STUDIES ON ROAD KILL DYNAMICS AND ITS POTENTIAL FOR WILD ANIMAL HEALTH INVESTIGATIONS: A CASE STUDY OF MIKUMI NATIONAL PARK

BY

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ABSTRACT

A study was conducted at Mikumi National Park (MINAPA) with the main aim of assessing the roadkill dynamics and its potential in the surveillance of the wild animal diseases in the park. Roadkill dynamics were assessed by recording the types, number and frequency of animals killed by road accidents identifying factors that predispose animals to highway accidents as well as identifying accident hotspot areas. Similarly, secondary data on roadkill counts for September-April months for 2003-2007 period provided by the park were used for comparison purposes. To assess the type of infestations and infections of wild animals in MINAPA, various types of samples were taken on road-killed animals and these were complemented by other samples (faeces and ectoparasites) taken away from the highway. A total of 314 roadkill cases were counted during the study period. Mammals (especially smaller-bodied ones) suffered more deaths (145) ($P<0.05$) followed by reptiles (107) and birds (62). The roadkill pattern observed for mammal and bird groups during the present study was comparable to counts recorded during the same months during the 2003 – 2007 period, but was higher ($P<0.002$) for reptiles. When categorized on monthly bases, total roadkill per month increased significantly ($P<0.05$) during December and January and least in April. Most of the bacteria isolates in samples collected *in-situ* from roadkills belonged mainly to the *Enterobactriceae* family (*Escherichia coli*, *Citrobacter spp*, *Klebsiella spp*, and *Proteus spp)* while internal and external parasites belonged to the Phyla *Platyhelminthes*, *Nemathelminthes* and *Arthropoda* of different families and genera. Faecal samples collected *ex-situ* showed that elephants had more egg counts whereas wildebeest had more coccidian oocyst counts than other animals investigated. *Rhipicephalus appediculatus* and *Amblyoma variegatum* were the main tick species found in the park. Undoubtedly, if this study is done over a long period of time, appropriate mitigation measures can easily be instituted to reduce the ever

increasing incidence of roadkills in the park at the same time providing a low cost disease surveillance approach to a wide range of species.

DECLARATION

I, EDSON RUGAIMUKAMU, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and it has not been submitted for a degree award in any other University.

Edson Rugaimukamu — 25/11/2009
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ABBREVIATION AND SYMBOLS

GPS	Global Positioning System
ml	Milliliter
NaCl	Sodium Chloride
G	Gram(s)
mm	Millimeter
%	Percent
m^2	Meter square
MINAPA	Mikumi National Park
Km	Kilometer
MSc	Master of Science
SUA	Sokoine University of Agriculture
TANAPA	Tanzania National Parks
GDP	Gross Domestic Product
V.I.C	Veterinary Investigation Center
TAWIRI	Tanzania Wildlife Research Institute
^oC	Degree Celsius
EDTA	Ethylene Diamine Tetracetic Acid
MR	Methyl red
VP	Vogeur Proskeua
pp	Page number
Spp	Species
EPG	Egg per gram
OPG	Oocyst per gram
i.e.	that is

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Wild animals are one of the valuable and most diversified natural resource in Africa and Tanzania (Baldus, 2009). This natural resource constitutes a unique natural heritage that is biologically and economically important to the country and the world at large. About 30% of Tanzania's arable land is now dedicated to conserving this natural heritage in vast protected areas some of which are of limits to human use (National Parks, Game Reserves and Open areas). This natural biodiversity attracts the involvement of both international and national conservation agencies with a mandate to maintain wildlife populations and their habitats in the natural state (Rodgers *et al.*, 2003). Some of Tanzania's protected areas now enjoy internationally recognition like the Selous Game Reserve, Serengeti National Park, Kilimanjaro National Park, Ngorongoro Conservation Area which have been designated as World Heritage Sites and the other two (Serengeti/Ngorongoro and Lake Manyara National Park) as Biosphere Reserves (Baldus, 2009).

The economic importance of wildlife sector in Tanzania is also apparent at local, regional, and national levels. The contribution of the Wildlife Sector to the Tanzanian GDP is currently estimated at between 7 - 10%, and wildlife-based tourism being one of the Tanzania's largest and most rapidly expanding sources of national revenue (DPG, 2009). Hence, any threat to this sector needs to be minimized if not avoided completely since it will automatically affect the nation socially or economically (Roskaft *et al.,* 2002). However, with the current increase in human population, economic struggles and cultural dependence on land, there is an increasing pressure on the preserved land and wildlife (Gryz and Krauze, 2008; Coelho *et al.*, 2008).

In any nation, roads and highway constructions are key development vehicles, but due to economic and political reasons, we now witness major highways built through protected areas contrary to conservation ethics with varying detrimental effects, some of which are receiving concern worldwide (Ament *et al.*, 2008; Elzanowski *et al.*, 2009). In Tanzania, highways are known to pass through three protected areas, namely Katavi, Serengeti and Mikumi National Parks (Elibariki, 2006). The impact of roads on wild lands and wildlife includes both the destruction of the habitats during construction and changes in the dynamics of the ecosystems (Trocme, 2006). The latter primarily include effects on animal populations whose home ranges are split by roads and mortality of individual due to collision with vehicle (Slater, 2002). Wilkie *et al.* (2008) reports that roads are closely linked to market accessibility, economic growth, natural resource exploitation, habitat fragmentation, deforestation, and the disappearance of wild lands and wildlife.

Mikumi National Park (MINAPA) is the fourth largest Park in Tanzania. The Park is bisected by a trans-national tarmac highway linking Dar es Salaam to Tunduma on route to Zambia and passes through the northern half of the Park. The highway was first paved in 1972, the length of the highway within the Park is 50 km and has width of 6.7m (Johnson, 2000; MINAPA, 2007). Wildlife killed on roads due to collision with vehicles (roadkill) is an issue that is increasingly becoming difficult to ignore and has been reported by Kidegesho (2001), Hamis (2002), Joseph (2006) and Magnus (2006). Almost all species of wild fauna are killed on the highway due to various reasons (MINAPA, 2007). Most of the road-killed animals left on the road are usually removed and thrown away by MINAPA staff but some are either scavenged or crushed completely by passing vehicles (Personal observation). None of the road-killed animals in MINAPA have previously been used to provide samples or any other materials which can be used for short or long-term conservation research programs. As animals are practically killed on everyday basis in MINAPA (Mofulu, F. personal communication), if used properly, these dead animals could indeed be a highly valuable source of samples for long-term surveillance health and other such studies.

The use of various samples collected from road-killed animals in MINAPA is indeed useful and possibly critical as specimen collection in wild animals for long-term conservation studies is usually a long standing logistical problem, since in most cases it largely depends on invasive methods requiring an animal to be captured (Mutayoba, 2002). This is usually a challenging activity because wild animals are not used to be handled by human beings (Gardipee, 2003). Past experience has shown that the invasive technique approach of obtaining specimens in wildlife research is also very expensive, needs to be done by experts and can have negative consequences to the well-being of the wild animals and people involved (Fumagwa, 2000).

The simplest method of obtaining samples is non-invasive approach which can be used singly or in combination in studying wild fauna without having to catch or even to observe them (Mutayoba, 2002). That approach is safe and cost effective and provides powerful means of collecting a large number of samples without disturbing the animals (Mutayoba, 2002). Samples which are normally being collected in non-invasive way are faeces, hair slip or feathers, urine, eggshells, snake skin and even bones from decomposed carcasses (Gardipee, 2003). Cropped animals for community use, naturally dead animals, and roadkills can be the best source of samples because a variety of specimen can be obtained as opposed to immobilized animals (Fumagwa, 2000). MINAPA roadkills can present an opportunistic way of getting samples in non-invasive way. They can be useful source of samples for wide-range of studies instead of getting thrown away by MINAPA staff.

Wildlife diseases are recognised by conservation biologists as an increasing challenge to the conservation of wildlife (Deem *et al.*, 2008). It is one of the limiting factors of species survival which can be amplified by anthropogenic changes on global scale (Deem *et al.*, 2008). According to Kock *et al.* (2009) the increase in livestock, in line with human demographic growth, causes close physical contact between people, livestock, and wild animals. As a result pathogens such as microbes and parasites benefit hugely from the dynamic state created by migration among them. Therefore, understanding the ecology of diseases is of paramount importance since it gives the general picture of the health status of animals in a given locale so that precautions and appropriate control measures can be established (Chapman *et al.,* 2009).

1.2 Problem Statement and Justification

Roadkill is one of the threats to wild animal survival and the ecosystem that needs to be monitored (Mata *et al.*, 2005). Elibariki (2006) reports the occurrence of loss of wildlife as roadkill and the paucity of information on the magnitude of the problem in MINAPA. The present study focused on the problems of animal-vehicle collisions in MINAPA. This was done by assessing the extent of road mortalities and their significance, likely collision points and assessment of factors determining collision risks. The data on roadkill patterns and the models that predict the most likely collision points can be of practical value. They can be used to predict the location of road sections with the highest collision probability and the specific crossing points with the highest risk and then mitigation measures can be sited accordingly.

As conservation programs expand and contact between humans, domestic and wild animals increases, disease transmission between them becomes a very significant problem. In the disease transmission process, wildlife plays a major role and is very important when addressing certain disease in domestic animals or humans (Arguirre and Tabor, 2008). Historically, diseases in wildlife have been of interest after they have had direct impact to livestock or human health (Arguirre and Tabor, 2008). According to Mlengeya and Lyaruu (2004), however, disease transmission has important implications not only for wild animal management, but also for public health, livestock development, and rural livelihoods. Therefore prompt detection of disease and its effective management will rely greatly on field surveys. Wildlife disease studies involve the collection of specimens from animals and these specimens can easily be obtained from road-killed carcasses. According to Gibbons (2008) some roadkills can directly provide materials for environmental education.

Although there has been substantial research with a wildlife disease component in Tanzania, Keyyu *et al.* (2003) report that there is paucity of information on wild animal parasites and other major infections and infestations of wildlife in Tanzania. Lack of knowledge on wild animal diseases and their dynamics invariably hamper efforts to control, prevent, or even eliminate those diseases that are threat to human health and biodiversity (Morner *et al.*, 2002; Mlengeya *et al.,* 2006; 2008).

Although the majority of living organisms are parasitic, infections and infestations are among the diseases which could help us to understand the health and disease dynamics of different animal species (Morner *et al.*, 2002). The present study took the advantage of collecting samples from roadkills for a period of 8 months to assess some of health issues related to wild animals found in MINAPA. This approach, if it is done for a very long period of time, it could provide the cheap means of conducting continued disease surveillance in long-term providing versatile ecosystem health information for the Park.

1.3 Overall study objective

To assess roadkill dynamics in MINAPA and its potential in the surveillance of the wild animal diseases in the Park.

1.3.1 Specific objectives

(a) To assess the types, number and frequency of animals killed by road accidents

(b) To identify accident hotspot and factors that predispose animals to highway accidents

(c) To assess the type of infestations and infections of wild animals in MINAPA (i) using road-killed animal samples and (ii) fresh faecal samples and ectoparasites collected away from the highway.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wildlife management and Infrastructure

Wildlife includes all non-domesticated animals, plants and other organisms in their natural habitat (Bolen and Robinson, 2002).Wildlife can be found in all ecosystems, deserts, rainforests, plains, and other areas including the most developed urban sites. While in popular culture the term usually refers to animals that are untouched by human factors, most scientists agree that wildlife around the world is impacted by human activities (Mbassa *et al.*, 2003; Wallgren *et al.*, 2007). The four major human-related activities include overexploitation, habitat destruction and fragmentation, impact of introduced new species and chains of extinction (Havlick, 2004). To minimise these impacts to wildlife and habitat, appropriate wildlife management strategies have to be put in place.

Wildlife management is the process of keeping certain wild animal populations, including endangered animals, at desirable levels determined by wildlife experts (Groom *et al.*, 2006). It deals with protecting threatened, non-threatened, endangered animal species and their habitats. Wildlife managers aim to use the best available science and technology to balance the needs of wildlife with their perception of the needs of people (Havlick, 2004). Wildlife management takes into consideration ecological principles such as carrying capacity of the habitat and is also concerned with the preservation and control of habitat aiming at minimizing external influences on the population and its habitat (Chapman and Reiss, 2000). This is appropriate in a National Park where one of the stated goals is to protect ecological processes (Chapman and Reiss, 2000). The construction of transport infrastructures, urban development and agriculture are some of the causes of fragmentation of wildlife habitat that are most quoted in literature (Sanz *et al.*, 2001). This goes against the wildlife management strategies since they cause impacts which alter the structure of the landscape and, therefore, the ecological processes associated with it (Sanz *et al.*, 2001).

Roads are common infrastructures for suburban and exurban development in developing countries and generate a wide range of environmental effects (Mata *et al.*, 2005; Row *et al.*, 2007). When built in wilderness or in a remote area close to wilderness, roads bring in items not native to a bioregion such as noise, garbage and also allows hordes of hunters, poachers, and trappers to access remote areas (Sanz *et al.*, 2001). Roads also bring not only pollution, but also bring with it the constant pressure of continued development (Bissonette, 2008). These developments cause an increased problem for terrestrial vertebrate conservation due to the increase of both road numbers and vehicle flow (Barrientos and Bolonio, 2009). Driving cars in the wilderness ends up killing animals purposely or accidentally (Mata *et al.*, 2005).

2.2 Wild animal roadkills and features that draw wildlife to the road or encourage lingering

Wildlife roadkill is the death of wild animals resulting from collision with a moving vehicle. It occurs because wild animals and people driving vehicles are on the road simultaneously, and cannot predict the behaviour of one another (Magnus *et al.*, 2004 Kelley *et al.*, 2007). According to Magnus (2006), the features that draw wildlife to the road or encourage lingering include areas where different land uses occur on opposite sides of the road. This represents diverse resources for wildlife, and therefore increases the likelihood that they will cross or spend time on the road. This includes areas where grasses and other herbaceous plants proliferate following previous fire burning, creating tender shoots which are attractive to herbivorous wildlife. Others are areas where standing water is likely to be present, especially in dry season (e.g. drains), and the sections of road where vegetation (habitat) is dense right up to the roadsides.

It is generally recognised that more wild animals are killed on roads where traffic is traveling quickly, but several other reasons predispose wild animals to become more susceptible to roadkill accident. According to Magnus (2006), aspects of road design also increase danger of wildlife to be knocked away. These designs include the areas where natural or artificial barriers occur on the roadsides after construction, which may make animal escape difficult (e.g. deep drains and guard rails), sharp corners and the areas of low visibility. Foods thrown from vehicles are also a major source of attraction to wildlife and carnivores are normally attracted by existing animal carcasses resulting from previous roadkill (Drews, 1995). Roads are also frequently used as open spaces for animals to socialize, especially primates, and for access to new territories for dispersing young (Magnus *et al.,* 2004).

Wild animals are also often present on roads simply through the action of crossing to the other side to gain access to crops, pasture, water or territories. In some conservation areas crossing roads is part of a regular migration route for some animals (Drews, 1995). Other animals are nocturnal leading to most encounters occurring in the dark or semi-dark periods. In addition, most nocturnal mammal species are dark in colour, resulting in low visibility for drivers (Drews, 1995). Driving at high speeds and inattentive driving also increases the likelihood of collision. Some roads are used by large numbers of heavy vehicles, which are difficult to slow down or maneuver quickly in response to wild animal crossing. In some situations drivers often have rigid schedule which discourage them from slowing down at wildlife killing hotspots or slowing to avoid accidents. Additionally, it is common for some drivers to be complacent about hitting a wild animal and there are instances of drivers deliberately attempting to hit animals and taking carcasses with them (Magnus *et al.,* 2004).

2.3 Animal Roadkill tragedy

Although there has been a substantial improvement on roadkill mitigation measures and fauna sensitive road design, a great tragedy is the quantity and species of wild animals that are struck and killed every year by speeding automobiles. Goff (1995) reported that more than half a billion animals, including 250,000 people, are killed every year on the planet's roads and highways. In America alone, each car kills an average of 3 to 4 vertebrate animals each year and cause endangerment of some species (Goff, 1995). These animal deaths are incredibly high because paved highways now dissect every major habitat in America (Gibbons, 2008). According to Havlick (2004), more than 100 black bears died on North Carolina roads in a single month and that a sampling of roadkill in just five States counted 15,000 dead reptiles and amphibians, 48,000 mammals, and 77,000 birds. In Australia, Taylor and Goldingay (2004) reports the occurrence of roadkill cases throughout the country at the rate of 0.3 roadkills/km or one roadkill every 3.8 km/week.

In Indiana, Plowman (2004) observed that during 274,210 miles of surveys, a total of 1,002 raccoons were killed by roadkill accidents during March 2004 only. This gave the index of 37 road-kill raccoons per 10,000 miles statewide. In Poland, the mortality of vertebrates was monitored on a local road running across Poland's Biebrza River Valley for 2 years (August 2005–July 2006). On the basis of the distance from the river and surrounding habitats, the road (of a total length 2,510 m) was divided into three stretches. The road was monitored on foot by two people every month, over a few consecutive days. A total of 1,892 roadkill representing at least 47 species were found. Of these, 90.7% were amphibians, 4.2% mammals, 3.1% birds and 2.0% reptiles. In Switzerland, Roedembeck and Voser (2008) concludes that roads act as a threatening factor for brown hare population and recommend establishing large un-dissected areas for these population and to protect these areas from being fragmented further. Elzanowski *et al.* (2009) conclude that in lowland Central Europe, the common toads, *Bufo bufo*, are the most common victims of vehicular traffic in suburban landscapes. In Tanzania, a study by Elibariki (2006) in MINAPA revealed that a total of 1199 roadkill cases were recorded, mammals being the leading group (468 cases) followed by birds (428 cases) and reptiles (311 cases).

2.4 Roadkill in Mikumi National Park and its causes

Wildlife highway mortalities have been reported to be a serious problem in MINAPA (Johnson, 2000; Kidegesho, 2001; Hamis, 2002; Joseph, 2006). A wild range of mammals, birds, reptiles, amphibians and arthropods are killed each year (Hamis, 2002). However, almost all wildlife capable of crossing the road are prone to highway accidents. According to Joseph (2006), mammals frequently killed in MINAPA including baboons, wildebeest, zebras, antelopes, jackals, and even elephants. The reasons for the high roadkill incidences in MINAPA are said to be high speed, careless driving, sliding of the tyres during wet season, animal behavior and water shortage during dry seasons (Joseph, 2006). Different categories of wild animal are killed for different reasons. Some are killed when searching for food and water (e.g., most herbivores), other are killed as they seek refuge on the warm road (e.g., reptiles), while others are killed as they eat the knocked down carcasses and thrown out garbage from the passing vehicles (e.g., primates and carnivores) (Elibariki, 2006).

Johnson (2000) conducted a survey on MINAPA highway to assess if there was relationship between roadkill of 13 large mammal species and four types of vegetation which are commonly found along the highway. The 13 large mammals investigated included baboons, black-backed jackals, reedbucks, buffaloes, elands, elephants, giraffes, impalas, lions, spotted hyena, warthogs, wildebeest and zebras. The four types of vegetation investigated included grassland of less than 1m height, grassland greater than 1m height, open woodland and woodland. He found that grassland of more than 1 m in height, eland, reedbuck, black-backed jackal, elephant, and wildebeest used area within 600 m of the highway while zebra in the same vegetation type used areas within 200 m of the highway. In woodlands, wildebeest used an area within 600 m of the highway. It was concluded that some different type of vegetation could influence the animal species distribution along the highway. This also had influence on the pattern of roadkill occurence in MINAPA.

Highway mortality patterns of large mammal (herbivore and carnivores) in MINAPA recorded by the Park Management between 1973 and 1988, during which 456 highway mortalities of large mammals were recorded, show that more roadkills are observed during the dry season than the wet season. Carnivores are most vulnerable to roadkill accident during the dry season in MINAPA due to their scavenging behavior. According to Johnson (2000), roadkills which occur during the dry season occur mainly due to search for water which makes animals to cross the road to access watering points.

2.5 Roadkill mitigation measures in MINAPA

Based on several research recommendations emanating from the work previously conducted to assess the impact of the highway on wildlife, the MINAPA management has taken various mitigation measures to reduce the incidence of roadkills. These include introduction of speed limit which is currently 70km/hr during the day and 50km/hr during the night period, introduction of speed bumps at specific location accross the entire 50 km sketch and large fines for careless drivers involved in accident in the Park (Elibariki, 2006). The implementation of speed bumps on highway started in 1999 and these speed bumps were designed and put purposely in areas with high rates of roadkill accidents (MINAPA, 2007). A total of 11 speed bumps now exist on the road, some are smaller and others are larger depending on the intensity of accidents in different vicinities (MINAPA, 2007). Despite these efforts many buses and other huge vehicles still go over majority of the bumps without slowing down (Personal observation). This suggests that this effort has not yet reduced significantly the incidences of roadkills especially that involve large vehicles. To date, roadkills are increasing year after year as better, faster and strong vehicles continue to be brought into the country and travel on this highway (MINAPA, 2007). Increasing the size of the bumps within the 50km stretch is not possible since it would curtail the movement of smaller vehicles in the Park.

Stern or large fines are another measure which was instituted in MINAPA to punish drivers who are involved in accidents in the Park including knocking down animals. These fines differed from one animal species to another. This measure was however difficult to implement since it requires a close monitoring and surveillance throughout the Park. Some of the drivers after hitting an animal have been observed to either trying to run away or some even take the animal away with them to conceal the accident (Elibariki, 2006). In addition to this, MINAPA also instituted the use of highway poster displays to warn or bring awareness to the drivers on what follows next. At the entrance gate in MINAPA, the road users are warned of occurrence of wild animals in the next 50 km road stretch. These warning signs are still being ignored by some irresponsible drivers. Though helpful, this measure has also failed to reduce the death of animals and incidences of roadkills are still increasing. Magnus (2006) conclude that it is important to consider that even if road signs do not reduce vehicle speed or collision rate significantly, they certainly have an effect on some individuals, and are therefore worthwhile.

2.6 Roadkill as source of health information in MINAPA

Traditionally, sample collection from domesticated animals for research investigations often involves the handling of these animals. This is because health monitoring in domestic animals focuses much more on individual animals in a herd and also because domestic species are relatively easy to handle (Fumagwa, 2000; Mlengeya *et al.*, 2002). This approach however, is relatively difficult in wild animal health investigations because the focus here is on the population rather than on individuals (Fumagwa, 2000). This has challenged wildlife experts and veterinarians to adopt new approaches in conducting surveys or research and do away with the traditional ways which requires handling of animals for specimen collection. The best alternative in doing research in the wild is to try as much as possible to obtain relevant and adequate information with minimum disturbance to the wildlife population (Mutayoba, 2002; Mlengeya *et al.*, 2002; Waits and Paetkau, 2005; Wasser *et al.*, 2004; 2005).

Although the information on population parameters is rarely collected from carcasses (Lovari *et al.*, 2007), cropped game animals for community use, dead animals caused by natural phenomena or accidents may serve as a good source of samples for research (Fumagwa 2000). There are currently a huge number of reports on the use faecal samples as the easiest alternative in wildlife research (Mutayoba, 2002; Waits and Paetkau, 2005; Wasser and Hunt, 2005; Gobush *et al.*, 2008). Faecal samples are usually readily available and can be collected in abundance. Using this type of samples has allowed researchers to move away from physical and chemical restraint on wildlife which is often used to obtain specimens for research and surveillances. Samples from road-killed animals can easily be obtained while fresh with fewer restrictions especially in studies which involve elusive species or endangered ones (Mutayoba, 2002; Wasser *et al.*, 2004).

2.7 Wildlife disease study and its importance

There are two main important reasons for studying wildlife diseases. First, there is substantial evidence that has shown that diseases can greatly impact local species populations by causing temporary or permanent declines in abundance (Dobson and Caper, 2000; Robert *et al.*, 2006; Smith *et al.*, 2009). Diseases of wildlife can cause significant illness and death to individual animals and can significantly affect other wildlife populations (Mlengeya and Lyaruu, 2004; Halpennty and Gross, 2008; Smith *et al.*, 2009). More importantly, these pathogens can interact with other driving factors such as habitat loss, climate change, overexploitation, invasive species and environmental pollution to contribute to local and global extinctions (Robert *et al.*, 2006).

Secondly, the increase in livestock in line with human demographic growth necessitates contact among wild animals, domestic animals and human beings. Zoonoses with a wildlife reservoir represent a major public health problem affecting all continents. Hundreds of pathogens and many different transmission modes are involved, and many factors influence the epidemiology of the various zoonoses (Morner *et al.*, 2002). The importance and recognition of wildlife as a reservoir of zoonoses is increasing (Mtambo *et al.*, 1997; 2000; Arguirre and Tabor, 2008). Cost-effective prevention and control of these zoonoses necessitate an interdisciplinary and holistic approach and international cooperation. Surveillance, laboratory capability, research, training and education, and communication are key elements in zoonotic disease control (Daszak, *et al.*, 2006).

Due to the above reasons, Deem *et al.*, (2008) report that infectious diseases have to be recognized by conservation biologists as an increasing challenge to the conservation of wildlife. The challenge here is how to detect and recognize the existence of the disease in a particular locale, since wild animals are not constrained by boundaries and can extend over large distance (Daszak, *et al.*, 2006). However, for animals that congregate in large numbers in the open, unusual outbreaks of mortality or diseases may be quite visible and early detected. In case of cryptic or more secretive species of wildlife or those located in remote areas and jungles, the presence of disease comes to light as a result of other biological collections of wildlife or from surveillance effort (Morner *et al.*, 2002). Also Lembo *et al.* (2008) have observed that knowledge of infection reservoir dynamics is critical for effective disease control, but identifying reservoirs of multi-host pathogens is challenging. For efficient wildlife disease monitoring, intensified disease study is preferred. Such study aims at collecting a certain number of samples from a target population over a long time to detect the prevalence of certain pathogens. These studies if used as an important component of wildlife management programs, can allow the management to stem specific interventions measure to curb animal catastrophes in their ecosystem (Morner *et al.*, 2002).

