.786$, Odds ratio = 1.004, $n = 60$). Nested PCR (F79) verses counts of grooming received results were also not significant ($p = 0.155$, Odds ratio = 0.976, $n = 60$)

6.3.5 Analysis of Packed cell volume (PCV)

Packed cell volume is known to be affected by several variables and consequently bivariate tests were carried out followed by multivariate analysis. The average packed cell volume of the entire group of animals was 41.91% +/- 4 (SD) (n = 64). The multivariate analysis was done using multiple regression and the predictor variables included were sex, age, and tick load (total tick counts). The year of darting was also included due to the differences in the tick stages by year, (Table 6.3.3).

Table 6.3.3: Summary of Multivariate Analyses on PCV

Response Variable – PCV, n = 59

Predictor variable	Adjusted R2	T	**B**	P	Direction
Age	-	-3.80	-.32	0.001	PCV declines with age
Sex	-	2.90	2.81	0.005	Males have higher PCV than females
Tickload	-	-2.72	-0.04	0.009	PCV declines with increase in tickload
Year	-	2.50	2.1	0.015	2008 animals had a higher PCV than 2007 animals
Combined model	35	-	-	<0.0001	

Adjusted R2(%) *- percentage explained by the model ,* ***T*** *– t-statistic value,* ***β*** *- regression co-efficient,* ***n*** *– number of animals sampled,* ***p*** *– probability,* ***Direction*** *– effect by the predictor*

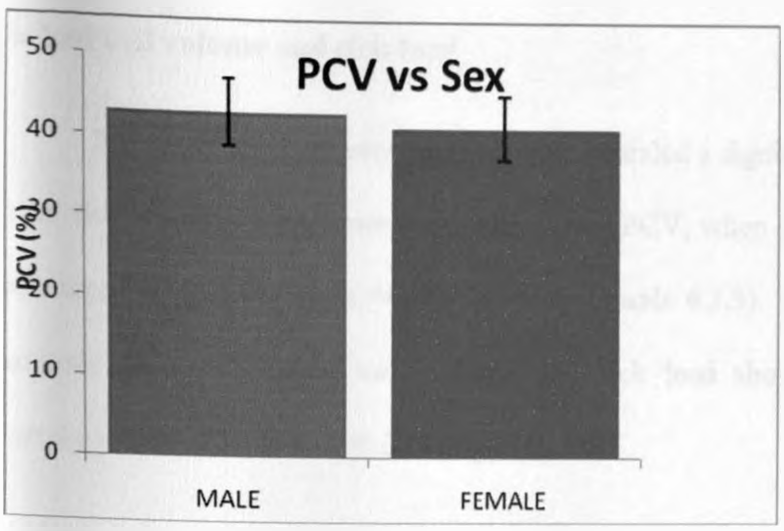
Packed cell volume and Sex

Multivariate regression analysis showed that PCV differed significantly by sex ($\beta = 2.81$, $p = 0.005$, $n = 59$) with males having a higher PCV than females when the other factors in the model were also taken into account (Table 6.3.3). The PCV for females was 41.19% +/- 4.0 (n=38) where as for males was 42.96%+/-4.1. (n = 26), (Figure 6.3.4).

Packed cell volume and Age

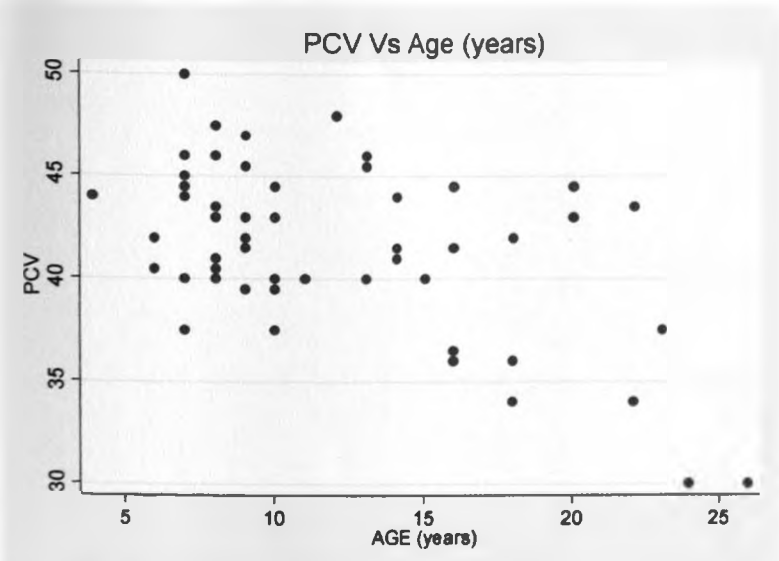
The multivariate analysis revealed that PCV significantly declined with age when other factors of the model were taken into account, ($\beta = -.32$, $p = 0.001$, $n = 60$), (Figure 6.3.5, Table 6.3.3). A simple correlation analysis done between PCV and age also showed a strong negative correlation ($r = -0.5169$).