2.8 Overview on some wildlife disease; what are the players?

2.8.1 Microparasites in wildlife health

Infections are among many of the diseases in wild animals which are caused by micro- and macroparasites, respectively (Hudson *et al.,* 2002). These disease conditions give serious problems in wildlife health management strategies. Both plant and animals can be infected with microparasite of different types (Dobson and Caper, 2000). Microparasites include viruses, bacteria, and fungi which are characterized by their ability to reproduce directly within individual hosts. They are small in size with relatively short generation time, allowing rapid increase of their populations within their host causing a crisis that leads either to the death of host or the development of immunity (Dobson and Caper, 2000; Hudson *et al.,* 2002). If organisms causes a disease in its host which leads to the death of the host, it is called a pathogen.

Among the bacteria which are commonly encountered in wildlife belong to the family *Enterobactericeae*. Member of this family are normal habitants of the intestinal tracts of vertebrates although within this family some species/strains are recognized as pathogenic (Bradley *et al.*, 2001). Some well-known examples of family members are: *Escherichia coli*, *Salmonella*, *Yersinia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Shigella* and *Proteus* (Wassenaar, 2009). In mammals the pathogenic strains may cause diarrhea and septicaemia in juveniles and rarely in adults. In birds, pathogenic strains may cause outbreaks of mortality, for example, in birds attracted to artificial feeding stations (Bradley *et al.*, 2001). *Klebsiella* develop a variety of pathological condition in animals as secondary infections as well as primary causes of suppurative lesion or of generalized infections (Wen liang and Yin ching, 2008). They have been isolated from inflammatory and suppurative processes in fowls, especially pneumonia and from cases of metritis in mares and sows. They have also been associated with atrophic rhinitis in swines and air sac infection in birds (Wen liang and Yin ching, 2008).

Proteus and *Citrobacter* species occur naturally in the environment of animals and man and particularly in the intestines (Mohanty *et al.*, 2008; Lipsky *et al.*, 2008). *Proteus* species also occur in animal manures, sewage, soil, and water and may be found associated with abortion and diseases of the newborn animals, otitis, peritonitis, dysentery in canine and swine as well as nervous disorders. (Wassenaar, 2009). *Citrobacter* species are also commonly found in soil and water and in many wildlife habitats (Mohanty *et al.*, 2008).

Other bacterial species encountered include *Bacillus subtilis, Staphylococcus epidermidis and Pasteurella multocida. B. subtilis* is mostly found in soil and is spread by water, wind and any moving object. Though *B. subtilis* is not pathogenic, sometimes it can be isolated in pure culture from internal organ such as liver. (Madigan and Martinko, 2005) *S. epidermidis* constitute part of the bacterial environment of animal and man world wide. It occurs commonly on the skin, anterior nares, saliva, intestine and faeces of many species of animals as well as in water, soil, and air. These are non-pathogenic strains of the *Staphylococcus* found in mans or animals (Ryan and Ray, 2004).

P. multocida is also another type of bacteria commonly isolated in many animals and are potentially pathogenic in domesticated and wild animals world wide (Blanchong *et al.*, 2006; Bredy, 2007; Al-Hassani *et al.*, 2007). *P. mult*ocida has been frequently identified in the respiratory tract and sometimes the intestine of apparently healthy animals and under certain predisposing conditions the organisms may multiply in those individual and cause severe and perhaps fatal infection (Boyce, 2004). Since typical organisms have been isolated from respiratory and digestive tracts of normal animals, the primary relationship of organism to the disease has been frequently doubted (Al-Hassani *et al.*, 2007). These organisms may act as secondary invaders to other diseases or to debilitative predisposing factors such as severe weather, long truck or train ride and faulty nutrition (Boyce, 2004). Ecologically, the bacteria spread through contaminated drinking water and waste. Inhalation is also another means of transmission of the *P. mult*ocida bacteria. Disease outbreaks have been shown to follow bird migration routes, especially the snow geese (Blanchong *et al.*, 2006). Wildlife biologists and veterinarians believe that these bacteria are transmitted by carrier birds or live in contaminated wetlands throughout the whole year (Blanchong *et al.*, 2006; Bredy, 2007).

2.8.2 Macroparasites in wildlife health

Macroparasites include helminthes (endoparasites) and arthropods (ectoparasites), both of which do not multiply directly within an infected individual but instead produce infective stages that usually pass out of the host before being transmitted to another host (Chapman and Reiss, 1995; Dobson and Caper, 2000). Compared to microparasites, they are relatively large, have long generation times, and are characterized by a great diversity of antigen so that immunity is transient and is a function of the history of infestation. Infestations tend to be chronic leading to morbidity rather than mortality (Hudson *et al.*, 2002). If macro and microparasites population increases in number, so that the host organism has a heavy parasitic load, then this is likely to weaken or even kill the host. Most wildlife populations have low levels of disease prevalence (Chapman and Reiss, 1995). But sometimes a disease will multiply and spread rapidly in a population causing unnoticed epidemics. An epidemic can be caused by a particular vigorous or virulent strain of macroparasites and may be assisted by some other factors such as environmental conditions (Halpennty and Gross, 2008). Disease epidemics are more likely to spread if the host species is very common or closely spaced together.

Parasites cause a multitude of clinical and subclinical problems to wildlife (Bliss, 2007). Clinical parasitism is a condition where parasite numbers have reached a point that the negative effects of parasitism are visible with the naked eye. Subclinical effects are difficult to see and measure, and usually manifest as reduced growth rates, reduced reproductive ability, reduced milk production for the young, and a reduced ability of an infected animal's immune system to fight off other disease conditions.

Endoparasites are worms which include nematodes, or roundworms; cestodes, or tapeworms; and trematodes, or flukes (Alexakis, 2009). The economical importances of these internal parasites differ significantly depending on species and its pathogenicity (Erick, 2009). The following are roundworms commonly found in domestic and wild species include:

(a) *Trichostrongylus*

The genus *Trichostrongylus* nematode belong to the family *Trichostrongylidae* (Alexakis, 2009). The species of this genus are small, slender, pale reddish brown worms without a specially-developed head end and have been reported as parasites of the gastrointestinal system in wild bovids and cervids (Samuel *et al.*, 2001; Horak, 2003). The parasites penetrate into the mucosa under the epithelium, rarely deeper, producing desquamation, which may be extensive in heavy infestations (Alexakis, 2009). The worms are not blood-suckers; nevertheless anaemia may be associated with heavy infection due to shortening of the life of the red cell, impaired erythropoiesis or to a reduction of the amino acid pool (Soulsby, 1983).

(b) *Haemonchus*

Haemonchus are parasites of the abomasums and are broadly distributed nematodes in ruminants throughout the world (Horak and Louw, 2005). They are commonly known as "stomach worm" or "wire worm" of ruminants and they are commonly pathogenic parasite (Samuel *et al.*, 2001). The principal feature of *Haemonchus* infection is anaemia, due to the blood-letting activities of the parasite (Soulsby, 1983). Nematodes of this genus are characterized by a prominent buccal tooth, well developed synlophe, and copulatory bursa in the male.

(c) *Moniezia*

The genus *Moniezia* belongs to the *Cyclophyllidae* family. The species in this genus occurs in the small intestine of ruminants in most parts of the world and only young are affected (Erick, 2009). A wide divergence of opinion exists regarding the pathogenic effects of the *Moniezia* species (Soulsby, 1983).

(d) *Raillietina*

The species in this genus are tapeworm that infects birds. Although they are small, they are very harmful parasites. They penetrate deeply into the mucosa and produce marked enteritis, which is frequently haemorrhagic in heavy infections. This causes the formation of nodules, which must be differentiated from tubercular nodules (Samuel *et al.*, 2001).

(e) *Metroliasthes*

The species in this genus also are tapeworm infects birds. They are rather rare occurring in the small intestine of fowl. The intermediate hosts are grasshoppers of genera *Chorthippus, Paraxya* and *Melanoplus* (Samuel *et al.*, 2001).

(f) *Heterakis*

Genus *Heterakis* belongs to the family *Heterakidae* and the member of this genus are medium- to small-sized with three lips around the mouth. They occur in the caeca of the fowl, guinea-fowl, pea-fowl, turkey and numerous other birds (Daniel, 2009). The infection is mildly pathogenic. However, they often carry a protozoan parasite *Histomonas meleagridis* which is the cause of blackhead disease (Chalvet-Monfray *et al.*, 2004).

(g) *Hymenolepis*

Genus *Hymenolepis* belong to the family *Hymenolepidae*. The genus contains a large number of species which occur chiefly in domestic and wild birds (Shen *et al.*, 2009). They are rather difficult to distinguish, but a generic diagnosis is sufficient for most practical purposes. However, the worms are narrow and thread-like in appearance (Soulsby, 1983; Duclos and Richardson, 2000).

(h) *Subulura*

Genus *Subulura* belongs to the family *Subuluridae*. Species in this genus occur in caeca of the fowl, turkey, guinea-fowl, and related birds in Africa (Soulsby, 1983). Pathogenesis is apparently not clear.

(i) Coccidia

Coccidia are exceptionally common protist parasites of both vertebrates and to lesser extent invertebrates (Mtambo *et al.*, 1997; 2000; Samuel *et al.*, 2001). Coccidia species which parasitise wild animals are those which belong to four genera contained in two families; *Eimeriidae* (*Cyclospora*, *Eimeria*, *Isospora*) and *Cryptosporidiidae* (*Cryptosporidium*) (Mtambo, 2000; Samuel *et al.*, 2001). Most the wild animals can be found to be infected with coccidian parasites at one or more times during their life and some may be infected during their entire lives with several species that constantly cycle through them. Given this ubiquitous nature of the coccidia, it is likely that most are probably harmless under natural wild condition (Arnastauskene, 2008). It is only when hosts are brought together in groups, enhancing transmission via their rapid, direct life cycle, that some species cause diseases. As a result, coccidiosis is recognized as a major health hazard only during intensive husbandry of domestic animals, in wild animals that are in captivity (e.g. zoos, breeding or research facilities), in wild animal populations when habitat is lost and crowding occurs or in wild animal species that have great reproductive potential and are protected by laws so that their population increase inordinately (Samuel *et al.*, 2001).

2.9 Wildlife Disease in Tanzania

Occurrence of major disease outbreaks in wildlife in Tanzania in the recent years seem to correlate with increase in the conflicts between people and wildlife around many of the protected areas due to fast increases in the human population and their activities (Mlengeya and Lyaruu, 2004). These activities include settlements, agriculture, livestock husbandry, deforestation, charcoal burning, tourism, and research. Disease transmission from livestock to wildlife and *vice versa* in Tanzania (Mtambo *et al.,* 1997; 2000; Cleaveland *et al.*, 2004) and elsewhere (Morner *et al.*, 2002; Ezenwa, 2004; Daszak *et al.*, 2006; Deem *et al.*, 2008) has become a more serious problem due to the increasingly constrained ecosystems.

Diseases which have continued to have a major impact on wildlife health includes sexually transmitted disease (STD) in baboons in Lake Manyara and Gombe of yet undefined etiology that seems to be associated with humid or damp conditions (Mlengeya and Lyaruu, 2004), ear disease in giraffes in Mikumi National Park and Selous Game Reserve that was first observed in 1999 in two giraffes but now has spread to affect giraffes throughout Mikumi National Park and is spreading further south into Selous Game Reserve, the largest wildlife area in Africa (Mlengeya *et al.*, 2002; Kagaruki *et al.*, 2003; Mlengeya and Lyaruu, 2004). Others disease outbreaks include a skin disease which seems only to affect giraffe in Ruaha National Park, possibly caused by *Dermatophilus spp.* and characterized by hair loss, followed by raising of the affected area, and later wrinkling, cracking, and encrustation. The disease was seen in the Park in 2000 and has continued to spread to other areas of the Park (Mlengeya and Lyaruu, 2004). In Gombe National Park chimpanzee have also been observed to suffer from human-related health problems including polio, scabies, pneumonias, helminthoses (Mlengeya and Lyaruu, 2004) which seem to pose a major threat to the surviving highly threatened chimp population. Fatal respiratory outbreaks of human-related metapneumovirus have also recently been described in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania (Taranjit, 2008).

Mlengeya and Lyaruu (2004) also described frequent seasonal deaths of sitatunga and bushbuck in Rubondo Islands National Park which have now been found to be associated with a combination of factors that include, high infestations with lice *(Melophagus spp)*, ticks *(Rhipicephalus appendiculatus* and *Amblyoma variegatum)*, and biting flies *(Hydrotea irritans* and *Stomoxys calcitrans*), heavy worm infestations, trypanosomoses, environmental changes, biotic and abiotic changes in the Lake waters and possibly genetic factors (Mlengeya *et al.*, 2008).

Other wildlife diseases which have been described but have low morbidity include foot-and-mouth disease in wildebeest in the Serengeti ecosystem, tuberculosis in lion, buffaloes, giraffes and wildebeest in many Parks (Mlengeya and Lyaruu, 2004, Cleaveland *et al.*, 2004;2007), and salmonellosis and anthrax outbreaks in elephants in Tarangire and Serengeti, respectively (Mlengeya and Lyaruu, 2004). Sleeping sickness seems to be endemic in and around northern National Parks and rabies is endemic in or around all Parks except Rubondo National Park (Mlengeya and Lyaruu, 2004).

However, the ability of Tanzania to address diseases in wildlife is still low because of the expansive nature of the conservation areas in the country, the diversity of species and inadequate specialized human resource and laboratories, funding and low awareness among decision makers of the impact of disease on wildlife (Mlengeya and Lyaruu, 2004). Mikumi National Park also lacks such capacities and hence no short or long-term disease surveillance programs are in place in the Park. Since roadkills in MINAPA is a rampant and long-standing phenomenon; objectives of present study included using these roadkills as a readily available source of variety of samples to address some of the health problems currently facing the wild animals in the Park.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This study was carried out for a period of eight months between September 2007 to April 2008, and was purposefully designed to meet the study specific objectives.

3.1 Study Area

3.1.1 History and site description of Mikumi National Park (MINAPA)

The present study was conducted in MINAPA (Fig. 1) which is the 4th largest National Park in Tanzania. The Park was gazetted in 1964 and named "MIKUMI" after the Village just beyond its border on the Dar es Salaam – Zambia highway. The village in turn, took its name from the local word "Mikumi", the Wavidunda vernacular name for the Borasus palms, which is also known as *mikoche* in kiswahili language and scientifically known as *Borassus flabellifer* (Anonym, 2000).

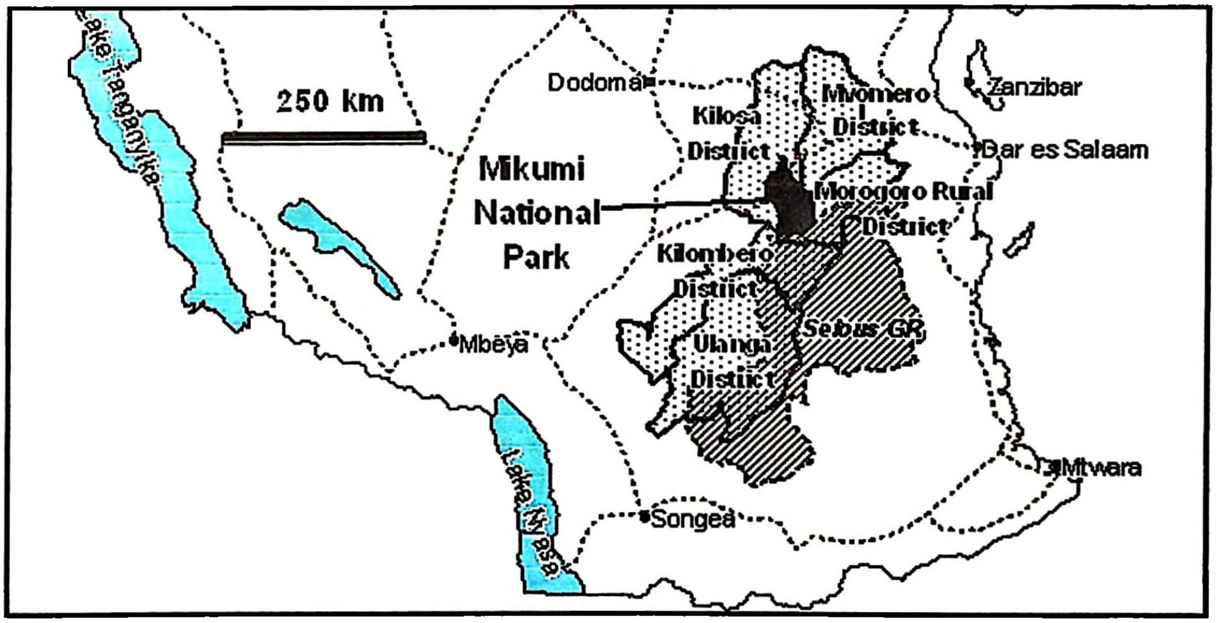


Figure 1: Map of Central-South Tanzania showing location of Mikumi National Park

Source: Animal Behaviour Unit (ABRU) –MINAPA

These trees are recognized by their straight, spindle-shaped pale trunk which grows in profusion in the Park, but rarely seen in the Mikumi village to date (MINAPA, 2007). MINAPA is located in Morogoro region, about 283km (175 miles) west of Dar es Salaam and 107 km (67 miles) from Morogoro town on the highway from Dar es Salaam to Zambia which bisects the Park for about 50 km (Fig.1).

The Park covers an area of 3,230 square kilometers and lies between latitude 7°00' and 7°50'S and longitude 37°00' and 37°30' E (Ereckson, 2001). It is enclosed by Uluguru Mountains to the north-east, Rubeho Mountains to the north-west, Udzungwa Mountains to the south-west and Selous Game reserve to the south. The Park shares one ecosystem with Selous Game reserve, thus allowing migratory animals such as elephants, buffaloes and zebras to move between these two protected areas.

3.1.2 Climate

MINAPA receives an average amount of annual rainfall of 508mm but this amount increases gradually with altitude (Moder, 1994). The Park has a bimodal rain season characterized by short rains between October and early November, followed by long rains lasting for five or six months, although the rainfall pattern is unpredictable. The rain pattern varies in different zones of the Park. Kimambo (2000) found that at the Park headquarters the average rainfall was 635mm per-annum but along the hills it was as high as 1067mm per-annum. Although there is a definite dry period in January and February, the wet months are associated with a hot, humid weather, where temperatures reach up to 30°C. Dry months are always cooler with temperatures between 20°C to 25°C. Annual average temperature is 25.5°C. The dry period is between June and September and temperatures range between 24°C in December and 20°C in July (Anonym, 2000).

3.1.3 Vegetation and landform

MINAPA lies within a horseshoe of mountains formed by the Uluguru mountain (east) and forest foothills of Vidunda (southwest), that have different vegetation and land form. These have been described by Kagaruki (2005) as follows:

(i) The Mkata river flood plane grassland that extends from Doma Game controlled Area to Vuma Hill forms the principal unit of the Park.

(ii) Miombo woodland, is the second largest unit and extends from Vuma hill to Kitangawizi.

(iii) Thickest vegetation encircled within the Miombo woodland.

(iv) Mixed woodland, which merges with wooded grassland, forms what has been named Secondary Seasonal Savannah. This forms the largest vegetation type within the Park.

(v) Acacia-Dalbegia woodlands, is found in the northern part of the Park, where it also occurs with patches of pure stand of *Dalbegia meloxylon* mixed with acacia species and a few *Lonchcarpus* species

(vi) *Combretum* woodland, is relatively smaller compared to the rest of the vegetation type in the Park and forming a continuation from the Acacia species.

(vii) High-elevation forest in patches within the Miombo woodland and along riverbanks (riverine vegetation), for example the forest in Matambiko, Makarakatu and Gombati

(viii) Montane rain forest occupies the highest peak of the Park, forming part of the eastern arc montane forest and covers the peak of Malundwe and Ngolwe mountains and

(ix) Other trees that occur in the Park are the giant baobabs especially in the south of the Park which is considered to be the largest tree in the area. *Hyphaene* and *Borassus* palms are dotted throughout the Park and along the water courses.

3.1.4 Animals

MINAPA is very rich in diversity and numbers of wildlife animals which include mammals, reptile, birds, and amphibians. The popular animals in the Park include the zebras (*Equus burchellii*), wildebeest (*Conochaetes taurinus*), impala (*Aepyceros melampus*), giraffes (*Giraffa camelopardalis*), buffaloes (*Syncerus caffer*), eland (*Tragelaphus oryx*), hippos (*Hippopotamus amphibious,* lions (*Panthera leo*) and the rare wild dogs (*Lycaon pictus).* More than 400 bird species have also been recorded in the Park.

3.2 Study design and data collected

This work constitutes two different but complementing studies which were designed to meet specific study objectives. The first study addressed the roadkill patterns in MINAPA and had three objectives: (i) to assess the types, number and frequency of animal killed by roads accidents, (ii) to identify the accident hotspots and (iii) to identify factors that predispose animals to highway accidents. In the case of these three objectives, two types of data were collected:

(i) Primary data were collected by the researcher and covered the period between September 2007 and April 2008. This involved counting and identifying all mammals, reptiles and birds which were killed by road accidents during that period on a daily basis. In addition, GPS locations were recorded at every point where roadkill accident occurred. Furthermore, all water points including permanent and temporary water holes and the locations designated as animal preference areas were also GPS marked.

(ii) Secondary data in this study were kindly provided by MINAPA Ecology Department. The data provided included the type and number of mammals, birds and reptiles killed by road accidents during the period of 4 years from 2003 to 2007 (4 years). To enable the harmonization of the secondary data with the primary data, only secondary data which was taken between April to September in each year were used. These were the months when the primary data was collected in this study. Additional secondary data which was provided by MINAPA included minimum and maximum ambient temperatures and rainfall pattern for the period between September 2007 to April 2008.

The second study involved the investigation of health dynamics of wild animals in the Park. In this study, a wide range of samples were collected on animals and birds killed by road accidents to assess the various types of infection and infestation of each animal and birds. As species diversity of roadkills was diverse, an attempt was done to complementing these *in-situ* - collected samples by collecting additional samples from the field. These samples included fresh faeces from few selected animals and ectoparasites. These additional *ex-situ* samples allowed objective health investigations for selected species as the numbers of roadkills within the study period were insufficient in numbers to provide statistically acceptable assessment of health dynamics of these animals. For *ex-situ* faecal sampling, the samples were collected 20m to 4km on each side of the highway. Ectoparasites were collected in five locations within MINAPA, namely Chamgole, Kisungura, Ikoya, Kikoboga and Mwanambogo.

3.3 Primary Study: Roadkill dynamics in MINAPA

3.3.1 Assessment of type, number and frequency of animal killed by road accidents

This study was conducted on a 50 km Dar es Salaam - Zambia highway which bisects MINAPA from near Doma village (Dar es Salaam side), at the eastern Park boundary to near Mikumi village (Mikumi village side) at the second gate where the Park ends (Plate 1). In this study all mammals, reptiles and birds killed by road accidents were identified and counted. These data were collected by an observer once a day starting from 6 a.m in the morning using a vehicle, GPS (Garmin GPS 36) and data sheets. The survey vehicle was a Toyota Landcruiser with good viewing windows. The vehicle was stopped at each roadkill item and the carcass was identified. The location from the GPS was recorded and entered in the data sheet. If identification was not possible, then it was recorded as unidentified mammal, bird or reptile. The carcass was removed to avoid double-counting on subsequent counting.

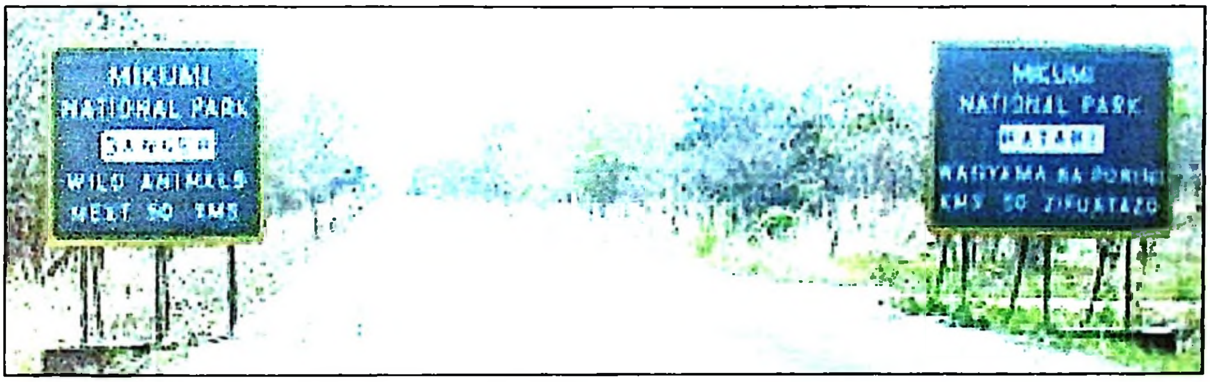


Plate 1: MINAPA exit site towards Mikumi village

3.3.2 Identification of roadkill hotspot

This study was also conducted on the Dar es Salaam Zambia highway from the first entrance where the Park starts (Doma side) towards the Mikumi village the second gate where the Park ends (50 km). This study also covered the period from September 2007 to April 2008. Data were collected by locating the areas at which roadkill accidents occurred most. These areas were termed as roadkill hotspots. To be able to locate these areas the road was divided into sections that covered the distance between adjacent speed bumps, which were represented by alphabets as A,B,C,D,E,F.G,H,I,J and K (Fig. 2). The first speed bump marked starting from the entrance gate situated closer to Doma village was designated as A and the last speed bump near the exit entrance next to Mikumi village as K.