Figure 6.3.4: PCV Vs Sex



Differences on PCV by sex, the error bars are +/-SD

Figure 6.3.5: PCV Vs Age

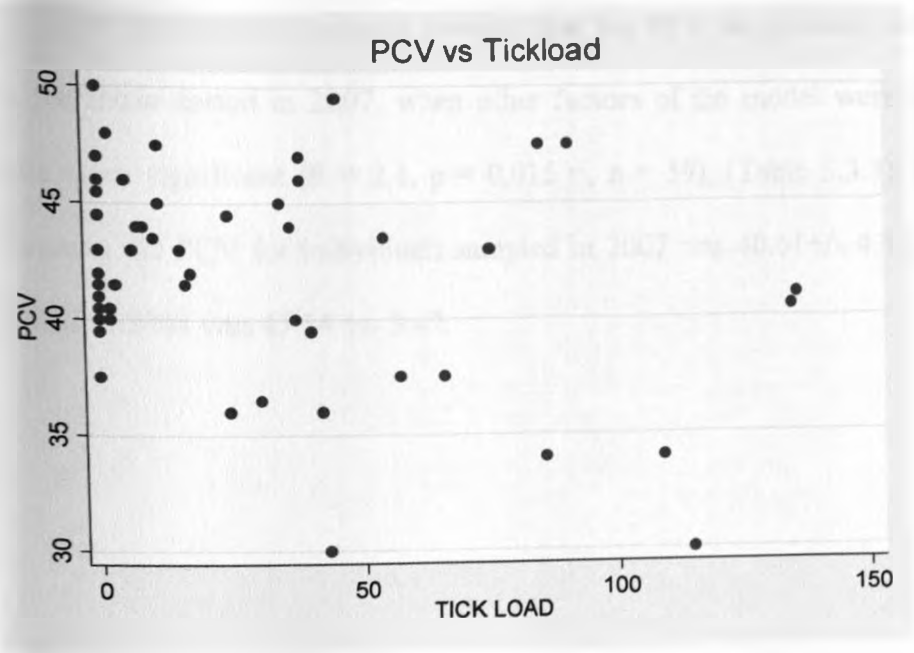


PCV for each animal compared against its age, each dot represents an animal.

Packed cell volume and tick load

Multivariate regression analysis also revealed a significant result, where higher tick loads (total tick counts) were associated with lower PCV, when the factors of the model were taken into account, (β = -0.04, p =0.009, n = 59), (Table 6.3.3). To illustrate this trend, a correlation analysis done of packed cell volume and tick load showed a negative correlation with a correlation co-efficient of -0.294 (Figure 6.3.6).

Figure 6.3.6: PCV Vs Tick Load

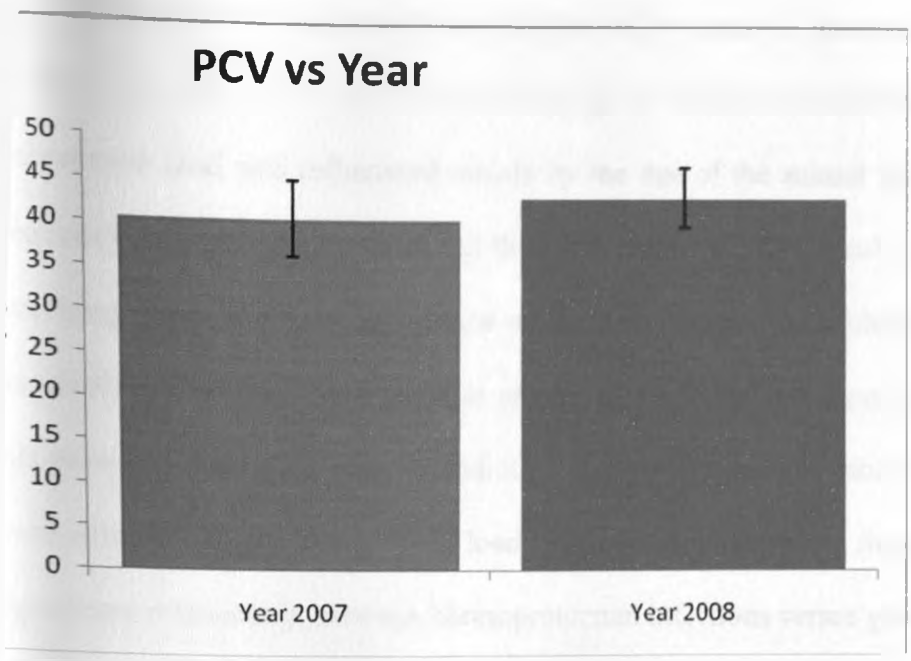


PCV for each animal compared against its tick load, each dot represents an animal.

'acked cell volume and year

The multivariate analysis revealed that the PCV for animals darted in 2008 was higher han for those darted in 2007, when other factors of the model were taken into account. The results were significant ($\beta = 2.1$, $p = 0.015$ =, $n = 59$), (Table 6.3.3). Figure 6.3.7 shows this difference, the PCV for individuals sampled in 2007 was 40.61+/- 4.43 and that for individuals sampled in 2008 was 43.14 +/- 3.47.

gure 6.3.7: PCV Vs Year



ifferences in PCV by the year of darting, the error bars are +/-SD

7.0 DISCUSSION

The results of this study revealed that the frequency of grooming received was influenced by the age, rank, sex and the social group to which an individual animal belonged. The ectoparasite load was influenced mainly by the age of the animal and to a lesser extent by the frequency of grooming received and the dominance rank. The results of the PCR haemoparasite screening showed a low prevalence of *Babesia* species or a closely related species in this population of baboons. The physical examination did not reveal any signs of acute infection by this parasite. Other physiological indices of the presence of infection such as packed cell volume were affected by the ectoparasite load, age and sex. However, the results did not reveal any significant relationship between haemoprotozoan infections verses grooming and verses tick load too.

Receipt of grooming in baboons and other non human primates appears to be beneficial both for social and health purposes. This study particular focused further on health benefits that wild baboons received from being groomed. The conclusion that can be made from these results is that sex, age, dominance rank, and group (possibly mediated by density effects) are the main contributors to variation in grooming received by different animals.

The frequency of grooming received was higher in females as compared to males in this study. This is in agreement with several previous studies indicating that the amount of grooming both received and given is higher in females compared to males. This means that any benefits received from grooming, both social and health are mainly enjoyed by females and one can speculate that males may be more stressed and be more predisposed to diseases transmitted by ectoparasites as is further discussed below. Studies by Saunders (1988), Kervene (1989) and Silk et al. (2006) have reported that grooming in females enhances reproductive success, providing an

additional incentive for forming strong social bonds. Males mainly engage in grooming bouts when soliciting mating opportunities and females groom males to get protection from other baboons. Males are less likely to groom each other thus the grooming they receive is mainly from females.

The ages of the animals darted in this study ranged from 4 to 26 years. This age included adults and a few subadults. The bivariate models show that age contributes to some extent to the amount of grooming received by animal, with the younger animals receiving more grooming as compared to the older animals. However, the multivariate results show that when other factors are included into the model, the effects of age on grooming received is reduced. This may be due to the fact that as an animal grows older, it is more inclined to conserve its energy for important activities such as feeding, foraging and resting than spend it on grooming and other social activities such as playing. The data in this study showed that the animals above 20 years received fewer bouts of grooming. Most of the animals started receiving high grooming frequencies between 7 and 8 years and this may be attributed to the fact that they started forming bonds as they socialize and struggle to acquire favorable ranks thus needing coalitionary support from each other. This means that the older animals are more likely to be predisposed to the disadvantages of receiving low grooming frequencies.