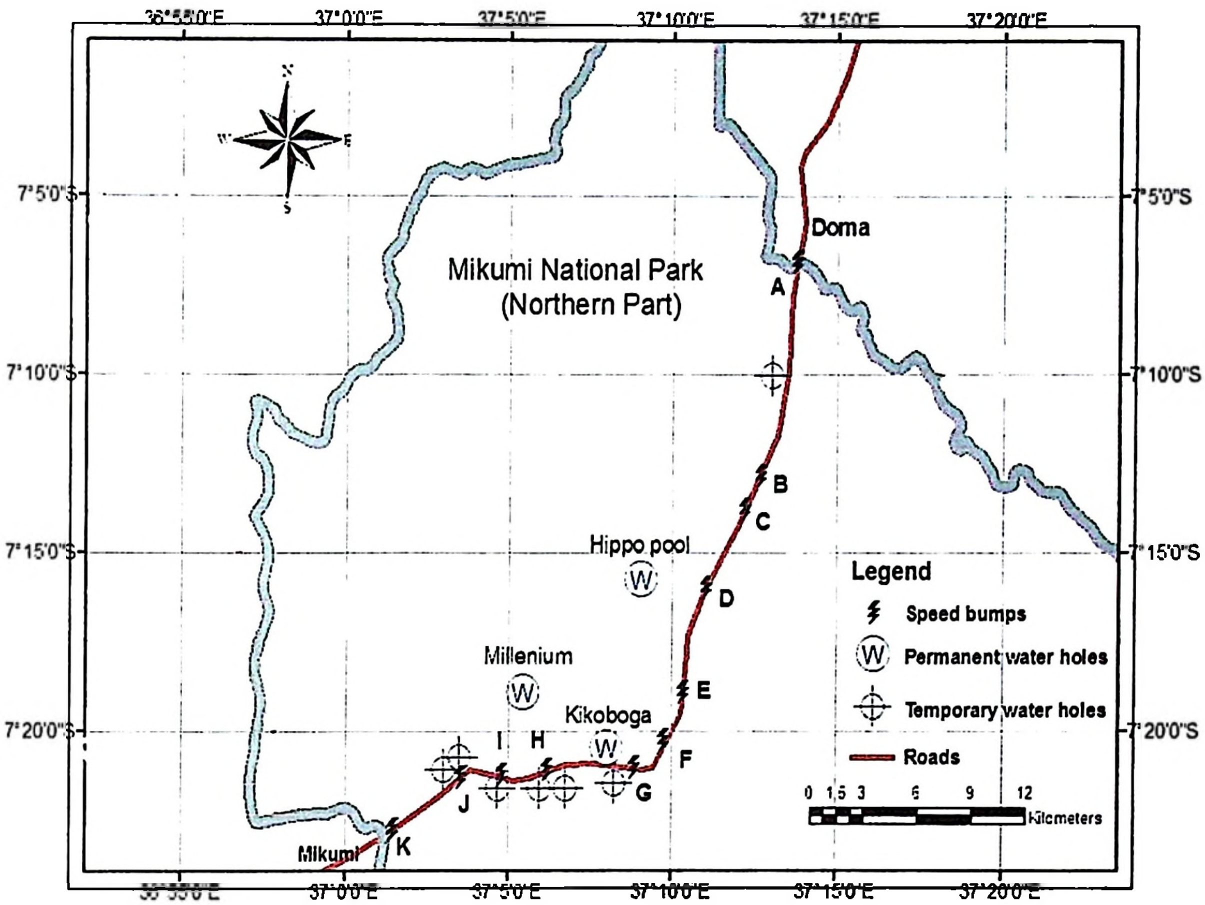


Figure 2: Northern part of MINAPA showing locations of road sections designated as A to K and location of water holes between road bumps

3.3.3 Assessment of factors that predispose animal to road accident

This study was also conducted on the 50 km Dar es Salaam – Zambia highway that bisects MINAPA. In this study, GPS locations were recorded at every point where accidents occurred, and all water points including permanent and temporary water holes along the highway were recorded (Fig. 2 and Appendix 1). Location designated as animal road crossing points were also marked (Appendix 1). In addition, data on temperature and rainfall pattern were also collected from the Park Headquarters (Appendix 6).

3.4 Studies on health dynamics of MINAPA animals

3.4.1 Study on the infection and infestation of the road-killed animals in MINAPA

Various samples for microbiological studies were collected aseptically from visceral organs (i.e. liver, lungs, spleen, and lymph node) (Plate 2). These specimens were stored into sterile universal bottle. The specimen were labeled and put into cool box and thereafter transported to SUA labs as soon as it was possible for further analysis. If not sent to SUA immediately, the samples were frozen at the mini laboratory present at Mikumi Veterinary Unit.

Plate 2: Collection of samples from African civet carcass for laboratory studies

Live worms and intestinal content were collected into clean containers. Ectoparasites also were carefully removed from dead animals and put in containers with 70% alcohol added as a preservative for ectoparasites and worms. Skin scrapings were also collected into clean sterile container but no preservative was added. All these samples were put into a cool box and immediately sent for analysis. Reference books by Soulsby (1983), Chowdhury (2001), and Manual of Veterinary Parasitological Laboratory Techniques, (1986) 3rd Edition were used as guide for collection, preservation and analysis of specimen.

3.4.2 Collection of *Ex-situ* samples for assessment of health of animals in the Park

Two types of samples were collected from the field, including fresh faecal samples and ectoparasites. A total of 442 fresh faecal samples were collected from selected animal species, namely buffaloes, wildebeest, giraffes, impala, and elephant. This included 95 samples for buffaloes, 92 samples for wildebeest, 99 samples for giraffes, 71 samples for impala and 85 samples for elephant. The samples were collected from the ground in the field. The most suitable samples were those collected fresh from animal, observed in the act of defaecation. Samples were collected into 30 ml wide mouth screw capped bottles which were filled to the top in order to exclude as much air as possible to diminish the rate of development and hatching of the worm eggs. The samples were put in the cool box and transported to SUA Parasitology laboratory for examination.

In addition to the collection of faecal materials, tick survey was also done for the purpose of complementing the *in-situ* study. This survey was done by using the cotton cloth of 1 meter square. This cloth was made stiff by putting a piece of wood in two opposite sides and the holding rope on one side. The cloth was dragged in one $1000m^2$ in each of selected five locations in MINAPA at a slow walking pace over the ground (Plate 3). Every $100m^2$ the drag was turned over and examined for ticks. The ticks attached on the cloth were collected into a container containing 70% alcohol. The identification of ticks was done at SUA. The surveyed location included Mwanambogo, Kikoboga, Kisungura Ikoya and Chamgole.

Plate 3: Tick survey using cotton cloth

3.5 Laboratory analysis

3.5.1 Isolation and Identification of microorganisms

Bacterial isolation and identification was done at SUA Microbiology laboratory using standard laboratory techniques as described by Barrow and Feltham (2004), and Carter and Lema (1998) (Appendix 7).

a) Isolation of microbes

Isolation of pure cultures was essential for the identification of bacteria and this was achieved by plating out on a solid culture medium in a Petri dish. After having isolated the microbes in pure culture, then microscopic examination was performed after staining with Gram stain and observed at 100x magnification. With this technique, the bacteria were differentiated as Gram positive or Gram negative depending on the individual reaction to this stain (Appendix 7).

b) Tests for metabolic products

The test for metabolic products was also performed to further identify the bacteria at the species level according to the technique described by Barrow and Feltham (2004). Pure culture of bacterial colonies of interest were taken aseptically and inoculated in special media for biochemical test. The mixture was incubated at 37°C. The colour change of the specific medium in specified patterns gave an indication on the type of bacteria which was identified (Appendix 7).

c) Identification of ecto- and endoparasites

The live-collected specimens of ticks and worms were identified using standard procedures described by Smith and Stinson (1998), Soulsby (1983), Carter and Lema (1998), Chowdhury (2001) and the Manual of Veterinary Parasitological Laboratory Techniques (1986). The above procedures involved egg-counting, faecal cultures for identification of larvae and identification of ticks. The counting of eggs and oocyst was done using the MacMaster method.

Since many nematode eggs are closely alike morphologically and cannot be easily differentiated in the identification process, the faecal culturing technique for hatching and developing into larval stage is usually a more suitable tool for their identification. This technique of culturing, recovery and identification of larvae from faeces was done according to Smith and Stinson (1998), Soulsby (1983), Carter and Lema (1998) Chowdhury (2001) and the Manual of Veterinary Parasitological Laboratory Techniques (1986). Culturing was done by breaking the faeces in fine particle using a motor and a pestle. The faeces were moistened with water until the correct consistency was obtained. The mixture was transferred into a wide-mouth glass jar, incubated at 27°C for 7 days, and the larvae were harvested.

3.6 Data analysis

The primary and secondary data including the GPS readings were entered and stored in the Microsoft Excel® spreadsheet. Both primary and secondary data were descriptively and quantitatively analysed and analysis was done using (SAS)-Statistical Analysis Systems® and Microsoft Excel®. Microsoft Excel® was used to generate tables, chart (lines and histograms) and Correlations (Appendix 3 and 5). P-values were also generated using ANOVA (Microsoft Excel) (Appendix 3, 4 and 5) and Duncan's Multiple Range Test (SAS) (Appendix 3). The GPS data were processed using the ArcView ® system and GIS image produced as a map (Fig. 2, 7, and 8 and Appendix 1 and 2).

CHAPTER FOUR

4.0 RESULTS

4.1 Assessment of type, number and frequency of animals killed by road accidents using primary data

4.1.1 General assessment on mammals, birds and reptiles killed by road accidents

The results on roadkill counts per study month for the period covered September 2007 to April 2008 show that a total of 314 roadkill cases were counted. Among these, 145 (46.2%) cases were mammals, 107 (34.1%) cases were reptiles and 62 (19.7%) cases were birds (Table 1).

Table 1: Mammals, Birds and Reptile species killed between September 2007 to April 2008

	2007				2008				
Species	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr	Total, Percentage and *Duncan Grouping
Mammals	9	6	14	25	39	20	29	3	145^{a} (46.2%)
Reptiles	2	2	13	24	37	27	2	-	107^{b} (34.1%)
Birds	2	2	9	22	15	3	6	3	62^{c} (19.7%)
***Total**	**13^{e}**	**10^{e}**	**36^{d}**	**71^{b}**	**91^{a}**	**50^{c}**	**37^{d}**	**6^{e}**	**314**

*Values in the rows and column bearing different superscripts are significant different (Duncan multiple range test) and superscript (a) being ranked the highest ($P<0.05$)

Duncan's multiple range test show that there was a significant difference in roadkill pattern between mammals, reptile and mammals ($P<0.05$). The Mammal group had the highest roadkill cases, followed by reptiles and birds (Table 1 and Appendix 3). Also the correlation analysis in roadkill counts among these three groups shows that there was a significant positive correlation ($P<0.05$) between them (Appendix 3) implying that an increase in roadkill counts in one group was associated with an increase in other groups.

Mammals and reptiles had significant higher correlation coefficient value of 0.736 (P=0.02). The increase in mammal roadkill counts was associated more with those of reptiles group. Birds and reptiles had significant and medium positive correlation coefficient of 0.649 (P=0.046), while mammals and birds had values of 0.647. On monthly bases, more road-kills were observed in January (91 cases), followed by December (71 cases) and lowest in April (6 cases) (Table 1 and Fig. 3).

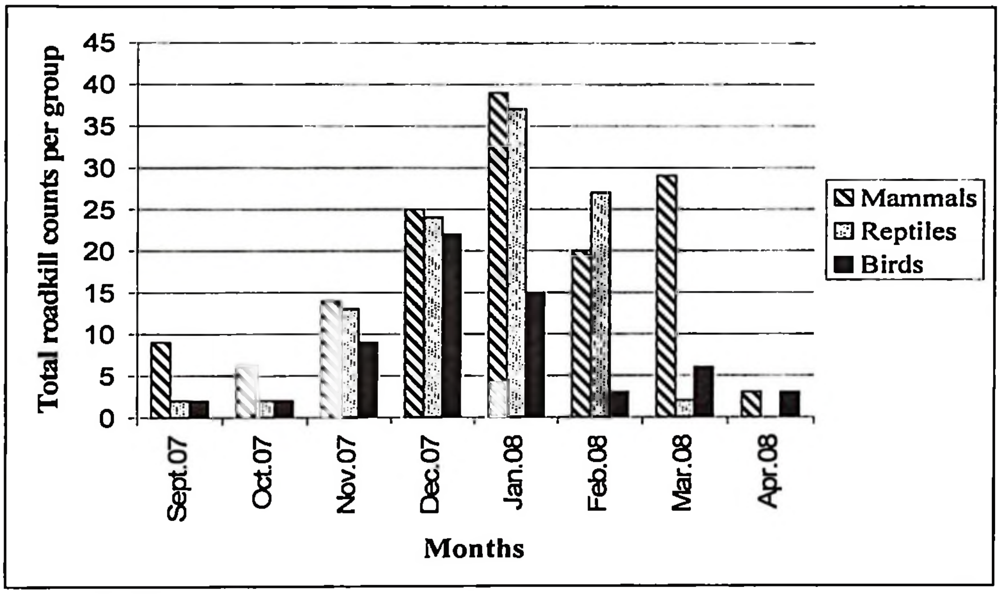


Figure 3: Number of roadkill cases per species between September – December, 2007 and January – April 2008

4.1.2 Roadkill within groups: Mammals

In case of the mammal group alone, the number of roadkill cases also differed significantly (P<0.05) among species within a group (Table 2 and Appendix 3). Out of 145 roadkill cases which occurred during the entire study period (between September 2007 to April 2008), impala (28), rodents (26) and hare (25) constituted the largest groups of animals killed by road accidents (Table 2) and these 3 groups did not show any significant difference in roadkill cases between them (P>0.05). The second group include the African civet (17), followed by buffalo (12). The remainder of the species had only 1 to 6 deaths. The number of roadkill cases per species for mammals and their significance to each other are also shown on Appendix iii and summarized in Table 2. It was also observed that out 39 cases of roadkill cases which occurred in January, the impala group had 11 cases (Table 2). Plate 4 show a freshly killed impala found dead on the MINAPA highway.

Table 2: Mammals killed by road accidents in year 2007 – 2008 along a 50km road stretching across MINAPA and their grouping

	2007				2008				
Species	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr**	***Total and Duncan Grouping**
African Civet	1	-	1	4	4	3	2	2	17^{b}
Baboon	1	-	-	-	-	2	-	-	3^{de}
Buffalo	-	3	-	4	2	1	2	-	12^{c}
Bush buck	-	-	-	-	1	-	-	-	1^{e}
Bush Baby	-	-	-	1	1	-	-	-	2^{e}
Bunded Mongoose	-	-	-	-	-	1	-	-	1^{e}
Elephant	-	-	1	-	1	-	-	-	2^{e}
Giraffe	-	-	1	1	1	1	-	-	4^{de}
Genet	-	-	-	-	-	2	4	-	6^{d}
Hare	3	-	2	2	8	4	5	1	25^{a}
Hyena	-	-	-	-	2	-	2	-	4^{de}
Impala	3	2	2	2	11	3	5	-	28^{a}
Jackal	-	-	-	1	-	1	-	-	2^{e}
Leopard	-	-	-	-	1	-	1	-	2^{e}
Lion	-	1	-	1	-	-	-	-	2^{e}
Porcupine	-	-	1	1	-	-	-	-	2^{e}
Reed buck	-	-	-	-	-	-	1	-	1^{e}
Rodent	-	-	4	8	6	2	6	-	26^{a}
Ratel	1	-	1	-	-	-	-	-	2^{e}
Warthog	-	-	1	-	1	-	1	-	3^{de}
Total	**9**	**6**	**14**	**25**	**39**	**20**	**29**	**3**	**145**

*Values bearing the same superscripts in this column do not differ significantly from each and superscript (a) being ranked the highest (P<0.05)

Plate 4: A freshly killed female impala (*Aepyceros melampus*) found dead on TANZAM highway

4.1.3 Roadkill within groups: Reptiles

In case of the reptile group, the roadkill pattern also differed significantly ($P<0.05$) between species ($P<0.05$). Black mamba had highest ($P<0.05$) death cases (32 cases) followed by Puff adder (17 cases) (Table 3 and Appendix 3). The rest of the species had counts between 1 to 6 cases. However the reptile group had 42 roadkill cases which could not be identified at species level as the carcass were found extensively crashed by passing vehicles on the highway. Most roadkill cases among reptiles occurred in January (37 cases) and out of which the Black mamba group recorded 12 cases (Table 3 and Plate 5).

Table 3: Reptiles killed by road accident in year 2007 – 2008 along a 50km road stretching across MINAPA and their grouping

	2007				2008				
Species	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	***Total and Duncan Grouping**
Black Mamba	1	-	1	9	12	9	-	-	32^{a}
Green Mamba	-	-	-	-	3	-	-	-	3^{cd}
Cobra	-	-	-	-	2	-	-	-	2^{d}
Puff adder	-	-	-	4	8	5	-	-	17^{b}
Python	-	-	-	-	4	-	-	-	4^{cd}
Monitor Lizard	-	-	-	-	1	-	-	-	1^{d}
Leopard tortoise	-	-	-	1	3	1	1	-	6^{c}
Unidentified snake	1	2	12	10	4	12	1	-	42
Total	**2**	**2**	**13**	**24**	**37**	**27**	**2**	**-**	**107**

*Values bearing the same superscripts in this column do not differ significantly from each other and being ranked the highest (P<0.05).

Plate 5: Road-killed black mamba on TANZAM highway

4.1.4 Roadkill within groups: Birds

A total of 62 birds were killed on MINAPA highway during the study period, of which 36 belongs to 15 identified bird species and the remaining cases could not be identified as their carcasses were found completely crushed (Table 4 and Appendix iii). Within bird species which where identified, roadkill cases also differed significantly ($P<0.05$). Helmeted guinea fowl suffered more death ($P<0.05$) (11 cases) followed by Owl group which had 7 (11.29%) cases. One Guinea fowl found dead on the highway and heavily crushed is shown in Plate 6.

Plate 6: Road-killed Guinea fowl on TANZAM highway

The rest of the bird species had counts between 1 to 4 cases. Most roadkill cases among birds occurred in December (22 cases), followed by January (15 cases) (Table 4).

Table 4: Birds killed by road accidents in year 2007 – 2008 along MINAPA highway and their grouping

	2007				2008				
Species	**Sep**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr**	***Total and Duncan Grouping**
Ashy starling	-	-	-	-	1	-	-	-	1^{d}
Crowned plover	-	-	-	-	1	-	-	-	1^{d}
Eagle	-	-	-	-	-	1	-	-	1^{d}
Fisher's love bird	-	-	-	-	1	-	-	-	1^{d}
Hart lab's turaco			-	-	1	-	-	-	1^{d}
Helm.Guinea fowl	2	2	1	2	-	1	2	1	11^{a}
Laughing dove	-	-	-	-	1	-	-	-	1^{d}
White browed coucal	-	-	-	1	3	-	-	-	4^{c}
Owl	-	-	1	2	1	1	1	1	7^{b}
Red rumped swallow	-	-	-	-	1	-	-	-	1^{d}
Striped swallow	-	-	-	-	1	-	-	-	1^{d}
Swallow	-	-	-	2	1	-	-	-	3^{c}
White tailed swallow	-	-	-	-	1	-	-	-	1^{d}
Franklin							1		1^{d}
Yellow throated long claw	-	-	-	-	1	-	-	-	1^{d}
Unidentified bird	-	-	7	15	1	-	2	1	26
Total	**2**	**2**	**9**	**22**	**15**	**3**	**6**	**3**	**62**

*Values bearing the same superscripts in this column do not differ significantly from each other and superscript (a) being ranked the highest ($P<0.05$)

4.2 Assessment of Roadkill Dynamics Using Secondary Data

The secondary data on number of road-kills for mammals, reptiles and birds in MINAPA was kindly provided by MINAPA Ecology Department. The monthly roadkill counts between 2003-2007 within mammal group are shown in Table 5, within the reptile group in Table 6 and within the bird group in Table 7. The targeted data used here was that collected during the period starting from September to April in each respective year. These months were selected to match the same months in which the primary data was collected in the present study in order to provide more insight in the pattern and trends of the road-kills in MINAPA. A total of 680 roadkill cases were recorded during the period of 4 years and the distribution of the road-kills per year between mammals, reptiles and birds is depicted in Table 8. When the total roadkill counts between these three groups were assessed a very significant difference were observed ($P<0.05$) (Table 8). Out of 680 roadkill counts recorded, mammals group had significantly ($P<0.05$) high counts of 365 cases, followed by birds (254 cases) and the least were reptiles (61 cases). The trends of road kills for mammals showed fluctuation between 2003 and 2007. The lowest counts were recorded in 2004/5 (61) and highest in 2007 (113). For the reptiles, the road kill counts between 2003 and 2007 were below 20 cases per year varying from 12 (2004/5) to 18 (2006/7). Similar pattern observed for reptiles was also recorded in the bird group. The highest counts were recorded in 2004/5 (71) and lowest (58) in 2003/4 (Table 8). However, when these differences were assessed statistically on yearly bases within group, no marked difference was observed ($P>0.05$) (Table 8).

Table 5: Monthly roadkill counts within mammal group between 2003 – 2007

	Mammal Group							
	Sept.	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**
2003/4	21	11	14	16	20	8	8	9
2004/5	3	5	7	9	17	2	8	10
2005/6	15	19	24	9	9	2	1	5
2006/7	20	20	23	33	9	7	1	0
2007/8	9	6	14	25	39	20	29	3

Table 6: Monthly roadkill counts within reptile group between 2003 – 2007

	Reptile Group							
	Sept.	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**
2003/4	2	0	0	4	6	1	0	3
2004/5	2	0	0	2	1	0	2	5
2005/6	1	2	1	2	6	1	0	2
2006/7	1	4	2	5	4	2	0	0
2007/8	2	2	13	24	37	27	2	0

Table 7: Monthly roadkill counts within bird group between 2003 – 2007

	Bird Group							
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
2003/4	13	0	0	4	18	5	7	10
2004/5	1	0	2	19	22	7	5	15
2005/6	8	11	13	5	8	11	4	4
2006/7	5	13	5	15	8	11	4	0
2007/8	2	2	9	22	15	3	6	3

Table 8: Summarised data showing the effect of roadkill cases within and between groups per years (2003/7) and the significance in P values

	Years and total roadkill					
Groups	**2003/4**	**2004/5**	**2005/6**	**2006/7**	**P-values**	***Total an P**
Mammals	107	61	84	113	0.2336	365^{a}
Reptiles	16	12	15	18	0.8219	61^{c}
Birds	58	71	64	61	0.9495	254^{b}
Total	**181**	**144**	**163**	**192**		**680**

*Values bearing different superscripts in this column do differ significantly from each other and superscript (a) being ranked the highest (P<0.05).

The results also showed that between individual groups, roadkill counts per year was not significantly different (P=0.7515) between mammals and birds, but was significant between mammals and reptiles (P<0.05) and between reptiles and birds (P<0.05) groups (Table 10). Roadkill counts within the respective groups per year, however, showed no significant difference (P>0.05) (Tables 9).

Table 9: Statistical analysis compared the effect of roadkill cases among groups (ANOVA)

Groups	F critical	F calculated	P value
Mammals vs Reptiles	10.1279	50.8152	0.005
Birds vs Reptiles	10.1279	141.0612	0.001
Mammals vs Birds	10.1279	3.6635	0.751

4.3 Assessment of roadkill dynamics using combined primary and secondary data

When secondary and primary data was combined, results show that a total of 994 roadkill cases were recorded for the period of 5 years (Table 10). This included 680 cases or 170 kills/year recorded by the Park for the period covering September –April from 2003 to 2007 and 314 roadkill cases recorded within the same months during the present study (2007/8). The three animal groups exhibited different patterns compared to that seen in the secondary data alone. When the primary data (2007/8) was included, a different roadkill pattern was observed between groups. Mammals and reptiles groups depicted an upward trend while the bird group had no change (Fig. 4).

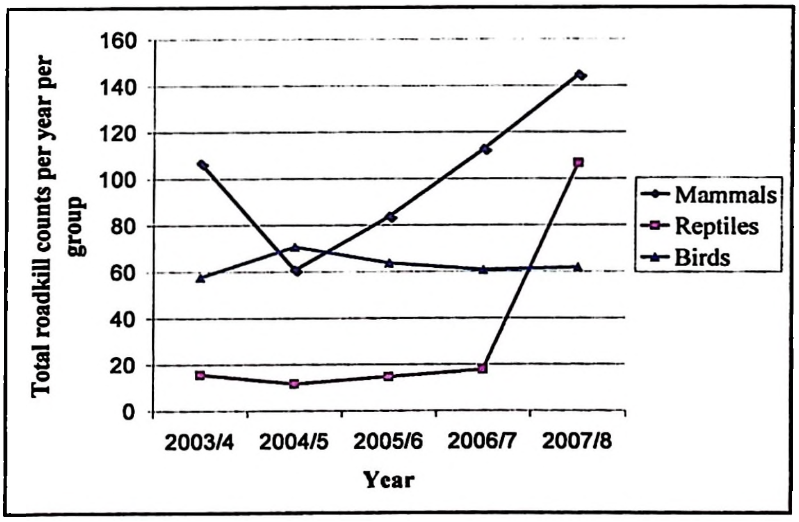


Figure 4: Roadkill pattern for Mammals, Reptiles and Birds for year 2003 to 2008

The results in Table 10 show that the roadkill counts for year 2007/8 (314 cases) ranked highest (P<0.05) compared to the roadkill counts for the other years. This difference was mainly caused by the significant in numbers of reptiles (107) (P<0.002) and to a lesser extent mammal (P<0.165) roadkill cases recorded during the present study period (Table 10 and Appendix 5). No significant difference was observed for the bird group (P=0.983) (Appendix 5).

Table 10: Combine primary and secondary data showing the effect of roadkill cases within each group per year from September 2003 to April 2008

	Secondary data (years and total roadkills)				Primary data		
Species	**2003/4**	**2004/5**	**2005/6**	**2006/7**	**2007/8**	***Total**	**P**
Mammals	107	61	84	113	145	510^{x}	0.165
Reptiles	16	12	15^{c}	18	107	168^{z}	0.002
Birds	58	71	64	61	62	316^{y}	0.983
***Total**	**181^{c}**	**144^{e}**	**163^{d}**	**192^{b}**	**314^{a}**	**994**	**P<0.05**

*Values bearing the different superscripts in the same row and column differ significantly from each other and superscript (a) and (x) being ranked the highest (P<0.05)

The correlation analysis in roadkill counts between these three animal groups, shows that there was a positive and negative correlation between them as follows; There was a significant positive correlation between mammals and reptiles (0.792) (p=0.02) and mammals and birds was significant negatively correlated at (-0.691) (p=0.031). The bird and reptiles was negatively correlated (-0.112), however the correlation was not significant (Appendix 5).

4.3.1 Assessment of roadkill dynamics using combined monthly primary and secondary data

When the combined primary and secondary data was catagorised on monthly basis for the period covering 2003 to 2008, results show that no clear trend was observed within months (Fig. 5). However, more roadkills cases were observed in December (194 cases) and January (219 cases) and least in April (77 cases) (Fig. 6).

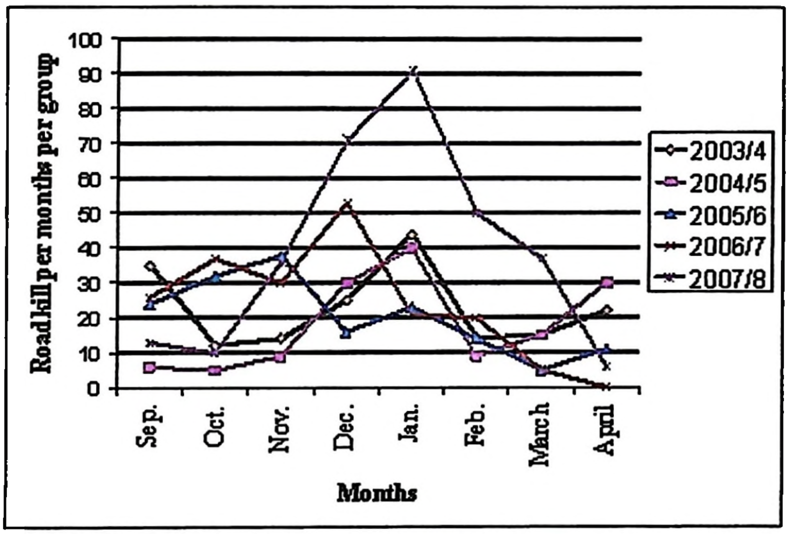


Figure 5: Mammals, reptiles and birds roadkill pattern for September 2003 to April 2008

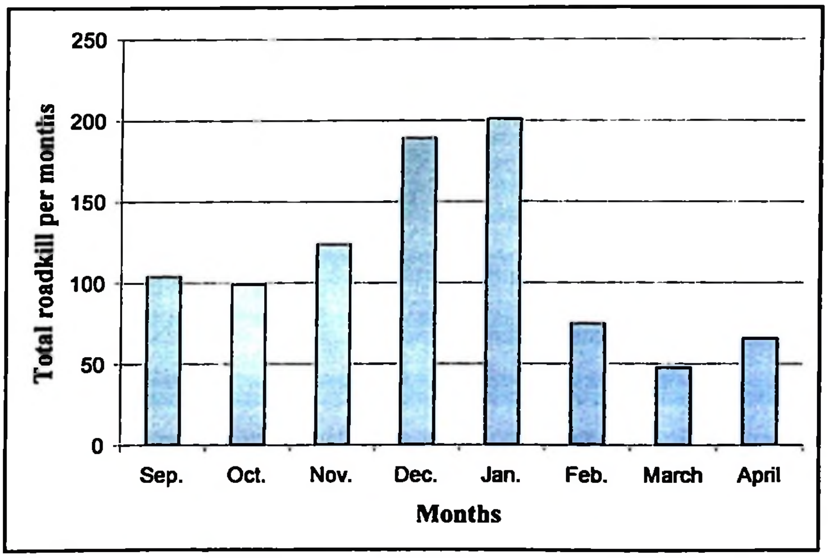


Figure 6: Monthly total roadkills (mammals, reptiles and birds) between September to April from 2003 to 200

4.4 Identification of accident hotspots and factors that predispose animal to road accident based on primary data

4.4.1 Identification of accident hotspots

Roadkill accidents in mammals, reptiles and birds during the 2007-2008 study period were recorded using GPS and the results are depicted in Fig. 7A for mammals, Fig. 7B for reptiles and Fig. 7C for birds. In all groups, these deaths were scattered along the entire 50-km road stretch.

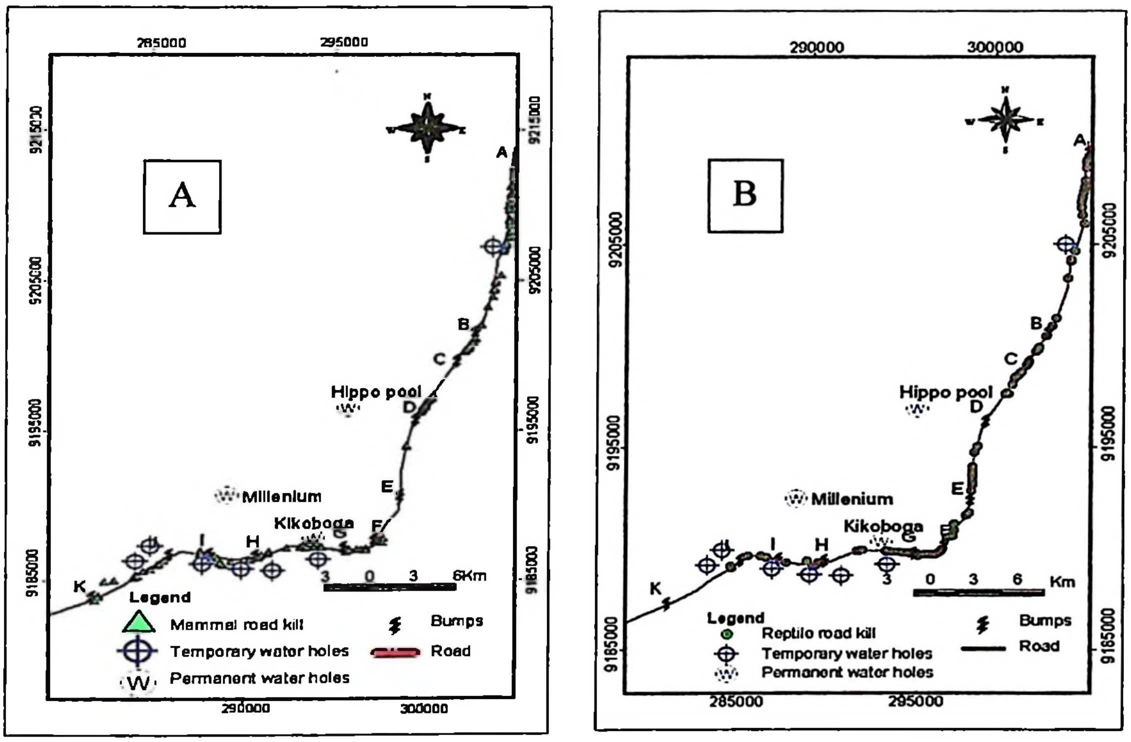


Figure 7: Roadkill accidents in mammals (A) and Reptiles (B) on MINAPA highway during the study period

When roadkills were assessed by the total number dying per section more deaths were recorded in section A-B and least in section I-J. Intermediate deaths were recorded in other sections (Fig. 7A, 7B, 7C and Table 11). When the roadkills were assessed based on total number dying per section per kilometer, the highest roadkill counts were recorded in section F-G (14.1 cases), and least cases were recorded in section I-J (2.8 cases) (Table 11). The average roadkill was 6 cases per kilometer. Sections between marks A to G had roadkills above average while sections between marks G to K had roadkill cases below average (Table 11).

Within groups, the highest number of roadkills per section per kilometer in mammals was recorded in section C-D (6.2 cases) and least in section I-J (1.25 cases) (Table 12). Other intermediate counts were recorded in other sections. For the reptile group, the highest roadkill counts were recorded in section F-G (5.4 cases) and least in section J-K (0.3 cases) (Table 12). For birds, the highest roadkill counts were recorded in section F-G (3.6 cases) and least in section I-J (0 cases) (Table 12).

Table 11: Roadkill sections, vegetation type, number of roadkill (mammals, reptiles and birds) per section and its total

Section	Vegetations	Section length in Km	Number Dying per Section			Total no. dying per section	Total no. dying per km
			Mammals	Reptiles	Birds		
A-B	-Wooded grassland -Open woodland (Miombo)	12.1	39	21	15	75	6.2
B-C	-Bushland -Open woodland	2.1	8	6	3	17	8.1
C-D	-Bushland	4.2	26	15	2	43	10.2
D-E	-Bushed grassland	5.1	9	10	13	32	6.3
E-F	-Bushed grassland	3.7	7	9	7	23	6.2
F-G	- Bushed grassland - Open grassland	2.2	11	12	8	31	14.1
G-H	- Bushed grassland	10.4	14	18	3	35	4.7
H-I	-Bushed grassland	4.2	7	8	6	20	4.8
I-J	-Bushed grassland	4.0	5	6	0	11	2.7
J-K	-Bushland -Open woodland (Miombo)	6.8	19	2	5	26	3.8

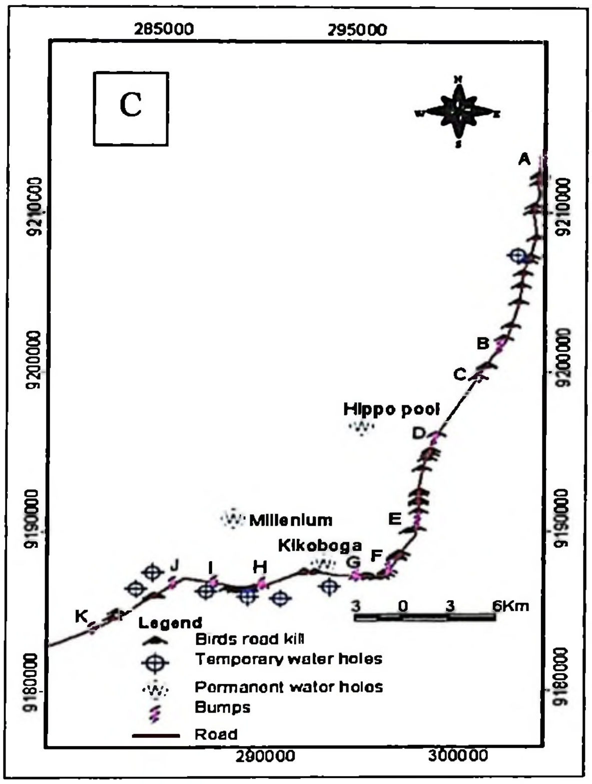


Figure 7C: Roadkill accidents in Birds on MINAPA highway during the study period

Table 12: Road sections, vegetation type and number of roadkill (mammals, reptiles and birds) per section per kilometer

Section	Vegetation(s)	Section length in Km	Number killed per Section		
			Mammals	Reptiles	Birds
A-B	-Wooded grassland -Open woodland	12.1	39 (3.2)	21 (1.7)	15(1.2)
B-C	-Bushland -Open woodland	2.1	8 (3.8)	6(2.8)	3(1.4)
C-D	-Bushland	4.2	26 (6.2)	15(3.5)	2(0.4)
D-E	-Bushed grassland	5.1	9(1.7)	10(1.9)	13(2.5)
E-F	-Bushed grassland	3.7	7(1.8)	9(2.4)	7(1.8)
F-G	- Bushed grassland - Open grassland	2.2	11(5.0)	12(5.4)	8(3.6)
G-H	- Bushed grassland	10.4	14(1.3)	18(1.7)	3(0.2)
H-I	-Bushed grassland	4.2	7(1.6)	8(1.9)	6(1.4)
I-J	-Bushed grassland	4.0	5(1.2)	6(1.5)	0(0)
J-K	-Bushland -Open woodland	6.8	19(2.7)	2(0.3)	5(0.7)

Numbers in brackets are roadkills/km

4.4.2 Impact of vegetation type on roadkill dynamics (primary data only)

In order to assess whether the roadkill pattern along MINAPA highway was associated with differences in the surrounding vegetation cover, an attempt was made to assess the vegetation types in each subsections A-K with respect to number died per section (Fig. 8 and Table 11 and 12). The assessment was done for mammals, reptile and birds. Generally, the entire highway has dominant vegetation type comprising of wooded grassland, open woodland (Miombo), bushlands and bushed grassland (Fig. 8). These types of vegetation are all distributed along the highway, each designated section having almost a different type of vegetation. Since these vegetation types serve as good sources of food/feed and shelter to different species of wildlife, it is envisaged that animal distribution within these vegetations types would occur differently depending on the species of wildlife and its feeding preferences.

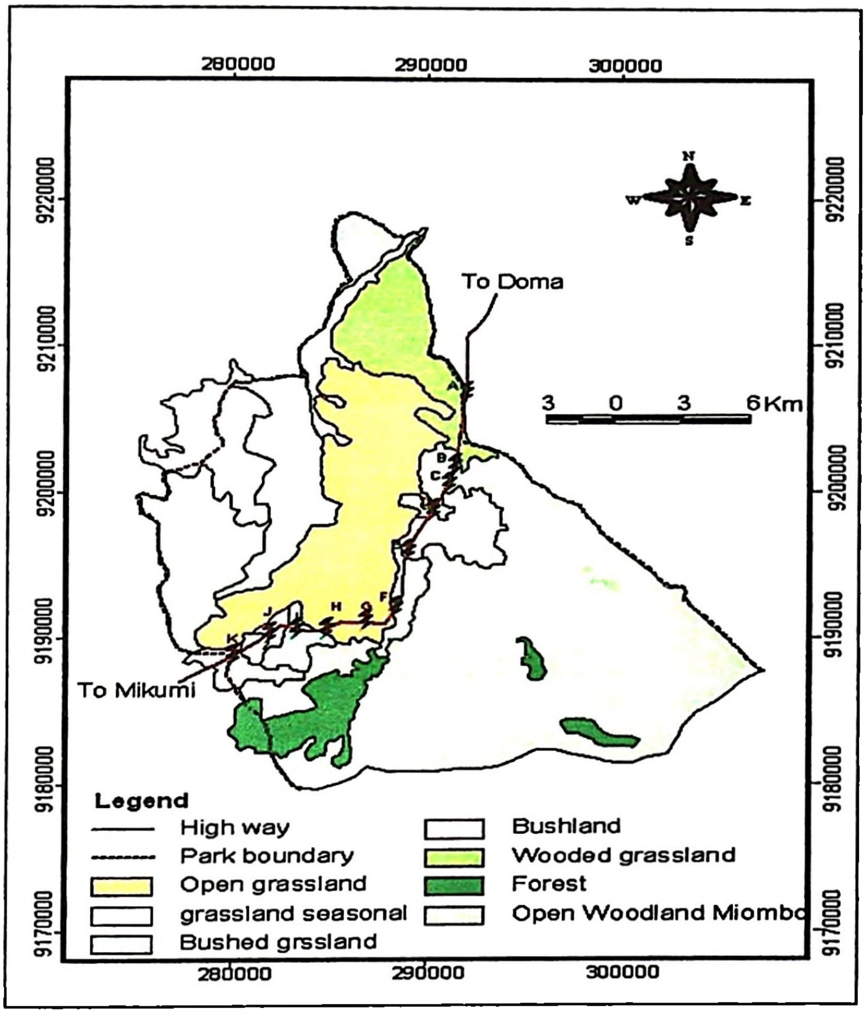


Figure 8: Vegetation type surrounding the MINAPA highway
Source: Animal Behaviour Unit (ABRU) MINAPA/GIS section SUA

The road sections, vegetation type, and animal species dying per section are summarized in Table 11 and 12. The results show that section F-G which had the highest cases of roadkill cases per kilometer of 14.1 cases (Mammals, Reptiles and Birds having 5.0, 5.4 and 3.6 roadkill cases per kilometer respectively) was dominated by open grassland and bushed grassland vegetation cover (Table 13 and 14). This was followed by sections B-C and C-D (8.1 and 10.2 roadkill cases per kilometer respectively) which was dominated by open woodlands and bushlands. Other sections which had rodkill cases per section per kilometer ranging from 2.7 (Section I-J) to 6.3 (Section D-E) was covered mainly by bushed grasslands areas (Table 13 and 14 and also and Fig. 7A, 7B, 7C and 8).

Table 13: Road section, vegetation and preferred section (mammals)

Section	Vegetation	Number of Temporary water holes	Number of permanent water holes	Preferred section for (Mammals)	Species died
A-B	-Wooded grassland -Open woodland (Miombo)	1	0	Hare Civets Jackals Elephants Baboons Buffaloes	Leopard Giraffes Lions Buffaloes Impala Porcupine Jackal Hare Rodents Civets Genet Mangoose
B-C	-Bushland -Open woodland (Miombo)	0	0	Hare Civets Baboons Warthogs Elephants	Hare Rodents
C-D	-Bushland	0	0	Hare Civets Baboons Warthogs	Buffaloes Rodents Hare Ratel Porcupines Baboons Civets Giraffes Leopard Jackal Impala Ratel
D-E	-Bushed grassland	0	1	Impalas Hare Warthogs Giraffes	Civets Hare
E-F	-Bushed grassland	0	0	Impalas Giraffes Zebras Elephants Buffaloes Civets	Buffaloes Civets Warthog Impalas Lion

Table 13: continue

Section	Vegetation	Number of Temporary water holes	Number of permanent water holes	Preferred section for (Mammals)	Species killed
F-G	- Bushed grassland - Open grassland	0	0	Impalas Giraffes Zebras Elephants Buffaloes Civets	Hare Rodents Giraffes Impala
G-H	- Bushed grassland	2	1	Baboons Impalas Giraffes Buffaloes Zebras	Buffaloes Giraffes Impala Rodents Bushbuck
H-I	-Bushed grassland	1	1	Baboons Giraffes Impalas Elephants Buffaloes Zebras	Elephant Impala Reedbuck Hare
I-J	-Bushed grassland	1		Baboons Giraffes Impalas Elephants Buffaloes	Impala Hyena
J-K	-Bushland -Open woodland (Miombo)	2		Baboons Giraffes Impala Elephants Buffaloes	Elephant Bushbaby Impala Hyena Rodent Civet

4.4.3 Impact of vegetation type on roadkill dynamics in different animal categories (primary data only)

In order to assess whether the roadkill pattern along MINAPA highway was associated with differences in the surrounding vegetation cover, an attempt also was done to assess the vegetation types in each subsections A-K with respect to number of different animal categories. Mammal was chosen as representative group as it was more defined group with regard to their body weight and the group was categorized as small (0-20kg), medium (20-

50kg) and large (50 and above kg) bodied mammals. Almost each designed road section had small, medium and large bodied mammal roadkill, these occurred in different proportions depending on the type of mammal and vegetation preference (Table 13 and 14). Also mammal preference to different designated sections differed depending on the section and type of mammal species involved (Table 13 and 14). Section A-B (Wooded grassland and open woodland) and C-D (Bushland) had highest number of different mammal species (12 species) killed by accidents compared to other sections and section B-C (Bushland and Open woodland), D-E (Bushed grassland) and I-J (Bushed grassland) had least number of roadkill species (2 species) (Table 13 and 14). The result also showed that 24 out of 39 roadkill cases in section A-B (Wooded grassland and open woodland), and 16 out of 26 roadkill cases in section C-D (Bushland) were small mammals and the predominant ones were civets, hare and rodents. Roadkill in the rest of the sections was associated more with large to medium sized mammals with very few deaths of small mammals.

Table 14: Road section and different categories of mammals killed

Section	Large mammals	No. killed	Medium mammals	No. killed	Small mammals	No. killed	Grand Total
A-B	Leopard	1	Impala	5	Hare	7	
	Giraffes	1	Warthogs	2	Rodents	10	
	Lions	1	Baboons	1	Civets	5	
	Buffaloes	2	Porcupines	1	Genet	1	
			Jackals	1	Mangoose	1	
Sub total		**5**		**10**		**24**	**39**
B-C	-		-		Hare	4	
					Rodents	4	
Sub total						**8**	**8**
C-D	Buffaloes	4	Porcupines	1	Rodents	5	
	Giraffes	1	Baboons	1	Hare	6	
	Leopard	1	Jackals	1	Ratel	2	
			Impala	1	Civets	3	
Sub total		**6**		**4**		**16**	**26**
D-E					Civets	5	
					Hare	4	
Sub total						**9**	**9**
E-F	Buffaloes	2	Warthogs	1	Civets	1	
	Lion	1	Impala	2			
Sub total		**3**		**3**		**1**	**7**
F-G	Giraffes	1	Impala	5	Hare	4	
					Rodents	1	
Sub total		**1**		**5**		**5**	**11**
G-H	Buffaloes	4	Impala	5	Rodents	3	
	Giraffes	1	Bushbuck	1			
Sub total		**5**		**6**		**3**	**14**
H-I	Elephant	1	Impala	1	Hare	3	
	Reedbuck	1	Baboon	1			
Sub total		**2**		**2**		**3**	**7**
I-J	-		Impala	4	-		
			Hyena	1			
Sub total				**5**			**5**
J-K	Elephants	1	Impala	8	Rodents	3	
	Bushbaby	1	Hyena	3	Civets	3	
Sub total		**2**		**11**		**6**	**19**
Total							**145**

4.4.3 Roadkill hotspots associated with permanent and temporarily water holes (using primary data only)

The present study also assessed the relationship between roadkill cases and the location of water holes which are closer to the highway. During this study, a total of 3 permanent and 7 temporary water holes were recorded and their GPS locations were taken. This data is depicted in Tables 13 and 15 as well as in Fig. 2, 7A, 7B and 7C. The GIS map (Fig. 2) show that these water holes are randomly distributed along the highway and the temporary ones lie within 20m of the vicinity of the road. Permanent water holes are located 1-5 kilometer away from the highway (Fig. 2 and Appendix 1). Fig. 2 also shows that 5 out of 7 temporary water holes and 2 out of the 3 permanent water holes are located within section G-K. The rest (1 temporary and 1 permanent) water holes are located within section A-G (Fig. 2). When this data was related to the roadkill pattern, sections between G-K which had more water holes had fewer roadkill cases per kilometer than overall average (below average of 6 roadkill case per kilometer) while section between A-G which had fewer water holes had roadkill cases above average (Table 15 and Fig. 2). These two variables were negatively correlated ($r = -0.6$, $p=0.037$) meaning that the increase in the number of water holes in a given areas along the highway reduced the incidence of roadkill cases and vice versa (Table 15).

Table 15: Number of roadkill per section per km and number of water holes per section

	A-B	B-C	C-D	D-E	E-F	F-G	G-H	H-I	I-J	J-K
Number of water holes section^{-1}	1	0	0	1	0	0	3	2	1	2
Number of roadkills section^{-11} km^{-1}	6.2	8.1	10.2	6.3	6.2	14.1	4.7	4.8	2.8	3.8

$r = -0.6$

The GIS map also shows that mammal roadkill cases seemed to clump together to particular areas. These areas are those which were mainly close to the watering point (Appendix 2). However this pattern did not show up clearly for reptile and bird groups. Mammal group had highest roadkill cases per kilometer in section C-D (more than 6 roadkill cases km^{-1} $section^{-1}$). This section is close to the Hippo pool watering point which suggests that many mammals cross this section to this watering point (Appendix 2).

4.4.4 Roadkill accident hotspot associated with change in weather (using primary data only)

(a) Rainfall

The results of the present study clearly suggest that there was a relationship between roadkill pattern and changes in rainfall pattern in MINAPA. The average rainfall in MINAPA for the period from September 2007 to April 2008 is shown in Fig. 9. September to November period were the dry months in MINAPA, and the average monthly rainfall within these months was less than 20mm. Short rains started in October and ended in early February and the average rainfall recorded was 70mm. The heavy rain (more than 80mm) started in mid February (Fig. 9). Total roadkill within the same period show that more roadkills occurred between September and January with 221 cases, (44.2 case $month^{-1}$) and least in February to April with 98 cases (31.2 cases $month^{-1}$). When the rainfall and total roadkill data were compared, it was observed that there were more roadkill cases during the short rain period (September to January), but as the rainfall increased (February, March and April) the number of animal killed per month on the highway decreased (Fig. 9).

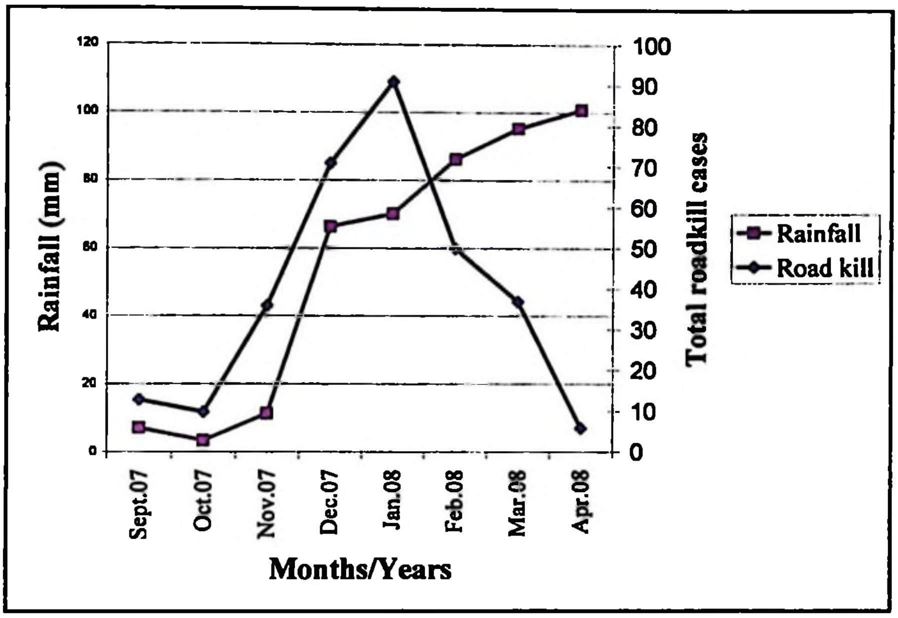


Figure 9: Total roadkill versus rainfall pattern from September 2007 to 2008 in MINAPA

The correlation analysis also showed that although these two parameters were positively correlated, this correlation was weak (r = 0.3). The same trend was observed when the rainfall pattern was assessed based on individual group roadkills. For the mammal group (Fig. 10), there were less than 10 roadkill cases per month during September and October (dry season) and this increased during November and December reaching peak in January (39 cases) which was the end of the short rain. February to April was the wettest months in MINAPA and during this period, the roadkill counts decreased reaching minimum of 3 cases in April. A similar trend was observed for the reptile (Fig.11) and birds (Fig. 12) groups.