Six social groups in the Amboseli population were studied and the results of grooming received between groups were significant. In addition to this, the multivariate analysis shows that group has the greatest effect on grooming. This could be possibly due to the size of each group such that animals belonging to the larger groups (Nyayo-70 and Weaver-100) receive less grooming as compared to the smaller groups (Omo- 30animals, Viola-50 and Linda- 60). This may reflect a true difference between the groups in the rate of grooming, or it may simply reflect the fact that we sampled the groups heterogeneously, with a larger fraction of females being

sampled in some groups than in others (this is specified in Table 5.1.1). For the purposes of this analysis, the relevant point is that we must control for the effect of the group each animal belongs to, in identifying sources of variance in grooming. However to narrow down of the actual effects of group on grooming, a further analysis may have to be carried out and currently, this is beyond the scope of this study

Dominance rank in primates has been known to have an effect on grooming (Seyfarth 1977; Lazaro-Perea 2004). In this study, dominance rank influenced the amount of grooming received by an animal. The analysis was done for each sex separately but the overall effect was that animals lower in the hierarchy received less grooming, for both males and females. In females these results support the results and model presented by Seyfarth (1977) that predicted that lower ranking animals would be more motivated to groom higher ranking animals than the reverse, because of the help that high ranking animals can give them. The results show that higher ranking animal had a lower tickload as compared to the lower ranking baboons. This may have been due to the higher frequency of grooming received by these baboons.

The major tick species collected and identified in this study is *R. simus* which has been previously associated with baboons. *R. simus* and *R. pulchellus* have not been associated with haemoparasitic infections but they are found in the same habitat with wild and domestic animals and thus may pick up infections from them and further transmit them to baboons. Further work should be done to determine what infections these tick may be harboring.

The bivariate results showing the differences in tick load between the sexes can be explained by the results that show the amount of grooming received by females is significantly higher than that in males. In addition to this, the spearman correlation shows that there is a weak but significant negative correlation between grooming and tickload. These results show the role

of the frequency of grooming received in the reduction of ectoparasite load. The age of the animals is also a major contributor to the tick load and this can be tied once again to grooming because the older the animal the lower the amount of grooming it receives and thus the higher the number of ticks on its body. The older animals mainly spend their time on foraging and resting and thus spend more time in the vegetations where ticks are waiting to attach to hosts. The oldest animal, Kathryn was amongst the animals with high tick counts. The effects of group on tickload were not significant; the expected results would be to have fewer ticks on baboons which belonged to groups with the highest amounts of grooming received. This can be due to different reasons: (1) there is an extensive overlap in home range across the groups but in some cases there are habitat differences among the groups which may result in differences in exposure to ticks, (2), this may be attributed to the quality of grooming received such that the baboons have been receiving high counts grooming but poor quality of grooming, (3), as speculated and suggested by Brain and Borhmann (1992), the pain involved in removing tick together with the associated unpleasant taste may lead to inhibition of grooming to remove more ticks and (4) other dynamics such as social group composition and density mediated effects could also play a role in the differences in tick load in the social groups.

Direct effects of the ticks on the animals were visible such as wounds caused by the clustering of the ticks on various parts of the body (under the armpits, ears, neck and back region). These are areas that benefit from being groomed such that individuals that received less grooming were more seriously affected. Previous studies in baboons in the Namib Desert showed that ticks caused direct harmful effects to the extent that some of the animals died (Brain 1991; Bohrmann and Brain 1992). The results also show that there was a difference in the stage of the ticks by year. The 2007 animals were more seriously infested by adult ticks as compared to the 2008 animals, which had a higher load of immature ticks/larvae than of adults. There was a significant

difference in the mean PCV with the 2008 animals having a higher PCV than the 2007 animals. This could be attributed to the amount of adult ticks found on the individuals which in turn could have been due to the heavier rains in 2007 followed by a dry period in 2008 (Refer to table 5.1.2). The results for the white blood cell counts were within the normal range for all the baboons when compared to the known ranges for captive olive baboons.

The packed cell volume data was collected to aid in determining both the direct and indirect effects that the ticks could have caused. The packed cell volume declined by age as revealed by the correlation analysis and illustrated by a scatter plot (Figure 6.3.6). The results also indicated that the PCV could have been affected by the tick load, animals with higher tick loads also had lower PCVs. The slight but significant variability of PCV by sex could have been due to the fact that female baboons loose blood during their menstrual cycles. PCV may also be affected by many other factors such as worms (Oesophagostomam, Strongyloides), (Archie B, unpublished data) and other parasites affecting the blood system in general, which may also predispose them to anemia. However, this study made an assumption that since the animals lived in the same environment, they must have all been predisposed to the same risk factors, for instance, PCR screening revealed that all the darted animals had *Hepatocystis kochi* infection (Tung et al, unpublished data). Never the less, PCV data may give the ideal results and in future field studies should be augmented with other tests such as analysis of haemoglobin levels using a portable analyzer and red blood cell counts all which could not be carried in this study.

Ticks have been known to transmit several diseases in domestic animals, including diseases that are easy to detect in animals such as Babesiosis and Theileriosis. In the vertebrate host the main signs of these piroplasmic infections are haemoglobinuria, fever, anaemia and death. In addition, Brondson et al. (1999), indicates that *Entopolypoides*, which has close phylogenetic characteristics to Babesiosis may be transmitted by ticks although proof of this is

yet to be demonstrated. However, it is not known whether ticks play any role in the transmission of these haemoparasites to baboons. Given that the study area (Amboseli) is inhabited by man, wild animals (baboons included) and domestic animals (cattle, sheeps and goats), the assumption that some of these parasites would be found on any of the above and thus would be transmitted amongst them was made.

Both blood smears and PCR screening were used to identify haemoparasites in the baboons, but the results of these two methods were somewhat dissimilar. The results from the blood smear screening revealed none of the tick-borne parasites (although a number were infected with *Hepatocystis*, a midge-borne malaria-like parasite). This could have been due to the absence of the parasites in the animals or due to their presence but in very low concentrations. is prevalent in this population and some studies have indicated that *Hepatocysitis kochi* provides animals with immunity against *Entopolypoides macaci* (Moore and Kuntz 1975; Jenneby et al. 2008). This might explain why *Entopolypoides* was not detected in this population. The PCR analysis of *Babesia microti* amplified using BmicF1 and BmicR1 revealed a low prevalence of *B. microti* infection, at 4%. The general primers (F34 and R323, F79 and R206) used for the amplification of two *Theileria* species and the seven *Babesia* species revealed a very high prevalence of infection, but it is difficult to determine exactly what these primers amplified. There is also a possibility that the first set of primers (BmicF1 and BmicF2) were less sensitive or the general primer set ((F34 and R323, F79 and R206) also cross amplifies parasites that are not *Babesia* species, such as *Hepatocystis kochi*. The PCR results were interpreted by simply viewing the amplicons and comparing their results with the expected base pair sizes as indicated by Caccio et al. (2000). The amplicons observed were between the ranges of 170 and 200 which all indicated the presence of *Babesia* species only. These results warrant for additional molecular diagnosis such as sequencing of the amplified PCR products to narrow down on the exact species

of parasites these animals are harboring. These results do not show any potential benefits of ectoparasite removal in baboons as regards haemoparasite transmission.

3.0 CONCLUSION

The results of this study strongly suggest that grooming represents a behavioral means by which baboons reduce tick infestations and thus may protect them from the direct effects caused by ticks. Whether this also reduces the transmission of tick-borne parasites is unclear from this study. This study also shows that differences in social behavior (grooming received, dominance ranks and social groups) among animals, age and sex of the animals may result in differences in vulnerability to tick infestations and possibly tick-borne diseases; this needs further study with a larger sample.

The presence of *B. microti* in baboons in this study, in spite of previous studies that have concluded the lack of natural occurring *Babesia* organisms in nonhuman primates, is a result that should be explored further. These results may have two main implications: the possibility of a low prevalence of *Babesia microti* in nonhuman primates, and its surveillance in the Amboseli popoulation due to its status as an emerging zoonoses in humans; and secondly, the continued use of non human primates as models for human parasitic diseases, with the caveat that xenografts should be handled with care in order to avoid transmission from baboons to man.

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