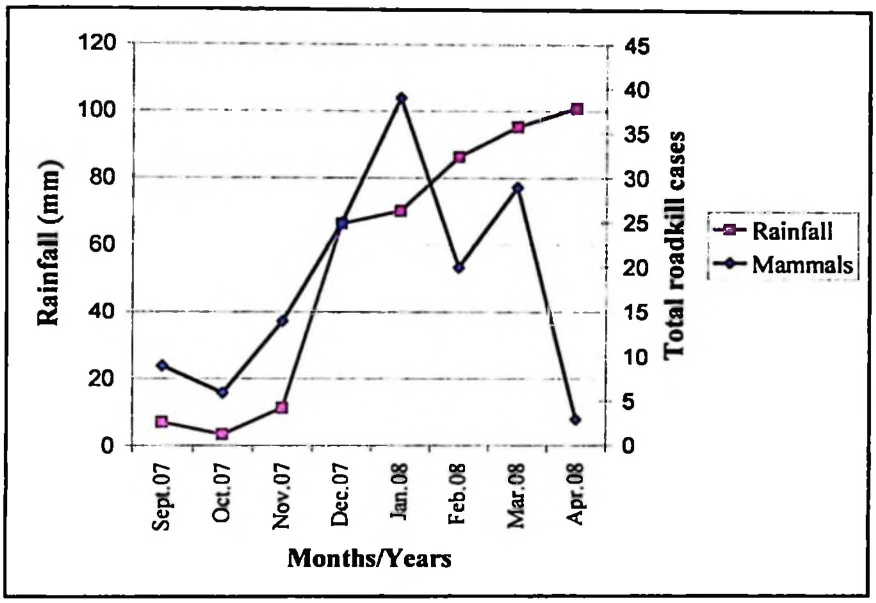


Figure 10: Mammal roadkill counts versus rainfall pattern from September 2007 to 2008

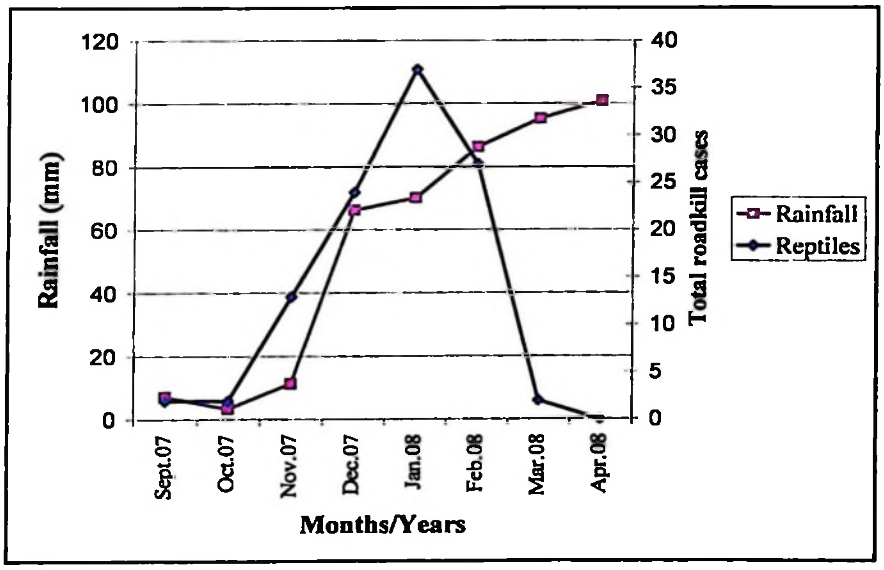


Figure 11: Reptile roadkill counts versus rainfall pattern from September 2007 to 2008

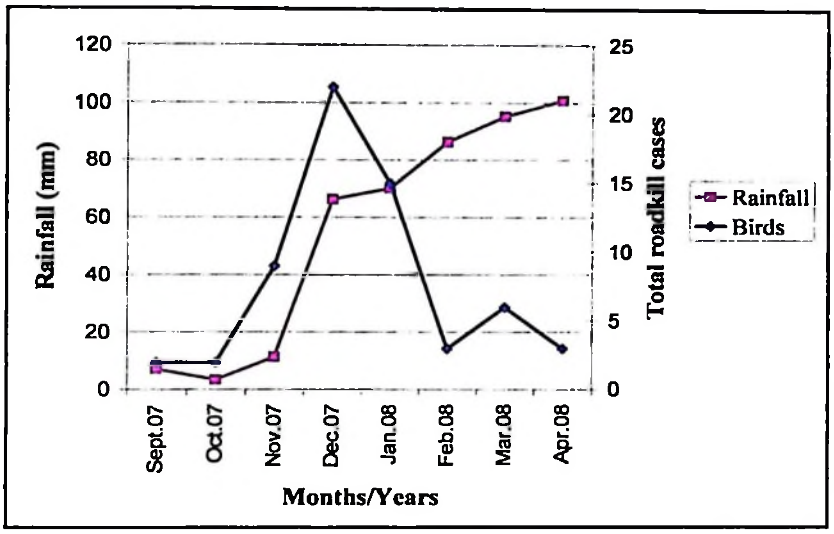


Figure 12: Bird roadkill counts versus rainfall pattern from September 2007 to 2008

(b) Temperature

The temperature change within the Park during the study period (September 2007 – March 2008) is shown in Fig. 13 and Appendix 6. The temperature ranged between 24.16±0.21 °C in September to 27.71 ± 0.34 °C in November which was the end of the dry season in the Park. The ambient temperature varied equivocally in the following months ranging from 29.12 ± 0.30 °C in December to 28.86 ± 0.25 °C in March 2007. When these data were compared with roadkill pattern within the same study months no clear trend was observed. (r=0.1) (Fig. 13).

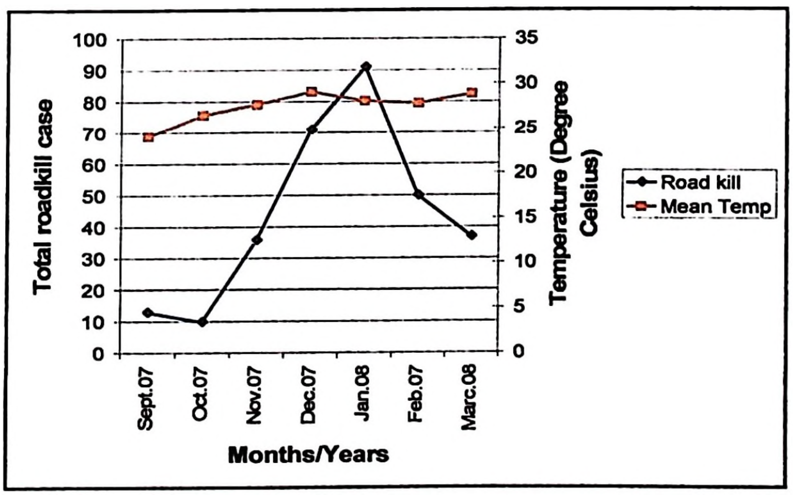


Figure 13: Roadkill cases versus Temperature range from September 2007 to 2008

4.5 Studies on health issues on wildlife in MINAPA

4.5.1 Assessment of the type of infections and infestations of road-killed animals

(a) Bacteriological studies

In order to ensure that good quality bacteriological information was obtained, 45 randomly selected roadkill animals were sampled for bacteriological examination. The animals sampled were mammals (28), reptiles (9) and birds (8) (total 45) and a total of 105 target specimens were collected from major internal organs (liver, lungs, spleen, intestines) and swabs (tracheal and ear swabs). Out of these 45 cases, 18 cases were intact, 8 cases were semi crushed and 19 cases were severely crushed animals (Table 19, 20 and Plate 7a, 7b, and 7c) Where it was not possible to identify individual organ due to the severity of accident, miscellaneous tissues (three different unidentifiable organs) were collected (Table 19 and 20). From these specimens, different microbes were isolated from different animals and organs, some of organs revealed no isolates at all. The majority of isolation in all groups belonged to *Enterobactericeae* family, which depending on route of infection can occasionally cause infection to domestic and wild animals. The isolated bacteria from mammal samples includes *Escherichia coli*, *Citrobacter spp*, *Klebsiella spp*, and *Proteus spp*, which are common gastrointestinal tract bacteria, and *Pasteurella multocida* which was isolated from tracheal swab of jackal in pure culture. *P. multocida* can live and multiply in the respiratory tract of healthy animals but under predisposing conditions, the organism may cause severe and perhaps fatal infections. *Staphylococcus epidermidis*, the main skin contaminants were isolated from different animals. *Bacillus subtilis*, which is commonly found in the soil, was isolated from a severely crushed rat possibly as soil contaminant (Table 19 and 20).

Table 16: Animal species, body condition, specimen taken and bacterial isolates

S/N	ANIMAL SPECIES	BODY CONDITIONS	SPECIMEN TAKEN	LAB. FINDINGS
		Mammals		
	Baboon	Semi crushed	Liver	No growth
1			Lungs	No growth
2	Baboon	Intact	Tracheal Swab Lungs Liver	Proteus spp
3	Baboon	Intact	Liver	*Escherichia coli*
4	Baboon	Semi crushed	Miscellaneous organs	*Escherichia coli*
5	Baboon	Intact	Ear swab Liver Lungs	*Staphylococcus epiderm.* No growth No growth
6	Baboon	Semi crushed	Liver	No growth
7	Civet	Intact	Liver Spleen Lung	No growth No growth No growth
8	Civet	Severely crushed	Miscellaneous organs	*Citrobacter spp* *Escherichai coli*
9	Civet	Severely crushed	Miscellaneous organs	*Proteus spp*
10	Civet	Severely crushed	Miscellaneous organs	*Staphylococcus epidermidis* *Klebsiella spp*
11	Civet	Severely crushed	Miscellaneous organs	*Escheriachia coli*
12	Hare	Severely crushed	Liver	No grows
13	Hare	Intact	Lungs Liver Spleen	No growth
14	Hare	Semi crushed	Intestinal content	*Escherichia coil*
15	Hare	Severely Crushed	Miscellaneous organs	*Proteus spp*
16	Hare	Intact	Liver	No growth No growth

Table 17: Animal species, body condition, specimen taken and bacterial isolates

S/N	ANIMAL SPECIES	BODY CONDITIONS	SPECIMEN TAKEN	LAB. FINDINGS
			Mammals	
17	Hare	Semi crushed	Liver Spleen Lung	No growth
18	Hare	Semi crushed	Lungs Intestinal content	No growth *Escherichia coli*
19	Impala	Intact	Miscellaneous organs	*Proteus spp*
20	Rat	Intact	Liver	No growth
21	Rat	Severely crushed	Miscellaneous organs	*Proteus spp* *Bacillus subtilis*
22	Hyena	Severely crushed	Miscellaneous organs	*Proteus spp* *Staphylococcus epiderm.* *Escherichia coli*
23	Ratel	Intact	Liver, Lung	No growth
24	Jackal	Intact	Tracheal swab Lungs Liver	*Pasteurella multocida* No growth No growth
25	Baboon	Intact	Liver	No growth
26	Eland	Intact	Liver Lung Spleen	No growth
27	Buffalo	Intact	Liver Lung Spleen	No growth
28	Lion	Semi crushed	Liver Lungs	*Escherichia coli*
			Reptiles	
1	Unid. Snake	Severely crushed	Miscellaneous organs	*Staphylococcus epidermidis*
2	Unid. Snake	Severely crushed	Miscellaneous organs	*Staphylococcus epidermidis*
3	Unid. Snake	Severely crushed	Miscellaneous organs	*Escherichia coli*
4	Black Mamba	Semi crushed	Intestinal content	*Escherichia coli*
5	Black Mamba	Semi crushed	Liver?	No growth
6	Puff Udder	Severely crushed	Miscellaneous organs	*Proteus spp*
7	Tortoise	Severely crushed	Miscellaneous organs	*Proteus spp*
8	Cobra	Severely crushed	Miscellaneous organs	*Proteus spp*
9	Unid snake	Severely crushed	Miscellaneous organs	*Escherichia coli*
			Birds	
1	Eagle	Intact	Liver	No growth
2	Ashy staring	Severely crushed	Miscellaneous organs	Proteus spp
3	Guinea fowl	Intact	Intestinal content	*Eschechria coli* *Citrobacter ?*
4	Unid. Bird	Intact	Liver	*Proteus spp*
5	Unid. Bird	Intact	Lungs	No growth
6	Owl	Intact	Lungs Intestinal content	No growth *Escheriachia coli*
7	Francolin	Severely crushed	Miscellaneous organs	*Escherichia coli* *Citrobacter spp*
8	Bird	Severely Crushed	Miscellaneous organs	Proteus spp

(a) Intact Impala

(b) Severely crushed

Black-backed Jackal

(c) Semi-crushed Black mamba

Plate 7: (a) Intact impala, (b) Severely crushed black-backed jackal and (c) Semi-crushed black mamba

In reptiles, bacteria isolates of interest were *Staphylococcus epidermidis, Escherichia coli* and *Proteus spp all* from semi to severely crushed snakes. No intact snake roadkills were found on the highway during the study period. Similarly, *Proteus spp, Eschechria coli* and *Citrobacter spp* were the main isolates from birds.

4.5.2 Assessment of the type of internal and external parasite in road-killed animals

Out of the 45 selected roadkill cases, 30 specimens were collected for parasitological examination. The target specimens collected were intestines or intestinal content, faeces, ticks and worms. Seventeen out of the 30 samples collected had various internal and external parasites and no parasites were observed in the remaining 13 samples. Parasites which were recovered and their animal origins are summarized in Table 21.

a) Recovering and identification of internal parasites

All helminthes recovered belonged to the phyla *Platyhelminthes* and *Nemathelminthes* but of different families and genus (Table 21). In mammals, Strongyle eggs were isolated from impala and buffalo faeces. Also *Haemonchus spp* and *Moniezia spp* were recovered from impala's intestinal contents and *Trychostrongylus spp* from buffalo. No parasite was recovered from reptile group. For avian species, majority of the worm recovered belonged to the genus *Heterakis*, *Hymenolepis*, and *Raillietina* (Table 21 and Plate 9a and 9b). More than one species of worms was found in two Guinea fowl (Table 21 and Plate 8). Other worms recovered in birds included those which belonged to the genus *Subulura*, *Ascaridia* and *Metrolisthes* (Table 21).

Table 18: Parasites recovered from road-killed animals

S/N	SPECIES	ANIMAL SPECIES	SPECIMEN TAKEN	LAB. FINDINGS
			MAMMALS	
1	**Helminths**	Buffalo	Faeces	Strongyle egg/ *Trichostriongylus spp*
2		Impala	Faeces	Strongyle egg/*Haemonchus spp*
3		Impala	Intestine	*Moniezia spp*
4		Hare	Intestinal content	No parasite
5		Hare	Intestine	No parasite seen
6		Impala	Intestines	No parasite seen
		Hare	Intestines	No parasite seen
7		Civet	Intestines	No parasite seen
8		Hare	Intestines	Na parasite seen
9		Ratel	Intestine	No parasite seen
	Ticks			
10		Buffaloes	Ticks	*Rhipicephalus appendiculatus*
11		Jackal	Ticks	*Rhipicephalus appendiculatus*
12		Jackal	Tick	*Rhipicephalus appendiculatus*
13		Giraffes	Ticks	*Amblyoma variegutum*
14		Civet	Tick	*Rhipicephalus appendiculatus*
15		Civet	Ticks	*Rhipicephalus appendiculatus*
16		Giraffe	Ticks	*Amblyoma variegutum*
17				
			BIRDS	
	Helminthes	Guinea fowl	Intestine	No parasite seen
18		Bird	Intestinal content	No parasite seen
19		Un id. bird	Intestine	*Heterakis spp*
20		Guinea fowl	Intestine	*Metroliathes lucida*
21				*Ascaridia spp*
22		Guinea fowl	Intestine	*Heterakis dispar* *Heterakis isolonche* *Hymenolepis spp* *Raillietina spp*
23		Guinea fowl	Intestine	*Railietina spp* *Hymnolepis spp* *Heterakis spp*
		Un id. bird	Intestine	*Metrolisthes spp*
24		Guinea fowl	Worm	*Subulura spp*
25		Un id bird	Intestines	No parasite seen
26		Pigeon	Intestines	No parasite seen
27		Un id. bird	Intestines	No parasite seen
			REPTILES	
29		Snake	Intestinal content	No parasite seen
30		Snake	Intestines	No parasite seen
31		Snake	Intestine	No parasite seen

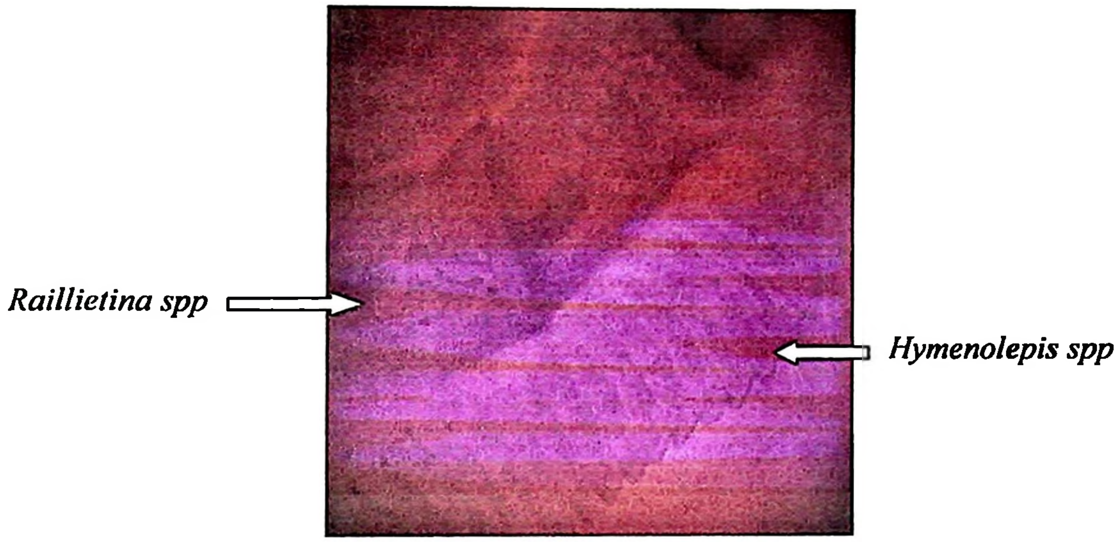


Plate 8: *Raillietina spp* and *Hymenolepis* species in the same Guinea fowl intestines

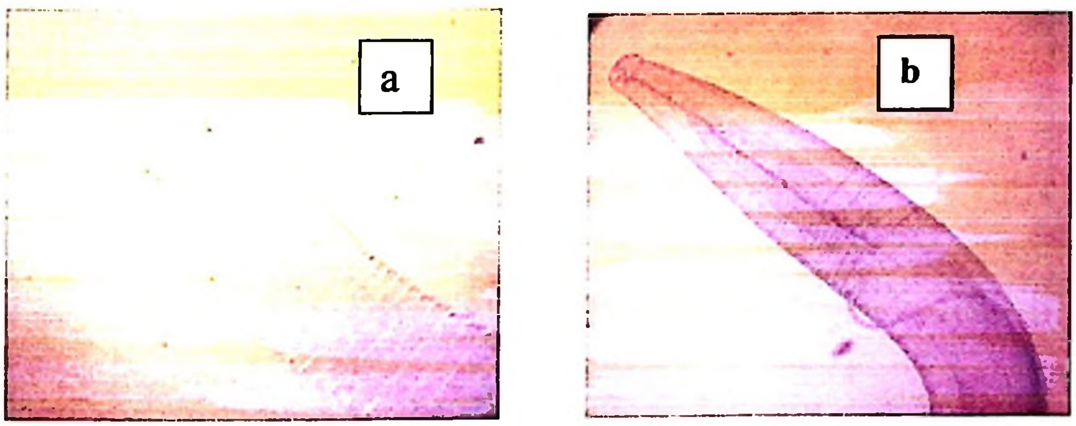


Plate 9: *Hymenolepis spp* (a) and *Heterakis spp* (b) recovered from Guinea fowl

(b) Recovering and identification of external parasites

Generally, all ticks recovered belong to the family *Ixodidae*. These ticks include the *Rhipicephalus appendiculatus*, *Rhipicephalus pulchellus*, *Amblyoma variegutum* and *Amblyoma gema* and all were recovered from mammal group only. No external parasites were recovered from bird and reptile groups (Table 21). Plate 10a and 10b shows the smashed jackal harbouring the *R. appendiculatus* tick on its ear. Also Plate 11a and 11b showing the smashed giraffe harboring *Amblyoma* tick.

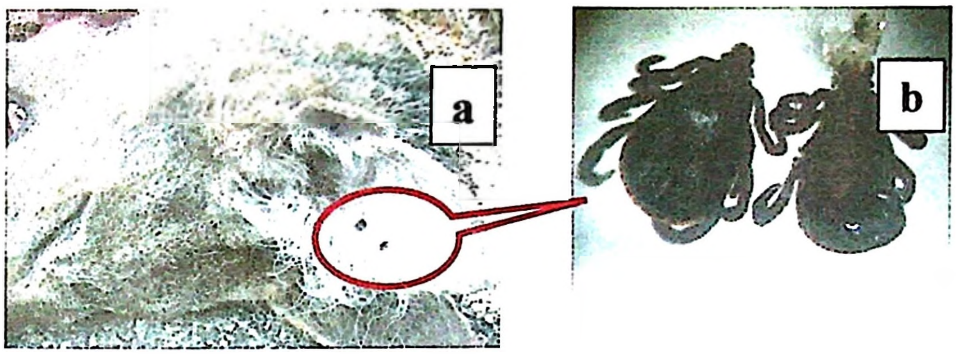


Plate 10: *R.appendiculatus* ticks on jackal's ear (a) and *R.appendiculatus* ticks (b) x10 magnification

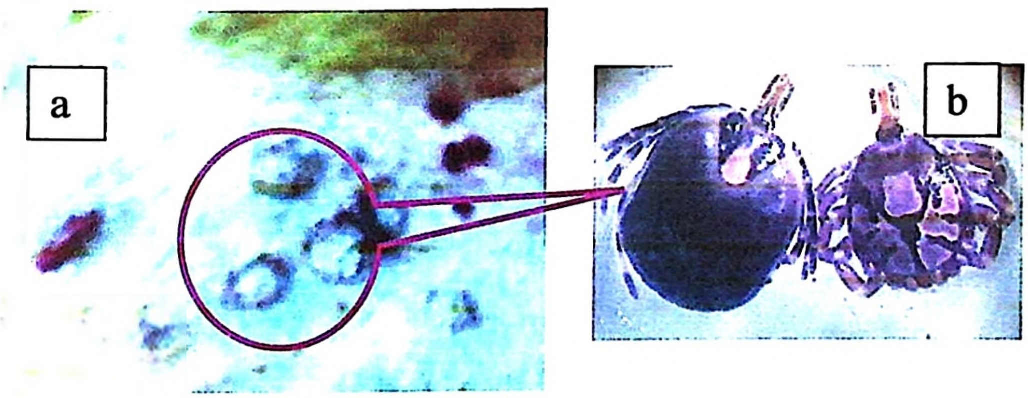


Plate 11: Smashed giraffe habouring *Amblyoma variegutum* ticks on anal area (a) and *Amblyoma variegutum* tick (b) x10 magnification

4.5.3 Worm recovery from field faecal samples for five selected species and tick survey (*ex-situ* study)

(a) Faecal egg and oocyst counts

Egg and oocyst counts were done on feacal samples collected *ex-situ* for five selected animal species. The animal species and number of samples collected (in parenthesis) are buffaloes (n = 95), wildebeest (n = 92), giraffes (n = 99), impala (n = 71) and elephants (n = 85) making a total of 446 samples (Table 22). Elephants seemed to harbour more helminthes than the other four species studied (Table 22 and Fig. 14). Out of 85 elephant faecal samples collected, 54 (64%) had worm eggs in their faeces and 29 of which (34%) had egg counts exceeding 200 eggs per gram. Six elephants had exceptionally high counts exceeding 1000 egg per gram. In other species, egg counts per gram were highest in wildebeest, followed by impala, giraffes and lowest in buffaloes (Table 22). Plate 12a and 12b show different types of eggs recovered.

Table 19: Helminthes egg counts in five selected species

Animal species	Total number of sample collected	Egg counts per gram	Number	Prevalence in %
		0	78	82.1
		1-100	10	10.5
Buffaloes	95	101-200	8	8.4
		> 200	0	0
		0	27	29.3
		1-100	24	26.09
Wildebeests	92	101-200	17	18.4
		> 200	24	26
		0	45	45.4
		1-100	22	22.2
Giraffes	99	101-200	12	12.1
		> 200	19	19.1
		0	29	40.8
		1-100	1	1.4
Impala	71	101-200	24	33.8
		> 200	19	26
		0	31	36.4
Elephants	85	1-100	15	17.6
		101-200	11	12.9
		> 200	29	34.11

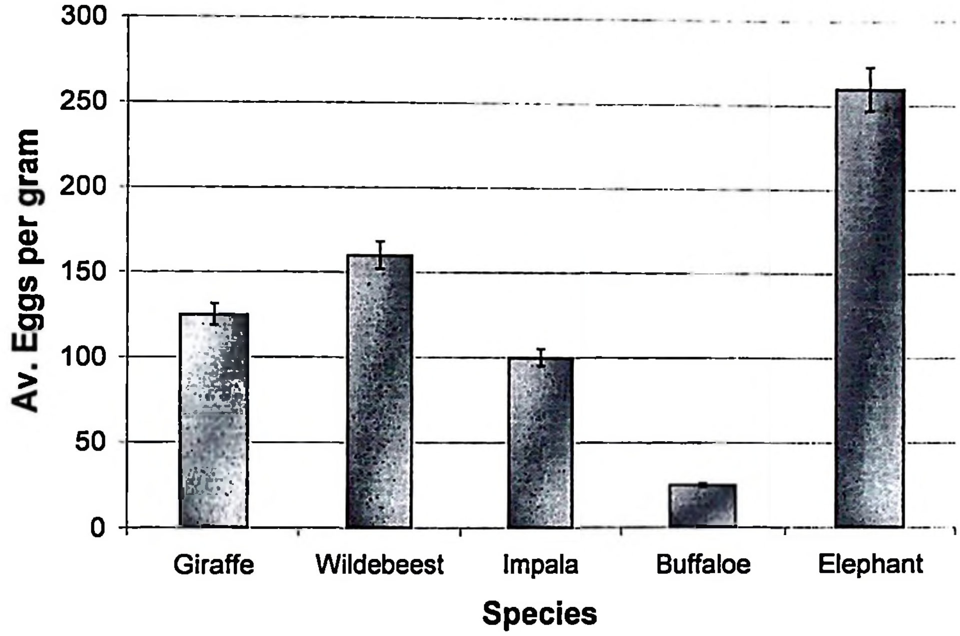


Figure 14: Average egg counts per gram

For coccidian oocyst, different species of coccidian were recovered from the same samples (Table 23). The results show that the wildebeest had the highest oocyst count (49 cases out of 92 samples), followed by Impala (4 cases out of 71 samples). No oocysts were observed in the elephants, giraffes and buffaloes samples (Fig. 15). Plate 13a and 13b show different types of coccidian oocyst recovered.

Table 20: Number of coccidian oocyst in five selected species

Animal species	Total number of sample collected	Egg counts per gram	Number	Prevalence in %
		0	95	100
Buffaloes	95	1 - 100	0	0
		101 – 200	0	0
		>200	0	0
		0	43	37.5
Wildebeests	92	1 - 100	19	9.7
		101 – 200	12	13.6
		>200	18	19.5
		0	99	100
Giraffes	99	1 - 100	0	0
		101 – 200	0	0
		>200	0	0
		0	67	94.3
Impalas	71	1 - 100	0	0
		101 – 200	1	1.4
		>200	3	4.2
		0	85	100
Elephants	85	1 - 100	0	0
		101 – 200	0	0
		>200	0	0

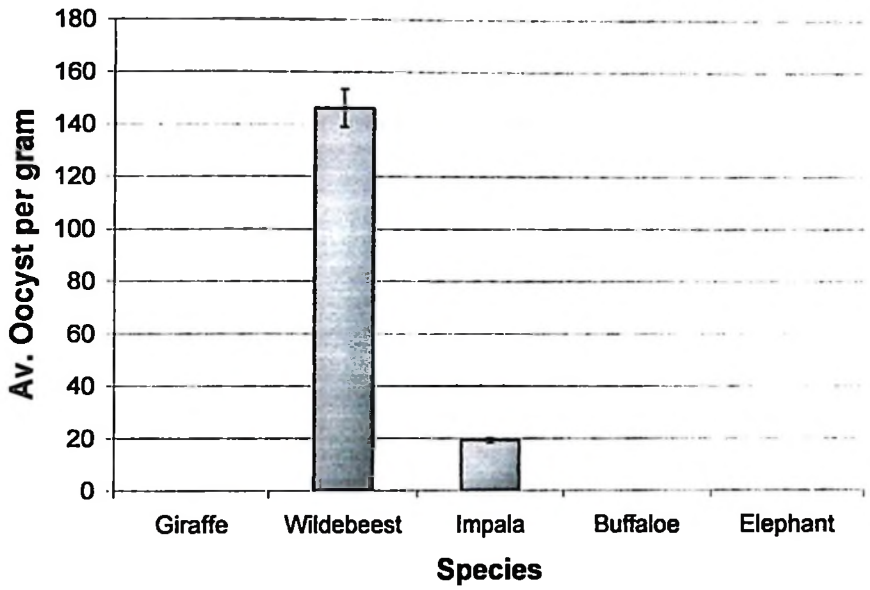


Figure 15: Average oocyst counts per gram

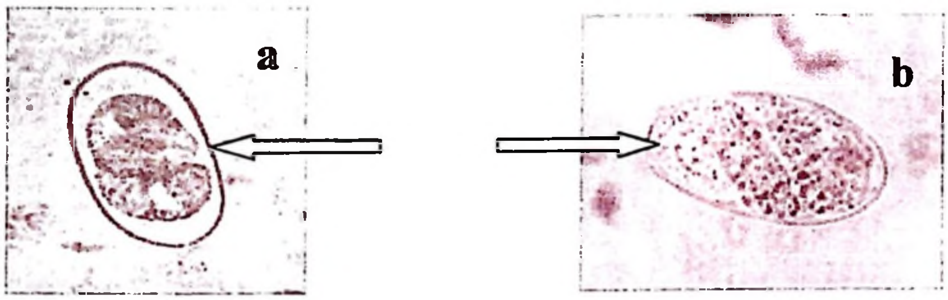


Plate 12: An egg recovered from impala (a) and elephant (b) feacal samples

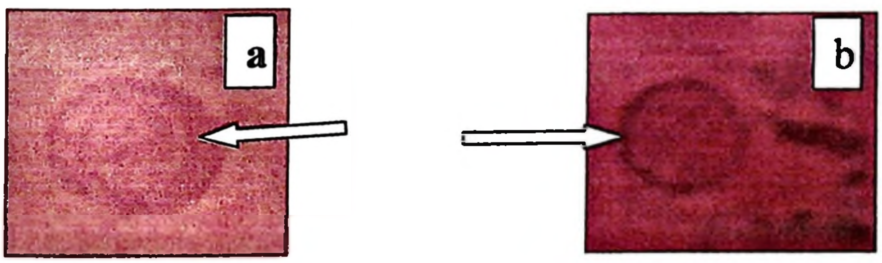


Plate 13: Coccidian oocyst recovered from impala (a) and wildebeest (b) feacal sample

(b) Identication of helminthes

To allow better identification of helminthes from eggs, all faecal samples which depicted the presence of helminthes eggs were pooled and cultured together for each respective species. The hatched larvae were examined under the microscope. All larvae from buffalo eggs culture were identified as *Haemonchus* and *Trichostrongylus* species (Table 24 and 25). The rest were described morphologically since they could not be identified due to lack of the identification key. Plate 14 show some of the larvae recovered from impala (14a) and buffalo (14b) samples.

Plate 14: Larva recovered from an egg of impala (a) and buffalo (b) feacal sample

Table 21: Laval description for identification

Animal Species	Larva Number	Description	Worm Identification
Buffalo	5	Head was narrow and rounded. Tail sheath was medium	**Haemonchus species**
Buffalo	3	Head was tapered. Tail sheath was short cone(Plate 26)	**Trichostrongylus species**
Giraffe	1	Total length was 720uM.Tail sheath was 60uM, Mouth part round, Oesophagus 120 uM. Intyestinal cell cuboidal	**Not identified**
Giraffe	1	Total length was 620uM.Tail sheath was 60uM, Mouth part round, Oesophagus 120 uM. Intyestinal cell cuboidal	**Not identified**
Impala	9	Total length 280u – 350uM MTail end is kinked. Tail sheath absent. Mouth part rounded. Intestinal cell columnar. Oesophagus not clearly marked (Palte 25)	**Not identified**
Elephant	1	Total length 830uM Tail sheath 180uM M Tail sheath filamentous. Mouth part round. Oesophagus length 120uM	**Not identified**
Elephant	1	Total length 980uM Tail sheath 140uM M Tail sheath filamentous. Mouth part round. Oesophagus length 110uM	**Not identified**
Elephant	1	Total length 940uM Tail sheath 170uM M Tail sheath filamentous. Mouth part round. Oesophagus length 120uM	**Not identified**
Elephant	1	Total length 1120uM Tail sheath 220uM M Tail sheath filamentous. Mouth part round. Oesophagus length 120uM	**Not identified**
Elephant	1	Total length 620uM Tail sheath 60uM M Tail sheath filamentous. Mouth part round. Oesophagus length 40uM	**Not identified**
Wildebeest	-	-	

Table 22: Overall worm identification results in MINAPA

Animal species	Number of pooled feacal sample	Larvae examined	Larvae identified	Larvae not identified/ described
Buffaloes	18	8	8	0
Impala	40	9	0	9
Elephant	40	5	0	5
Wildebeest	40	0	0	0
Total	**178**	**24**	**8**	**16**

(c) Tick survey results

Tick surveys were done to determine the types and prevalence of tick species in MINAPA. The survey was conducted on the ground in five different locations which included Mwanambogo, Kikoboga, Kisungura, Ikoya, and Chamgole. Tables 26 and 27 summarizes the tick prevalence at all location studied. In Mwanambogo and Kikoboga only 2 tick species namely *Rhipicephalus appendiculatus* and *Amblyoma variegutum* were identified. Chamgole had only *Rhipicephalus appendiculatus* and *Amblyoma gemma,* while at Ikoya location 3 species of ticks were identified as *Rhipicephalus appendiculatus, Rhipicephalus pulchellus and Amblyoma variegutum. Rhipicephalus appendiculatus* was the most prevalent tick species in the Park accounting for about 86% prevalence. *Amblyoma gema* was the least common tick (0.8% prevalence) while *Rhipicephallus pulchellus* and *Amblyoma variegutum* accounted for about 4.9% and 8.1% prevalence, respectively.

Table 23: Tick survey results for selected five locations in MINAPA

LOCATION	TICK SPECIES	SUB TOTAL		TOTAL
		ADULT	NYMPH	
Mwanambogo	*Rhipicephalus appendiculatus*	10	20	30
	Amblyoma variegutum	3	-	3
Kikoboga	*Rhipicephalus appendiculatus*	20	7	27
	Amblyoma variegutum	5	-	5
Kisungura	*Rhipicephalus appendiculatus*	14	13	27
	Amblyoma gemma	1	-	1
Ikoya	*Rhipicephalus appendiculatus*	16	3	19
	Rhipicephalus pulchellus	6	-	6
	Amblyoma variegutum	2	-	2
Chamgole	*Rhipicephalus appendiculatus*	2	-	2
TOTAL		**79**	**43**	**122**

Table 24: Overall tick survey results in MINAPA

Tick species	Total	Prevelance in %
Rhipicephalus appendiculatus	105	86
Rhipicephalus pulchellus	6	4.9
Amblyoma variegutum	10	8.1
Amblyoma gemma	1	0.8

CHAPTER FIVE

5.0 DISCUSSION

The present study addressed the roadkill dynamics on MINAPA highway and used the samples collected from highway kills to study the types of infections and infestations which might be affecting different types of wildlife in the park. There have been several previous studies which have investigated roadkills in MINAPA (Johnson, 2000; Kidegesho, 2001; Hamis, 2002; Joseph, 2006), but none of these used a study design similar to in the present investigation to address several compounding factors which might be associated with this problem in the Park. In addition, the previous reports which addresses numerous and emerging wildlife health challenges in Tanzania did not use the roadkill as an alternative potential source of health information in National Parks. For example, previous health-related studies on wildlife in MINAPA (Keyyu *et al.*, 2003; Kagaruki *et al.*, 2005) used opportunistic sampling procedures but did not target roadkilled animals as potential source of samples in the Park.

The present investigation covered a period of 8 months (September 2007 to April 2008) and was aimed at complementing the previous studies by combining the use of GPS information to map several sites on and around the highway which could help provide more insight on the magnitude of roadkill problems in the Park. In addition to the identification of all cases of all road-killed mammals, reptiles and birds on MINAPA highway during the study period, all locations where these kills occurred were also GPS identified. This quantification enabled a clear assessment of roadkill pattern between and within these 3 groups of animals.

5.1 Roadkill dynamics in MINAPA

5.1.1 Roadkill pattern

The roadkill pattern differed significantly among different groups of animals under investigation during the study period. Mammals were a predominant group killed by road accidents followed by reptiles and birds. Similar findings have previously been reported in MINAPA (Elibariki, 2006) and in other areas (Bennett, 1991; Taylor and Goldingay 2004; James *et al.*, 2005; Gryz and Krauze, 2008). Mammals possibly suffered more mortality on the highway due to fact that this group is made up of complex group of many species with different behavior and this group is also ecologically more successful terrestrially with few species which can fly (e.g. bats) and other can live aquatic life (e.g. hippos) (Kabigumila, 1993). Most of their activities including foraging and reproduction are done on land. This could have contributed much on the road crossing and roadkill accidents observed in MINAPA. As Seiller *et al.* (2004) previously showed that the denser the traffic and the denser the population, the more road-kills may be expected. This factor possibly also contributed to some extent to the roadkill accident for reptiles but less so the bird group which is adapted for arboreal flying. Secondly, roadkill dynamics in mammals in areas where roads/highway bisects parks are also possibly a function of their abundance in a particular ecosystem and traffic volume (Seiller *et al.*, 2004). This picture is true in MINAPA where mammals are abundance and diverse although bird group could higher.

Within groups, it was observed that different species were dying in different patterns. For the mammal group, impala, rodents, hares and civets were predominantly killed than others. This has also been reported previously by Elibariki (2006) in MINAPA. The high roadkill accident rates for these species possibly occurs because these species of animals are usually more attracted to the topography of a road due to the following reasons. Impala in addition to their huge numbers are normally attracted to the grasses growing on the side of the road and when crossing the road they get crushed by passing vehicles (Elibariki, 2006). The proliferation of rodents killed on the road is possibly associated with their insect feeding habits (Seiller *et al.*, 2004; Popov, 2009) and these kills usually attract scavengers such as civets, which in turn are often struck by cars (Seiller *et al.*, 2004). For the bird group, guinea fowl was predominantly killed by road accident than other species and this could possibly be due to the gregarious nature of these species as part of their behavior. Gregariousness is one of the functional structures of animal communities and do varies along the different environmental gradients. Impalas, rodents, hares and guinea fowls are abundant and potentially gregarious animals that appear to prefer crossing the road in groups. With other factors such as traffic volume, this behavior could contribute much on their high roadkill cases (Wallgren *et al.*, 2007).

The correlation analysis of roadkill counts among these three groups showed that there was a high positive correlation between them as follows; mammals and reptiles ($r = 0.736$), bird and reptiles ($r = 0.649$) and mammals and birds ($r = 0.647$) (See appendix 3). These positive correlations in roadkill counts between the three groups could be due to the fact that they share the same ecosystem including food and shelter. Reptile and mammal which showed a stronger positive correlation between themselves also showed almost similar killing patterns in MINAPA. Both groups are terrestrial animals and usually follow the same route when searching for food and shelter.

Roadkill counts obtained from secondary data covering the same months of September to April from 2003 to 2007 showed that a total of 680 roadkill cases were recorded which was about 170 cases/year. During the present study, almost twice this number (314 cases) were recorded. Similarly, secondary data also showed that mammals were the predominant group killed by road accidents followed by birds and reptiles an inverse of what was observed in the primary data. Furthermore, secondary data also showed that mammal and bird groups have no significant difference in their roadkill counts but both differed significantly from the reptile group. In addition, total roadkill counts within and between groups per year were not significant. The mammal and reptile groups showed strong positive correlation (r = 0.792) while mammals vs birds showed a strong negative correlation (r = -0.691) and bird vs reptiles showed a weak negative correlation (r = -0.112) (See appendix 5). As noted in the primary data, secondary data also agreed with the suggestion that the movement pattern of mammals and reptiles were possibly similar in MINAPA and thus they were killed in the same pattern. The reason why the yearly counts of roadkill recorded between September - April during 2003-2007 period were lower and there was negative correlation roadkill counts between mammals vs birds when compared to those observed in the present study is unclear. Statistical analysis showed that higher roadkill counts observed in the present study were mainly associated with significant large number of reptiles and to a lesser extent, mammals killed on the highway during the present study period when compared to the data collected over the previous 4 years by the park management. No significant difference was observed, however, for the bird group counts between years. It is postulated that this difference in roadkill counts between the primary and secondary data might be attributable to poor recording system, low commitment of personnel frequently allocated to this activity in the park (usually a driver and ranger) and lack of reliable working gear such as transport and funds. In most cases this activity is usually done only when funds permits (Mofulu F. personal communication).

When the roadkills were catagorised on monthly basis for a period covering 2003 to 2008, (combined primary and secondary data) results show that no clear monthly trends were observed within and between groups. In all cases however, more roadkills were observed in December and January and least in April. The increase of roadkill in December and January may be associated with increased traffic volume on TANZAM highway during these holiday months (Seiller 2004), dispersal of young and perhaps the change in activities resulting from mating (Inbar and Mayor 1999; Schlacher *et al.*, 2007; Gryz and Krauze, 2008) or change in foraging pattern of most mammals during this period (Elibariki, 2006). In MINAPA, this period is characterised by sprouting of new grass following a previous period of controlled-burning during the dry season; a yearly routine management exercise to control ticks and tall grass in the park (Hamis, 2002; Elibariki, 2006).

5.1.2 Roadkill accident hotspot

To allow good estimation of roadkill accident hotspot areas along the highway, GPS data was taken on all locations along the highway were roadkills were found. The results of this exercise showed that roadkills occurred continuously throughout the entire 50 km highway stretch. However, deaths were more clumped in some areas. Such wildlife roadkill patterns has recently also been described elsewhere (Coelho *et al.*, 2008). However, there were few areas along the highway which seemed to be more prone to roadkill accidents. Designated sections between A to G had road kills above the average of 6 case km^{-1} while sections between G to K had the road kill cases below the average. Designated section F-G alone registered about 14.09 roadkill cases per km while section I-J had the least number of roadkill/km which was 2.75 cases. This indicates that animals are killed more on the Doma side (Dar es Salaam side) of the road compared to Mikumi side. The section A-G on the Doma side is located far away from the Park headquarters and does not usually enjoy an equal routine ranger patrols compared to sections G-K which is well guarded by Park staff and the movement of Park staff to and from Mikumi town is also high.

Based on the results obtained in the present study, there were indications which suggest that mammals, reptiles and birds roadkill cases occurred preferentially more on different sections of the highway. Reptile and birds deaths occurred more on section F-G while mammals died more on section C-D. The least cases for mammal and bird kills occurred on section I-J while that of reptile occurred on section J-K. There are usually different reasons for variability in roadkill cases among different wild animal groups. First, this variability depend much on types of animal group involved e.g. mammals, reptiles or birds, and also on animal category e.g. small or large mammal (Seiller *et al*., 2004). The present study suggests that, different animal species and category were killed in different patterns due to their difference in their body size, behavior, abundance and life styles (Row *et al*., 2007). Small mammals had generally highest number of roadkill compared to other mammal category and these deaths were more random in nature, while large mammal roadkill pattern occurred in discrete sections. This was another indication that different animal species might have different road crossing preferences on the highway that passes through MINAPA. It is also possible that more deaths were observed in small mammals in MINAPA due to their high abundance and gregarious compared to other groups making them more prone to being killed when collision occurs.

5.1.3 Vegetation cover and roadkill accident hotspot in MINAPA

The dominant vegetation type found along the MINAPA highway include wooded grasslands, open woodlands (miombo), bushlands, bushed grasslands and open grasslands. These vegetation patterns in MINAPA have also been described elsewhere (Leyequien *et al*., 2007; Melletti *et al*., 2008; Stokke and Toit, 2008). It is well known that different wild animal species prefer different vegetations types (Ereckson, 2001; Wallgren *et al*., 2007; Leyequien *et al*., 2007; Melletti, 2008; Stokke and Toit, 2008). These different preferences are usually determined by different functional traits such as feeding type, body mass, activity patterns and gregariousness (Melletti *et al.*, 2008; Stokke and Toit, 2008). Such functional traits often vary with different species of animals and these variations tune different animals to different vegetation types (Wallgren *et al.*, 2007). Although the total number of animals killed per species per section was relatively small during the present study, data shows that in general, small mammals were killed more in all sections except in section I-J and J-K which has bushlands vegetation. No obvious trend was observed for large mammals as few deaths were recorded in this group during the present study. This is possibly due to the fact that animal-vehicle collisions, especially those involving large mammals, are often traumatic and usually produce damage to vehicles and can cause physical injuries or death (Seiller *et al.*, 2004). Hence, more often drivers are usually cautious and try their best to avoid them.

5.1.4 Roadkill accident hotspot associated with weather pattern in MINAPA

Water is crucial for life existence and hence it is not surprising that wildlife lifestyle greatly depends on the reliable supply of water. Roadkill frequencies are otften weather dependant (Seiller *et al.*, .2004; Newmark *et al.*, 2008). In MINAPA, there are clear dry and wet seasons (Ereckson, 2001). The present study started in September during the dry season and minimal rains was observed up to the end of October and all temporary watering points in close proximity to the highway were dry. During this period, most of the vegetation surrounding the highway was very dry and grass had not sprouted from the previous early burning exercise which was done by the park during the July and August months. During this period, roadkills in all groups (mammals, birds and reptiles) were few since most of these animals were mostly found in areas far away from the highway which had better grazing and watering areas.

Short rains started in November and culminated in mid-in January. This was followed by heavy rains in February to April. The marked sprouting of fresh vegetation around the highway and filling up of the temporary watering points which were mostly close to the highway concomitantly resulted in increased wildlife activity close to the highway. Most likely this contributed to the high frequency of roadkills which were observed during November, December and January in the present study for this and previous years. In other previous studies, Ereckson (2001) has shown that December and January months, rainfall is regular in MINAPA, temperature are hot and humidity is high, water is available but rainfall may be intermittent limiting location and grasses and herb species grow rapidly forcing many animals wonder more freely and the roadkill cases increase.

During March and April, there was heavy rainfall in MINAPA and there was plenty of water in temporary and permanent water areas and animal movement in search of water was limited. Consequently, the frequency of animal crossing the road decreased, as a result roadkill numbers went down. This agrees with other reports done in MINAPA (Ereckson, 2001; Newmark *et al.*, 2008) and elsewhere (Roedenbeck and Voser, 2008). It is also likely that increase in the grass tallness around the highway which was observed during this period contributed also to the observed low roadkills during these months since such vegetation is known to reduce the visibility of most animals and many get scared of being scavenged, hence out of survival instincts move from the highway to the open areas away from the highway (Seiller *et al.*, 2004; Newmark *et al.*, 2008).

Ambient temperatures in MINAPA ranged between 24.16 ± 0.21°C in September to 27.71 ± 0.34 °C in November which was the end of the dry season in the Park. In the following months, mean temperatures increase unequivocally ranging from 29.12 ± 0.30 °C in December to 28.86 ± 0.25 °C in March. When these data were correlated with roadkill pattern within the same study months no clear trend was observed suggesting that changes in ambient temperatures *per se* did not affect the roadkill dynamics in the park

5.2 Assessment of health issues associated with wild animals and birds in MINAPA

5.2.1 Types of infections of roadkilled animals and birds in MINAPA

Most of the bacterial isolates obtained in samples obtained from fresh and crashed mammals mainly belonged to *Enterobactericeae* family. This included *E. coli*, *Klebsiella*, *Citrobacter* and *Proteus*. Usually these organisms are normal habitants of the intestinal tracts of vertebrates and also found in water and soil. Some members of this family, however, have species/strains which are recognized as pathogens and cause diarrhea and septicemia in juveniles and more rarely in adult animals (Bradley *et al.*, 2001). Due to the origin of the specimen taken especially in semi-crushed and severely crushed animals, there was a high possibility that most of the organ samples which were taken were already contaminated with material from intestinal contents. This is supported by having about 50% isolation of *Proteus* species which occur naturally in the environment of animals. Of great interest was the isolation of *Pasteurella multocida* in a sample collected from the respiratory tract of the intact health jackal. *P. multocida* organisms are potential pathogen for many species of domesticated and wild animals world wide (Blanchong *et al.*, 2006; Bredy, 2007; Al-Hassani *et al.*, 2007). These bacteria are usually frequently found in the respiratory tract and sometimes in the intestine of apparently healthy animals and under certain predisposing conditions, the organisms may multiply causing severe and perhaps fatal infection (Boyce, 2004). In birds and reptiles, the bacteria which were isolated are *E. coli*, *Proteous spp*, *Citrobacter spp* and *Staphylococcus spp* which were most likely contaminations from the intestinal contents and surroundings since majority of these birds and reptiles were found severely crushed.

5.2.2 Types of infestations of roadkilled animals and birds in MINAPA

All helminthes recovered belonged to the Phyla *Plathelminthes* and *Nemathelminthes* of different families and genus (see Table 21). Internal worms identified in both mammals and birds belonging to the Phylum *Plathelminthes (Moniezia, Metroliathes Hymnolepis and Raillietin spp) and Nemathelminthes (Herakis, Ascaridia, Trichostriongylus, and Subulura spp).* Though most of the animals and birds in which these worms were identified seemed to be in good health, many species of *Nemathelminthes* are blood suckers and are frequently associated with severe anemia and ill health in severely affected individuals (Horak and Louw, 2005; Alexakis, 2009). Some *Plathelminthes* feed on gut contents interfering with availability of some essential nutrients in their hosts (*Moniezia sp)* causing nutritional disorders especially in youngs (Erick, 2009). Some species of *Raillietina* like those observed in guinea fowl in MINAPA do penetrate deeply into the mucosa and consequently producing marked enteritis, which is frequently haemorrhagic in heavy infections in birds (Samuel *et al*., 2001). M*etroliasthes* were also recovered in guinea fowls in MINAPA and though are rare in many species of birds, they are parasite occurring in the small intestine of fowls and usually transmitted via grasshopper intermediate hosts of genera *Chorthippus Paraxya* and *Melanoplus* (Samuel *et al*., 2001).

Another important worm affecting wild animals are nematodes of genus *Trichostrogylus* and *Haemonchus spp.* The species of genus *Trichostrogylus* has been reported as parasites of the gastrointestinal system in wild bovids and cervids (Samuel *et al*., 2001; Horak, 2003). The parasites penetrate into the mucosa under the epithelium, rarely deeper, producing desquamation, which may be extensive in heavy infestations (Alexakis, 2009). The worms are not blood-suckers; nevertheless anaemia may be associated with heavy infection, this being due to shortening of the life of the red cell, impaired erythropoiesis or a reduction of the amino acid pool (Soulsby, 1983; Smith and Stimson, 1998).

Haemonchus are parasites of the abomasums and are widely distributed nematodes in ruminats throughout the world (Horak and Louw, 2005). The principal feature of *Haemonchus* infection is anaemia, due to the blood-letting activities of the parasite (Soulsby, 1983).

Another worm recovered belonged to Families *Heterakidae* and *Hymenolepidae* of the Genuses *Heterakis* and *Hymenolepis,* respectively. The species of genus *Heterakis* occur in the caeca of the fowl, guinea-fowl, pea-fowl, turkey and numerous other birds (Daniel, 2009). The infection is mildly pathogenic. The genus *Hymenolepis* contains a large number of species which occur chiefly in domestic and wild birds (Shen *et al.*, 2009). Another species isolated belonged to the Family *Subuluridae* in Genus *Subulura*. Species in this genus occur in caeca of the fowl, turkey, guinea-fowl, and related birds in Africa (Soulsby, 1983). However, their pathogenesis is apparently not clear.

Ectoparasites were isolated from mammals only and were all three-host ticks belonging to the Phylum *Arthropoda (R. appendiculatus and A. variegutum)*. *A. variegutum* act as an intermediate host of *Cowdria ruminantium* which causes fatal heartwater disease in cattle, sheep and goats and it is parasitic to all domestic and many wild animals (Kirk and Hamlen, 2000) producing bad wounds in perineal, genital regions, interdigital and fetlock regions where it frequently attaches (Olwoch *et al.*, 2008). Wounds and inflammation of the extremity are normally very painful and therefore affect the movements of affected wild animals leading to severe debility and increases the chance of such animals to ease predation (Mlengeya *et al.*, 2008). *R. appendiculatus* tick is essentially parasitic to cattle, equines, sheep, goats, wild antelopes and has also been found on dogs and wild rodents. It attaches mainly under the tail, groin and ears. It is a chief vector of *Theileria parva* causing East Coast Fever (ECF) in cattle, *Babesia bigemina* causing Babesiosis of cattle and a number of other vectors which cause diseases in dogs and sheep and possibly wildlife (Soulsby, 1983, Dobbelaere and Heussler, 2004). *Theileria spp* infection has been reported in some wildlife species such as greater kudu, grey duiker, sable and roan antelopes (Dobbelaere and Heussler, 2004).

Due to study time limitations, and diversity of roadkill under investigation, it is quite possible that many endo- and ectoparasites were missed especially in reptiles. There is a need to continue this work in future in order to provide more insight on the helminthes and ectoparasite dynamics affecting several species of wildlife in MINAPA.

5.2.3 Assessment of the type of infestations based on samples collected ex-situ

Since the species diversity of roadkills was diverse and sample collections per species was insufficient to provide statistically acceptable assessment of infestation (endo- and ectoparasites) dynamics on these animals, this study was done on more samples at least for a few selected species. Fresh fecal samples were collected 20m to 4km on either side of the highway from five selected species of animals namely buffaloes, wildebeest, giraffe, impala and elephant. Ectoparasites (ticks) were collected in five locations in the park namely, Chamgore, Kisungura, Ikoya, Kikoboga and Mwanambogo.

Elephant fecal samples were observed to harbour more helminthes followed by wildebeest, giraffes, impala and lowest buffaloes. Bliss (2007) reported that worm transmission among different animal species normally occur through areas where wildlife find water since moisture is important in parasite development and for movement of infective larvae onto the vegetation. Ponds and other watering areas are sites often where wildlife congregates and therefore contamination is at a higher level compared to wide-open areas. Elephants are water loving animals and normally do congregate around watering points and this is possibly why they had more worm counts compared to others animals.

Results of the present study also show that different species of coccidian were recovered from those five selected species. Several species of coccidian have been isolated in wild animals in MINAPA (Mtambo *et al.*, 1997) and elsewhere (Duszynski *et al.*, 1999; Samuel *et al*, 2001; Ezenwa, 2003; Arnastauskene, 2008; Brunnell, 2009). Wildebeest had highest oocyst count (49 cases out of 92 samples) followed by impala (3 cases out of 71) and none in others. The occurrence of coccidia infection in wildebeests and impalas in MINAPA is possibly due to the fact that these two species of animals have great reproductive potential and due to their protection, their populations increase rapidly and often occur in large and sometimes overcrowded groups (Samwel *et al.*, 2001; Brunnell, 2009). Due to the overcrowding nature of these species rapid coccidia transmission among them become easier (Ezenwa, 2003; Arnastauskene, 2008). Wildebeest and impala are also gregarious and territorial which also increases the occurrence of parasitism in these species. Hosts with both traits (gregarious and territoriality) have been observed to harbored significantly more parasite than hosts with only one or neither trait (Ezenwa, 2004).

A tick survey done in Mwanambogo, Kikoboga, Kisungura, Ikoya, and Chamgole resulted in the collection of various adults and nymphs, and all were Ixodidae ticks belonging to two genera; *Rhipicephalus* and *Amblyoma*. In Mwanambogo and Kikoboga only two similar tick species were identified as *Rhipicephalus appendiculatus* and *Amblyoma variegutum*. Chamgole had only *R. appendiculatus* and *A. gemma*, while at Ikoya location *R. appendiculatus, R. pulchellus and A. variegutum* were collected. *R. appendiculatus* was the dominant species found in all 4 locations. Similar observations on tick distribution in MINAPA has previously been reported by Kagaruki *et al.* (2005). These results also show that tick species were not evenly distributed throughout the park. This might be associated with several factors including environmental limitation such as temperature and relative humidity within different locale in the park. For example, most of the ixodid ticks lose water when exposed to low relative humidity and extreme temperature (Olwoch, *et al*., 2008). Other limitation includes the presence of suitable host. Since animals in the park are not evenly distributed, it is likely that ticks distributions might follow same pattern like that of their potential host. It was interesting however, to note that only two species of Ixodidae ticks (*R. appendiculus* and *A. variegatum*) were found on roadkills which is possibly a function of their high abundance in the park, as it has been reported in a study by Kagaruki *et al.* (2005).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings of the present study, the following conclusions can be drawn:-

(a) Although there has been a substantial amount of work undertaken on roadkill mitigation measures, this study revealed that roadkill cases in MINAPA are still occurring in significant numbers and are increasing from year to year.

(b) Roadkills counts seemed to be more in smaller than larger mammals group possibly due to their abundance and diversity as well as to their marked differences in their functional trait such as feeding type, body mass, activity patterns and gregariousness.

(c) The incidences of roadkills are high during the dry season and peaks during December and January following a spell of short rains. Roadkill incidences go down during wet season from February to April.

(d) Although roadkill do occur anywhere on the 50 km stretch of the Tanzania – Zambia highway which bisects MINAPA, some areas seem to be more vulnerable to the road accidents. These include section C-D and F-G. Generally more roadkills seem to occur more on the Doma side (Dar es Salaam side) of the road compared to Mikumi side.

(e) Water availability during the dry season seems to be among the major factors which influence incidences of roadkill in MINAPA especially for the mammal group.

(f) It was generally more difficult to get suitable samples for health-related studies (microbiological or parasitological) from reptiles and birds roadkills as majority of these roadkills were found severely crushed. This is possibly related to their small body sizes making it easier for most drivers to run over them without paying much attention and care.

(g) Bacteria isolated from different body tissues of most roadkills seemed to have originated mainly from GIT contents possibly as spill-overs following heavy vehicle fatal collisions but the finding of pathogenic *P. multocida* in an intact jackal respiratory tract was of great health importance.

(h) Internal and external parasites found from *in-situ* and *ex-situ* parasitological studies belonged to the Phyla *Plathelminthes*, *Nemathelmenthes*, *Arthropods and Apicomplexa*. The internal parasite recovered belonged to the Phyla *Plathelminthes Nemathelminthes and Apicomplexa* and the magnitude of infestations was at different levels. For external parasites, both in-situ and ex-situ studies revealed only two genera (*Amblyoma* and *Rhipicephalus*) of the *Ixodiae* family.

6.2 Recommendations

The results obtained in this investigation have provided valuable information which will undoubtedly prove useful to MINAPA and other wildlife policy makers in Tanzania when making decisions on how best to improve the existing mitigation measures to minimize roadkill incidences in the park. In addition, the results also show that roadkilled animals could be useful in providing information on health status of various wildlife in MINAPA. The present study recommends the following:

(a) Roadkill data collection in MINAPA should be improved either by allocating more funds for the team which is working now or designing the special unit for that particular work. Also training personnel with the required skills is of paramount important.

(b) Since sections F-G and C-D were observed to be more vulnerable to road accidents, there is a need to conduct a longer planned study to confirm this observation and thereafter take appropriate mitigation measures to reduce the incidences of roadkills in these sections.

(c) There is a need by the park management to step up more organized measures or patrols on the stretch of the highway starting from Doma to Park Headquarters during the months of November, December and January, the period when the incidences of roadkills is highest in this section.

(d) Disease implications of the wild animals should be considered more widely by intensifying programmes of disease surveys and monitoring. Roadkill carcasses should be used as part of these surveys in MINAPA.

REFERENCES

Alexakis, L. (2009). *Trichostrongylus spp.* [http://www.cdfound.to.it/HTML/tricho.htm] site visited on 26/5/2009.

Al-Hasani, K., Boyce, J., McCarl, V.P., Bottomley, S., Wilkie, I. and Adler, B. (2007). Identification of novel immunogens in Pasteurella multocida. *Microbial Cell Factory* 18(6): 3.

Ament, R., Clevenger, A.P., Yu, O. and Hardy A. (2008). An Assessment of Road Impacts on Wildlife Populations in U.S. National Parks. *Journal of Environmental Management* 42(3): 480 – 496.

Anonym, G. (2000). *Mikumi National Park Guidebook.* African publishing group, Harare, Zimbabwe. 30pp.

Arguirre, A. and Tabor, G.M. (2008). Global factors driving emerging infectious disease: Impact to wildlife population. *Annals of the New York Acamy of Sciences,* 1149:1 – 3.

Arnastauskene, T. (2008). Coccidia and coccidioses of domestic and wild animals in Lithuania. *Journal of Environmental Management* 41(5): 590 – 640.

Baldus, R.D. (2009). Conservation and Sustainable Use of Natural Resources in Forestry and Wildlife. [www.wildlife programme.gtz.de] site visited on 10/2/2009.

Bolen, E. G. and Robinson, W. L. (2002). *Wildlife Ecology and Management* (4th Edition), Benjamin Cummings Publishers, Uk. 107-109pp.

Barrientoa, R. and Bolonio, L. (2009). The presence of rabbits adjacent to roads increases polecat road mortality. *Journal of Biodiversity and Conservation* 18(2): 402 – 418.

Barrow, G.I. and Feltham, R.K.A. (2004). *Cowan and Steel's Manual for the Identification of Medical Bacteria* 3rd Edition. Cambridge University Press, UK. 7 – 72pp.

Bennett, A.F. (1991). Roads, roadsides, and wildlife conservation: a review. *Nature Conservation* 2: 99 – 118.

Bissonette, J. (2008). Data issues describing road mortality hotspot and predictive models. *Journal of road ecology* 6:26 – 06.

Blanchong, J.A., Samuel, D.M., Goldberg, D.R., Shadduck, D.J., and Lehr, M.A. (2006). Persistence of Pasteulrella multocida in wetlands following avian cholela outbreaks. *Journal of Wildlife Diseases* 42(1): 33 – 39.

Bliss, D.H. (2007). The control of Gastro-Intestinal Nematode parasites of hoofed wildlife in North America, *Wildlife Society Bulletin* 43: 78 – 90.

Boyce, J.D., (2004). How does *Pasteurella multocida* respond to the host environment?. *Current Opinion in Microbiology*. 9(1): 117 – 122.

Bradley, G.A., Orr, K., Reggiardo, C. and Glock, R. (2001). Enterotoxigenic Escherichia coli infection in captive Black footed Ferrets. *Journal of Wildlife Diseases* 37(3): 617- 620.

Bredy, J.P. (2007). The effects of six environmental variables on *Pasteurella multocida* populations in water. *Journal of Wildlife Diseases* 25(2): 232 – 239.

Brunnell, T. (2009). The Importance of Faecal Indices in Assessing Gastrointestinal Parasite Infestation and Bacterial Infection in the Hedgehog (*Erinaceus europaeus*). [http://www.tonibunnell.co.uk/parasite.htm] site visited 25/5/2009.

Carter, Y.J. and Lema, O.E. (1998). *A Practical Laboratory Manual for Heath Centers in Eastern Africa*. African Medical Research Foundation (AMREF), Kenya 25 – 55pp.

Chalvet-Monfray, K., Sabatier, P., Chauve, C. and Zenner, L. (2004). A mathematical model of the population dynamics of Heterakis gallinarum in turkeys (Meleagridis gallopavo), *Journal of Poultry Science* 83(10): 1629-1635

Chapman, J.L. and Reiss, M.J. (2000). Ecology, *Principles and Applications, 2nd Edition*, Cambridge University press, UK. 15 – 78pp.

Chapman, C., Gillespie, T. and Golber, T. (2009). Primates and the Ecology of their Infectious Diseases: How will Anthropogenic Change affect host parasite interaction?. *Journal of Evolution Anthropology: Issues, News and Review* 14(4): 134 - 144.

Chowdhury, N. (2001). *Helminths of Wildlife*, Science Publication Inc. UK. 10-22pp.

Cleaveland, S, Laurenson, K. and Mlengeya, T. (2004). Impacts of Wildlife Infections on Human and Livestock Health with Special Reference to Tanzania. [http://www.wcs-ahead.org/book/chapter07] site visited on 20/6/2007.

Cleaveland, S., Mlengeya, T., Kaare, M., Haydon, D., Lembo, T., Laurenson, K. and Packer, C. (2007). The Conservation Relevance of Epidemiological Research into Carnivore Viral Diseases in the Serengeti. *Conservation Biology* 21(3): 612 – 622.

Coelho, I.P., Kindel, A. and Coelho, A. (2008). Roadkill of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, Southern Brazil, *Journal of Wildlife Research* 54: 639 – 699.

Daniel, T. (2009). Caecal worm or *Heterakis gallinae*. [http://poultrykeeper.com/turkeys/health/caecal-worms-or-heterakis-gallinae.html] site visited 28/5/2009.

Daszak, P., Tabor, G., Kilpatrick, A., Epstein, J. and Plowright, R. (2006). Conservation medicine and a new agenda for emerging disease. *Annals of the New York Academy of Sciences* 1026: 1 – 11.

Deem, S.L., Karesh, W.B. and Weisman, W. (2008). Putting Theory into Practice: Wildlife Health in Conservation. *Journal of Conservation biology* 15(5): 1224 – 1233.

Dobbelaere, D. and Heussler, V. (2004). Transformation of leukocytes by *T. parva* and *T. annulata*, *Annual Review of Microbiology*. 53: 1-42.

Dobson, A. and Carper, R. (2000). *Global warming and potential changes in host-parasite and disease-vector relationships - Global warming and biodiversity*. Yale University Press, UK. 77pp.

Drews, C. (1995). Roadkill of animals by public traffic in Mikumi National , Tanzania, with notes on baboon mortality. *African Journal of Ecology* 33: 89 – 100.

Duclos, L. M and Richardson, D. J. (2000). "Hymenolepis Nana in Pet Store Rodents." *Comparative Parasitology* 67(2):197-201

Duszynski, D., Wilson W.D., Upton S.J. and Levine N.D. (1999). Coccidia (Apicomplexa: Eimeriidae) in the Primates and the Scandentia, *International Journal of Primatology* 20(5): 761 – 797.

Elibariki, H. (2006). Wild Animal Deaths on the Highway; A case of Mikumi National Park. Special Project Report for award of BSc (WLM) Degree at Sokoine University of Agriculture, Morogoro, Tanzania 11 - 27pp.

Elzanowski, A., Ciesiołkiewicz, J., Kaczor, M., Radwańska, J. and Urban, R. (2009). Amphibian road mortality in Europe: a meta-analysis with new data from Poland, *European Journal of Wildlife Research* 55(1): 33 – 34.

Ereckson, A. (2001). Population Structure and Ecology of the Mikumi National Park Elephant Population, Dissertation for Award of Masters of Phylosophy Degree at Anglia Polytechnic University, UK. 23 – 67.

Erick, I. (2009). Parasites. [http://au.merial.com/disease_information] site visited on 30/5/2009.

Ezenwa, V. (2003). Habitat overlap and gastrointestinal parasitism in sympatric African bovids, *Parasitology* 126(4): 379 – 388.

Ezenwa, V. (2004). Host social behavior and parasitism infection: a multifactorial approach, *Journal of behavior ecology* 15(3): 446 – 454.

Fumagwa, R. D. (2000), Appropriate research methods for sustainable veterinary biodiversity monitoring In *Tanzania Wildlife Research Institute (TAWIRI), Proceedings of the First Annual Scientific Conference* (Edited by Kayumbo, H. K.) December 12-13, Arusha, Tanzania 41 - 45pp.

Gardipee, F. (2003). Development of faecal DNA sampling methods to assess genetic population structure of Great Yellowstone Bison. Dissertation for Award of MSc Degree at Montana University, Missoula, Montana. 22-30pp.

Gibbons, W. (2008). Roadkill offers lessons in Animal Ecology. [http://www.uga.edu/srel/ecoviews/ecoview080713.htm] site visited on 22/4/2009.

Gobush, K.S., Mutayoba, B.M., and Wasser, S.K. (2008). Long-Term Impacts of Poaching on Relatedness, Stress Physiology, and Reproductive Output of Adult Female African Elephants. *Conservation Biology* 22 (1): 590 – 1599.

Goff, P. (1995). Car Cultures's War on the Environment: The loss of farmland, forest and wildlife. [http:www.satyamag.com/letter.html] site visited on 24/2/2008.

Groom, M. J., Meffe, G. K. and Carroll, C. R. (2006) *Principles of Conservation Biology* (3rd ed.). Sinauer Associates Publishers, Sunderland. 97 -100pp.

Gryz, J. and Krauze, D. (2008). Effect of roadkill mortality on vertebrate, *European journal of wildlife*, 54(4): 707 – 714.

Hamis, I. H. (2002). Impact of infrastructure on wildlife conservation. Special project report for award BSc (WLM) Degree at Sokoine University of Agriculture, Morogoro, 8-9 pp.

Halpennty, C. and Gross, R.M. (2008). Macro- and microparasite infection profiles of hatchery fish before and after release from a conservation hatchery (Steelhead trout, Oncorhynchus mykiss). *Ecoscience* 15(4): 417 – 422.

Havlick, D. (2004). Road Kill, *Winter* 5(1): 33 – 67.

Horak, I G. (2003). Parasites of domestic and wild animals in South Africa. The *Onderstepoort Journal of veterinary research* 70(3): 175 – 186.

Horak, I.G. and Louw, J.P. (2005). Parasites of domestic and wild animals in South Africa. VI. Helminths in calves on irrigated pastures on the Transvaal Highveld. *The Onderstepoort Journal of veterinary research* 156(6): 67 – 93.

Hudson P.J., Rizzoli A., Grenfell B., Heesterbeek H. and Dobson A. (2002) *The ecology of Wildlife Diseases*, Oxford University pres Inc. New York. USA 4-5pp.

Inbar, M. and Mayer, R.T. (1999) Spatio-temporal trends in armadillo diurnal activity and road-kills in central Florida. *Wildlife Society Bulletin* 27: 865 – 872.

James P. Gibbs, J.P. and Gregory, W. (2005). Can road mortality limit populations of pool-breeding amphibians?. *Journal of Wetlands Ecology and Management* 13(3): 281 – *289.*

Johnson, M. (2000) Geography 485 Jeff Durrant. [www.geog. byu. Edu/durrant/courses/geog485/fall2000/Tanzania.html] site visited on 17/1/2008.

Joseph, F. (2006). The effect of highway to conservation of Mikumi National Park Morogoro Tanzania. Special Project Report for award of BSc (WLM) Degree Sokoine University of Agriculture, Morogoro, 13pp.

Kabigumila, J. (1993). Feeding habits of elephants in Ngorongoro Crater, Tanzania. *African Journal of Ecology* 31: 156 – 164.

Kagaruki, L.K., Fyumagwa, R., Mofuru, F. and Lyaruu, V. (2003). A study of Giraffe ear problems in Mikumi National Park: Tick and fly survey In *TAWIRI Proceeding of the Fourth Annual Scientific Conference,* (Edited by Keyyu J.D, *et al.*) 4-5 December 2003, Arusha Tanzania, 178 – 180pp.

Kagaruki, L.K., Fyumagwa, Batamuzi, E., Mlengeya, T., Lyaruu, V. and Mofuru, F. (2005). Giraffe ear disease problems in Mikumi National Par, Tanzania;: Tick and fly association. In *TAWIRI Proceeding of the Fifth TAWIRI Scientific Conference,* (Edited by Keyyu J.D. *et al.*) 1-3 December 2005, Arusha Tanzania, 310 – 311pp.

Kelley, L., Goodrich, J., Miquelle, D., Smirnov, E., Quigley, H. and Hornocker M. (2007). Effects of Roads and Human Disturbance on Amur Tigers. *Conservation biology* 16(1): 97 – 108.

Keyyu, J. D, Manduji, D.G, Kilewo, M, Moranda, M, Msalilwa, L. P. Kassuku, A. A, (2003). Internal Parasites of Wildlife in selected Protected Areas in Tanzania. *In TAWIRI Proceeding of the fourth Annual Scientific Conference,* (Keyyu J.D. *et al.*) 4-5 December 2003, Arusha Tanzania. 203 - 207pp.

Kideghesho, J. R. (2001). Mikumi National Park, under the pearl of human hands. *Kakakuona* 20: 8 - 15pp.

Kimambo, F. E. (2000). Patch selection by grazing herbivores at Mikumi National , Morogoro, Tanzania, Dissertation for award of MSc Degree at Sokoine University of Agriculture, 24 – 46 pp.

Kirk, J. and Hamlen, H. (2000). Heartwater: Another Potential Foreign Animal Disease. [http://www.vetmed.ucdavis.edu/vetext/IN-DA/INF-DA_HEARTWATER. HTML] site visited on 22/4/2009.

Kock, R., Kebkiba, B., Heinonen, R. and Bedane, B. (2009). The domestic/Wildlife interface: Issues for disease control, Conservation, Sustainable food production and emerging disease. *Annals of the New York Academy of Science* 969: 24 – 33.

Lembo, T., Hampson, K., Haydon, D., Craft, M., Dobson, A., Dushoff, J., Ernest E., Hoare, R Kaare, M., Mlengeya, T., Mentzel, C. and Cleaveland, S. (2008). Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. *Journal of Applied Ecology* 45(4): 1246 – 1257.

Leyequien, E., Verrelst, J., Slot, M., Schaepman-Strub, G., Heitkönig, I. and Skidmore, A. (2007). Capturing the fugitive: Applying remote sensing to terrestrial animal distribution and diversity. *International Journal of Applied Earth Observation and Geoinformation* 9(5): 1 – 20.

Lipsky, B. A., Hook, E. W. and Smith, A. A. (2008). Citrobacter infections in humans: Experience at the Seattle Veterans Administration Medical Center. *Journal of Infectious Disease*, 2:746-760.

Lovari, S., Sforzi, A., Scala, C. and Fico, R. (2007). Mortality parameters of the wolf in Italy: does the wolf keep himself from the door?. *Journal of zoology* 272(2): 117 - 124.

Madigan, M. and Martinko, J. (2005). *Brock Biology of Microorganisms* (11th ed.). Prentice Hall. Uk. 35-40.

Magnus, Z., Kriwoken, L., Moone,y N. J. and Menna, E. (2004). Reducing the incidence of wildlife roadkill: Improving the visitor experience in Tasmania. [http://www.crctourism.com.au/CRCBookshop/Documents] site visited on 2/3/2008.

Magnus, Z. (2006). Wildlife road killroadkill mitigation information kit; A guide for local Government and land managers.[http://www.crctourism.com.au/CRCBookshop/Documents] site visited on 2/3/2008.

Manual of Veterinary Parasitological Laboratory Techniques, (1986) 3rd Edition, Crown copyright. Uk. 23 – 311.

Mata, C., Hervas, I., Herranz, F., Sua'rez, J. and Malo, J. (2005). Complementary use by vertebrate of crossing structures along a fenced Spanish motorway. *Biological Conservation* 122: 231 – 242.

Mbassa, G.K., Young, S., Kauzeni,P. and Nkangaga., J. (2003). Quantitative impact analysis of wildlife conservation strategies: A study of impact of community – based conservation in Gonabis Wildlife Management Area and rare Antelopes in Game Reserves. *TAWIRI proceedings of the fourth annual scientific conference*, 4-6 December 2003, Arusha, Tanzania 50-64pp.

Melletti, M., Penteriani, V., Mirabile, M. and Boitani, E. (2008). Effects of habitat and season on the grouping of forest buffalo resting places. *African journal of ecology* 47(1): 121 – 124.

MINAPA, (2007). General Management Plan (2007-2010), 24 - 27pp.

Mlengeya, T.D., Mtambo, M.M.A, Msolla, P.M., Batamuzi, E.K., Fyumagwa, R. Lyaruu, V., Mofulu, F., Semuguruka, W.D., Maselle, R.M., Kilewo, M.K., Moronda, M., Silayo, R.S., Minga, U.M. and Kambarage, D.M. (2002). A severe infectious otitis in Giraffes in Mikumi National Park and Selous Game Reserve, Tanzania: A report of the problem and its management challenges, In: *TAWIRI Proceedings of the 3rd annual scientific Conference*; December 3-5, 2002 Arusha, Tanzania 283 - 290pp.

Mlengeya T. and Lyaruu, V. (2004). Experiences with and the challenges of Wildlife Health Management in the National Parks of Tanzania. [http://www.wcs-ahead.org/book/chapter07] site visited 20/6/2007.

Mlengeya, T., Batamuzi, E. and Mutayoba, B. (2006) Report on the Sitatunga and Bushbuck healthy study in Rubondo Island National, Northernwestern Tanzania. 1-22 pp.

Mlengeya, T., Batamuzi, E. and Mutayoba, B. (2008). A final report on the Sitatunga and Bushbuck healthy study in Rubondo Island National , Northernwestern Tanzania. 1- 50pp.

Moder, H.J. (1994). Animal habitat isolated by road and agriculture field. *Biological conservation* 29: 27 – 28.

Mohanty, S., Singhal, R., Sood, S., Dhawan, B., Kapil, A. and Das, B. K. (2007). Citobacter infection in a tertiary care hospital in Northern India, *Journal of infection*, 54(1): 58-64.

Morner, T., Obendorf, D.L., Artois, M. and Woodford, M.H. (2002). Surveillance and monitoring of wildlife diseases. [http://www.sgm.ac.uk/pubs/micro_today/pdf/110302] site visited 24/6/2007.

Mtambo, M., Sebatwale, J.B., Kambarage, D.M., Muhairwa, A.P., Maeda, G.E., Kusiluka, L.J. and Kazwala, R.R. (1997). Prevalence of Cryptosporidia spp. Oocyst in cattle and wildlife in Morogoro region, Tanzania. *Journal of Preventive Veterinary Medicine* 31(3-4): 185 – 190.

Mtambo, M.A. (2000). Occurrence of Cryptosporidium and Cyclospora species oocyst in stool specimens of various wildlife species in Tanzania: Impact of human – livestock – wildlife interaction, In: *TAWIRI proceeding of 1st annual scientific conference* (Edited by Kayumbo, H.Y.) December 12-13, 2000, Arusha, Tanzania, 72 - 77pp.

Mutayoba, B.M., (2002). Noninvasive approach to wildlife genetic and endocrine studies: A review to their potential and constraints, In: *TAWIRI proceedings of the third annual scientific conference* (Edited by Semuguruka, W.D. *et al.*), Arusha, December 3-5 2002. 317 - 331pp.

Newmark, W., Boshe, J., Sariko, H. and Makumbule, G. (2008). Effects of a highway on large mammals in Mikumi National Park, Tanzania. *Front Ecology Environmental* 6(6): 321 – 328.

Olwoch, M., Reyers, B. and Jaarsveld, A.S. (2008). Host-parasite distribution patterns under simulated climate: implications for tick-borne diseases. International Journal of Climatology 23(4): 207 – 230.

Plowman, B. (2004). March 2004 Raccoon Roadkill Survey, *Journal of Wildlife Management and Research note* 868: 312 – 317.

Popov, V., (2009). The Small Mammals (Insectivora, Lagomorpha, Rodentia) of Bulgaria. [http://www.worldwildlife.org/bsp/publications/europe/bulgaria/bulgaria15.html Summary] site visited on 23/4/2009.

Robert, S., Kaliński, A., Wawrzyniak, J., Bańbura, M., Skwarska, B., Zieliński, P. and Bańbura, J. (2006). Effects of experimental reduction in nest micro-parasite and macro-parasite loads on nestling hemoglobin level in blue tits *Parus caeruleus*. *Acta Oecologica* 30(2): 223 – 227.

Rodgers, A., Melamari, L. and Nelson, F. (2003). Wildlife Conservation in Northern Tanzanian Rangelands. In: *The Symposium of Conservation in Crisis: Experiences and Prospects for Saving Africa's Natural Resources*; held on December 10-12, 2003 at Mweka College of African Wildlife Management, Moshi, Tanzania. 23 - 50pp.

Roedembeck, I. and Voser, P. (2008). Effects of roads on spatial distribution, abundance and mortality of brown hare (*Lepus europaeus*) in Switzerland, European. *Journal of Wildlife Research* 54(37): 425 – 437.

Roskaft, E., Stokke, S., Mwakalebe, G., Lyamuya, R. and Melealle, W. (2002) Diversityof Ungulates and Primates in Serengeti National Park in Space and Time. In: *Tanzania Wildlife Research Institute, Proceedings of the third Annual Scientific Conference* (Edited by Semuguruka, W.D. *et al.*), December 3 – 5, 2002, Arusha, Tanzania. 66 – 75pp.

Row, J., Blouin-Demers, G. and Weatherhead, P.J. (2007). Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). *Journal of Biological Conservation* 137(1): 117 – 124.

Ryan, K. J. and Ray, C. G. (2004). *Sherris Medical Microbiology* (4th edition). McGraw Hill. Uk. 54-60pp.

Samwel, W.M., Pybus, J.M. and Kocan, A.A. (2001). *Parasitic disease of wild animals*, 2nd Edition, Manson Publishing Ltd, London. 10 – 305pp.

Sanz, L., Serrano, M. and Puig J. (2001) Integration of freeways: How does it affect roadkill? In: _*Proceedings of the international conference on environment and transportation*, USA 522 – 528pp.

SAS (2000). *Statistical Analysis System. SAS/STAT User's guide. 5th Edition.* Statistical analysis Institute. Inc. Nc. USA.1028pp.

Seiller, A., Helldin, J. and Seiller, C. (2004). Road mortality in Swedish mammals: results of a drivers' questionnaire. *Wildlife Biology* 10: 225 – 233.

Schlacher, T., Thompson, L. and Price, S. (2007). Vehicles versus conservation of invertebrates on sandy beaches: mortalities inflicted by off-road vehicles on ghost crabs. *Journal of Marine ecology* 28(3): 354 – 367.

Shen, I., Li, W., Qian, T. and ZhiYong, L. (2009). *Investigation on Hymenolepis nana infection in wild small mammals in Xiaguan,* Dali, Yunnan. Xinjiang Institute for Endemic Disease Control and Research press. Yunnan, China. 35 – 40pp.

Slater, F.M. (2002). An assessment of wildlife road casualties: the potential discrepancy between numbers counted and numbers killed. *Web Ecology* 3: 33 – 42.

Smith, H. and Stimson, W. (1998). *Parasitology.* Cambridge University Press., UK.188pp.

Smith, K.F., Whitehouse, A.K. and Pedersen A.B. (2009). The role of infectious diseases in biological conservation. *Animal conservation* 12(1): 1 – 12.

Soulsby, E.J.L. (1983). *Helminthes, Arthropods and Protozoa of Domesticated Animals, 6th Edition.* William Clowes and Sons Limited, London 359 – 750pp.

Stokke, S. and Toit, J. (2008). Sex and size related differences in the dry season feeding patterns of elephants in Chobe National Park, Botswana. *Ecography* 23(1): 70 – 80.

Tanzania Development partners group (DPG), (2009). The wildlife sector. [www.tzdac.or.tz] site visted on 5/5/2009.

Taranjit, K., (2008). Human virus in Chimpanzee. [http//medicineworld.org] site visited on 22/5/2008.

Taylor, B.D. and Goldingay, L.R. (2004). Wildlife roadkills on three major roads in north-eastern New South Wales. *Journal of Wildlife research* 31: 83 – 91.

Trocme, M. (2006). Habitat Fragmentation due to Linear Transportation Infrastructure: An overview of mitigation measures in Switzerland, In: *Swiss Transport Research Conference* March 15 – 17, 2006, Switzerland. 67-75pp.

Waits, L.P. and Paetkau, D. (2005). Noninvasive sampling for wildlife biologists: A review of application and recommendation for accurate data collection. *Journal of Wildlife management* 69(4): 1419 – 1433.

Wassenaar, T. (2009) Enterobacteria species. [www.bacteriamuseum.org] site visited on 26/5/2009.

Wasser, S.K., Shedlock, A.M., Comstock, K., Ostrander, E. A., Mutayoba, B. and Stephens, M. (2004). Assigning African elephant DNA to geographic region of origin: applications to the ivory trade. *Proceedings of the National Academy of Sciences* 101: 14847 – 14852.

Wasser, S.K. and Hunt, K.E. (2005). Noninvasive measures of reproductive function and disturbance in the barred owl, great horned owl, and northern spotted owl. *Annals of the New York Academy of Sciences* 1046: 109 – 137.

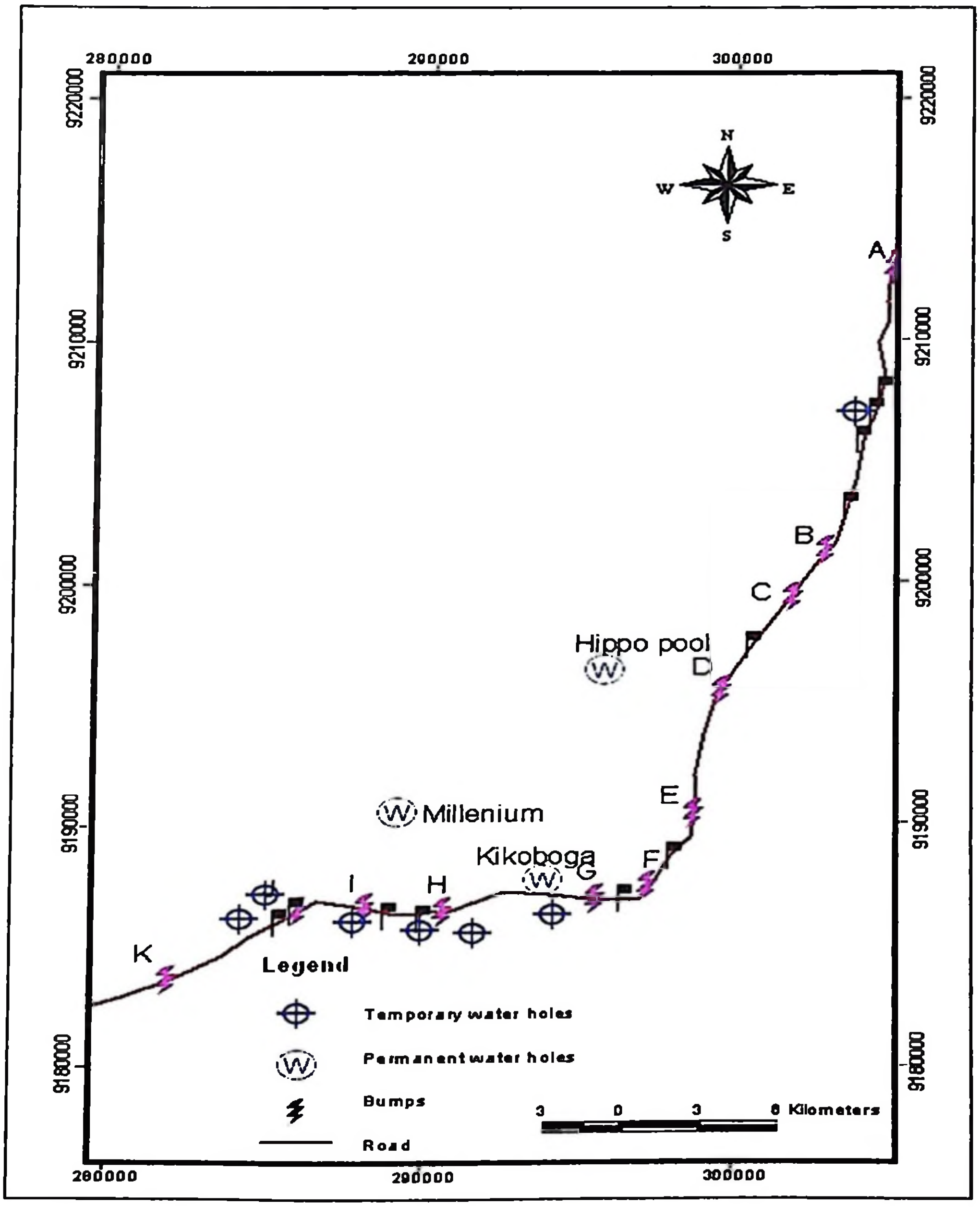
Wallgren, M., Skarpe, C., Bergström, R., Danell, K., Granlund, L. and Bergström, A. (2007). Mammal community structure in relation to disturbance and resource gradients in southern Africa. *African journal of ecology* 47(1): 136 – 145.

Wen liang, Y. and Yin ching, C. (2008). Microbiology and pathogenesis of Klebsiella pneumoniae infection in [http://www.uptodate.com/patients/content/topic.do?topicKey=~B3PEE80AwDqfw5B] site visited on 7/8/2008.

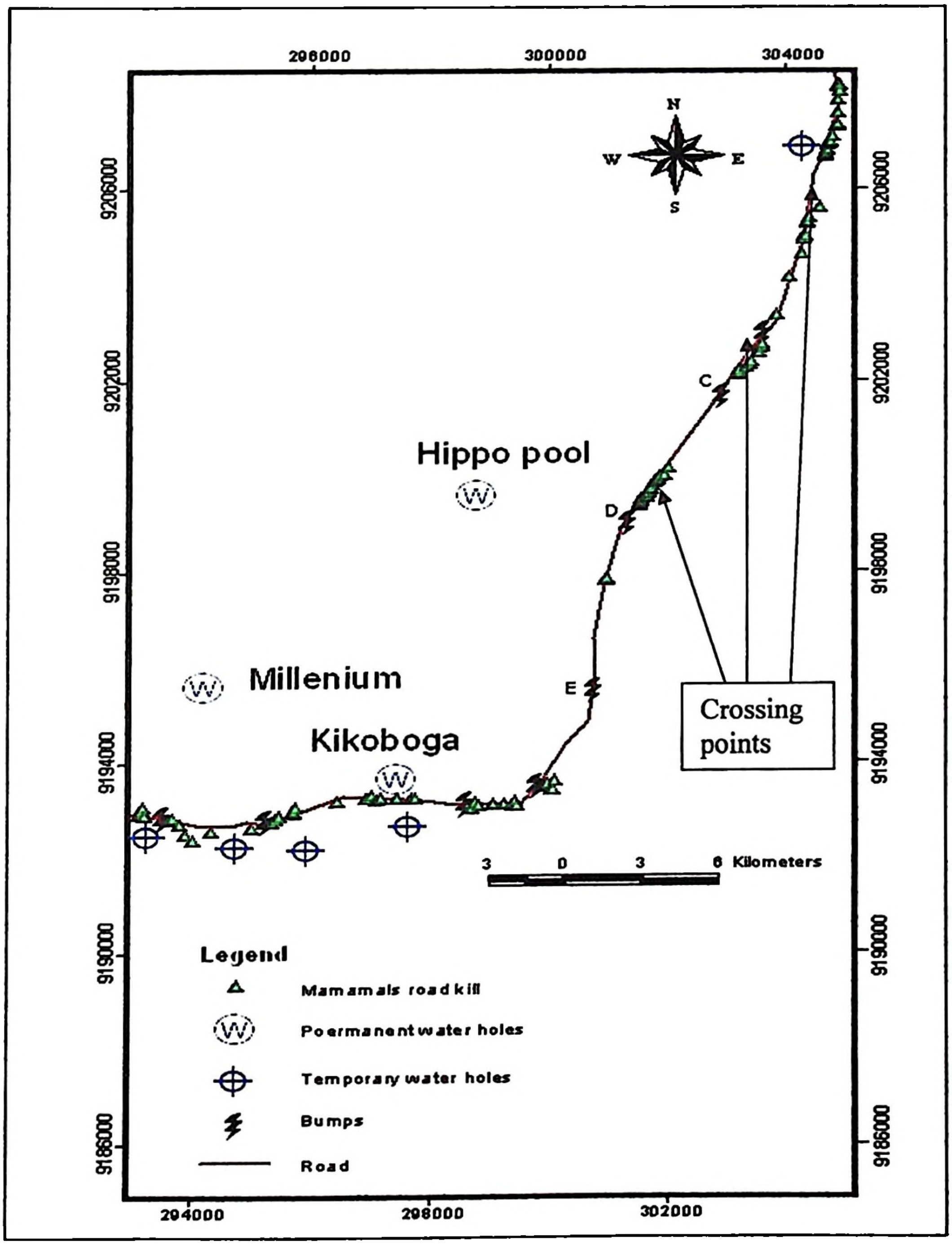
Wilkie, D., Shaw, E., Rotberg, F., Morelli, G. and Auzel, P. (2008). Roads, Development, and Conservation in the Congo Basin. *Conservation Biology* 14(6): 1614 – 1622.

APPENDICES

Appendix 1: GIS Map showing the road sections, watering points and possible crossing sections



Appendix 2: GIS Map showing mammal roadkills, road sections and watering points



Appendix 3: Statistical data analysis for primary data (Total)

Data for Mammals, Reptiles and Birds killed (Sept. 2007 – April 2008)

	Sept.	Oct.	Nov.	Dec.	Jan	Feb	Mar.	April	
Mammal	9	6	14	25	39	20	29	3	145
Reptile	2	2	13	24	37	27	2	0	107
Bird	2	2	9	22	15	3	6	3	62
Total	**13**	**10**	**36**	**71**	**91**	**50**	**37**	**6**	**314**

ANOVA analysis for Mammals, Reptiles and Birds died 2007/8

Anova:

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	8	145	18.125	154.4107
Row 2	8	107	13.375	203.4107
Row 3	8	62	7.75	53.07143

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	431.5833	2	215.7917	4.193522	**0.037404**	3.738892
Columns	2155.833	7	307.9762	5.984962	0.00226	2.764199
Error	720.4167	14	51.45833			
Total	**3307.833**	**23**				

Correlation Analysis for Mammals, Reptiles and Birds died 2007/8

	Mammals	Reptiles	Birds
Mammals	1 (p=0.001)		
Reptiles	0.736452 (p=0.02)	1 (p=0.001)	
Birds	0.647413 (p=0.046)	0.648629 (p=0.046)	1(p=0.001)

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Killings

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	8

Numbers with the same letter are not significantly different.

Duncan Grouping	Mean	N	Specie
A	145.000	1	Mammals
B	107.000	1	Reptile
C	62.000	1	Birds

Statistical data analysis for primary data (mammals)

<u>Mammals</u>

The SAS System

The GLM Procedure

Class Levels Values

Specie 20 Africanc Baboon Buffalo Bunded Bushbaby Bushback Elephant Genet Giraffe Hare
Hyena Impala Jackal Leopard Lion Porcupn Ratel Reed Rodent Warthog

Number of observations 40
The SAS System 10:28 Monday, March 18, 2002 22

The GLM Procedure

Dependent Variable: Killings

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3133.100000	164.900000	65.96	<.0001
Error	20	50.000000	2.500000		
Corrected Total	39	3183.100000			

R-Square	Coeff Var	Root MSE	Killings Mean
0.984292	21.51209	1.581139	7.350000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Specie	19	3133.100000	164.900000	65.96	<.0001

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Killings

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 20
Error Mean Square 2.5

Number with the same letter are not significantly different.

Duncan Grouping		Mean	N	Specie
	A	28.000	2	Impala
	A	26.000	2	Rodent
	A	25.000	2	Hare
	B	17.000	2	Africanc
	C	12.000	2	Buffalo
	D	6.000	2	Genet
E	D	4.000	2	Jackal
E	D	4.000	2	Giraffe
E	D	4.000	2	Hyena
E	D	3.000	2	Baboon
E	D	3.000	2	Warthog
E		2.000	2	Leopard
E		2.000	2	Bushbaby
E		2.000	2	Elephant
E		2.000	2	Ratel
E		2.000	2	Lion
E		1.000	2	Bushback
E		1.000	2	Reed
E		1.000	2	Bunded

Statistical data analysis for primary data (Reptiles)

Reptiles

The SAS System

The GLM Procedure

Class Level Information

Specie	7	B/mamba Cobra G/mamba L/Tortoi M/Lizard Puffadde Python

Number of observations 7

The SAS System

The GLM Procedure

Dependent Variable: Killiongs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1550.857143	258.476190	113.08	<.0001
Error	7	16.000000	2.285714		
Corrected Total	13	1566.857143			

Duncan's Multiple Range Test for Killiongs

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	2.285714

Number of Means	2	3	4	5	6	7
Critical Range	3.575	3.717	3.793	3.836	3.860	3.872

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Specie
A	32.000	2	B/mamba
B	17.000	2	Puffadde
C	6.000	2	L/Tortoi

	C			
D	C	4.000	2	Python
D	C			
D	C	3.000	2	G/mamba
D				
D		2.000	2	Cobra
D				
D		1.000	2	M/Lizard

Statistical data analysis for primary data (Birds)

Birds

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
Specie	15	A/starli C/plove Coucal Eagle Franklin G/Fowl L/bird L/dove Owl R/swallo S/swallo Swallow Turaco W/swallo Y/claw

Number of observations 15

The SAS System

The GLM Procedure

Dependent Variable: Killings

Source	DF	Sum Squares	Mean Square	F Value	Pr > F
Model	14	239.2000000	17.0857143	32.04	<.0001
Error	15	8.0000000	0.5333333		
Corrected Total	29	247.2000000			

R-Square	Coeff Var	Root MSE	Killings Mean
0.967638	30.42903	0.730297	2.400000

The GLM Procedure

Duncan's Multiple Range Test for Killings

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.533333

Number with the same letter are not significantly different.

Duncan Grouping	Mean	N	Specie
A	11.0000	2	G/Fowl
B	7.0000	2	Owl
C	4.0000	2	Coucal
C			
C	3.0000	2	Swallow
D	1.0000	2	Franklin
D			
D	1.0000	2	C/plove
D			
D	1.0000	2	L/bird
D			
D	1.0000	2	L/dove
D			
D	1.0000	2	A/starli
D			
D	1.0000	2	R/swallo
D			
D	1.0000	2	S/swallo
D			
D	1.0000	2	Eagle
D			
D	1.0000	2	Turaco
D			
D	1.0000	2	W/swallo
D			
D	1.0000	2	Y/claw

Appendix 4: Statistical analysis for the roadkill secondary data

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
2003/4	3	180	60	2077
2004/5	3	144	48	997
2005/6	3	163	54.33333	1260.333
2006/7	3	192	64	2263
Mammals	4	365	91.25	562.9167
Reptiles	4	61	15.25	6.25
Birds	4	253	63.25	34.91667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between years	436.25	3	145.4167	0.634084	**0.619712**	4.757063
Between groups	11818.67	2	5909.333	25.76744	**0.001134**	5.143253
Error	1376	6	229.3333			
Total	**13630.92**	**11**				

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
2003/4	3	181	60.33333	2074.333
2004/5	3	144	48	997
2005/6	3	163	54.33333	1260.333
2006/7	3	192	64	2263
2007/8	3	314	104.6667	1726.333
Mammals	5	510	102	1000
Reptiles	5	168	33.6	1688.3
Birds	5	316	63.2	23.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between years	5972.933	4	1493.233	2.450401	**0.130617**	3.837853
Between groups	11766.93	2	5883.467	9.654788	**0.007364**	4.45897
Error	4875.067	8	609.3833			
Total	**22614.93**	**14**				

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
2003/4	2	123	61.5	4140.5
2004/5	2	73	36.5	1200.5
2005/6	2	99	49.5	2380.5
2006/7	2	131	65.5	4512.5
2007/8	2	252	126	722
Mammals	5	510	102	1000
Reptiles	5	168	33.6	1688.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Years	9493.6	4	2373.4	7.536996	**0.037949**	6.388233
Mammals and Reptiles	11696.4	1	11696.4	37.14322	**0.003666**	7.708647
Error	1259.6	4	314.9			
Total	**22449.6**	**9**				

Appendix 5: Statistical analysis for the roadkill combined primary and secondary data

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
2003/4	2	74	37	882
2004/5	2	83	41.5	1740.5
2005/6	2	79	39.5	1200.5
2006/7	2	79	39.5	924.5
2007/8	2	169	84.5	1012.5
Reptiles	5	168	33.6	1688.3
Birds	5	316	63.2	23.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Years	3278.4	4	819.6	0.918422	**0.531873**	6.388233
Reptile and Birds	2190.4	1	2190.4	2.454505	**0.192252**	7.708647
Error	3569.6	4	892.4			
Total	**9038.4**	9				

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
2003/4	2	165	82.5	1200.5
2004/5	2	132	66	50
2005/6	2	148	74	200
2006/7	2	174	87	1352
2007/8	2	207	103.5	3444.5
Mammals	5	510	102	1000
Birds	5	316	63.2	23.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Years	1611.4	4	402.85	0.648868	0.6573	6.388233
Mammals and Birds	3763.6	1	3763.6	6.062012	0.069533	7.708647
Error	2483.4	4	620.85			
Total	**7858.4**	**9**				

Correlation Analysis for Birds, Reptiles and Birds for combined primary and secondary data

	Mammal	Reptile	Birds
Mammals	1 (p=0.001)		
Reptiles	0.792129 (p=0.02)	1 (p=0.001)	
Birds	-0.691(p=0.031	-0.112 (p=0.245)	1 (p=0.001)

Appendix 6: Table showing monthly rainfall records in MINAPA (April 2007 – Sept 2008)

	Sept.07	Oct.07	Nov.07	Dec.07	Jan.08	Feb.08	Mar.08	Apr.08
Rainfall (mm)	7.3	3.6	11.5	66.5	70.3	86.5	95.4	101

Table showing Temperature records in MINAPA (April 2007 – Sept 2008)

	Spt. 07	Sept. 07	Sept. 07	Oct. 07	Oct. 07	Oct. 07	Nov. 07	Nov. 07	Nov. 07
Dates	Min Tem	Max Temp	Av Temp	Min Tem	Max Temp	Av Temp	Min Tem	Max Temp	Av Temp
1st	17	30.6	23.8				19.2	34.4	26.8
2nd	18.1	31.3	24.7				18.1	35	26.55
3rd	17.5	29.9	23.7				19.4	34.8	27.1
4th	16.9	30.7	23.8				17.3	36.3	26.8
5th	17.2	30.6	23.9				18.9	37.2	28.05
6th	18.1	30.7	24.4						
7th	18.4	31.8	25.1	18.6	32.4	25.5			
8th	20.4	31.7	26.05	17.3	33.6	25.45	19	36.9	27.95
9th	20.9	30.9	25.9	19.8	33.9	26.85	18.5	37	27.75
10th	18.4	30.2	24.3	20.3	31.8	26.05			
11th	16.3	30.7	23.5	17.7	33	25.35			
12th				19.4	33.1	26.25			
13th				17.6	33	25.3			
14th				20.7	32.6	26.65			
15th				18.2	34.6	26.4			
16th				19.7	34	26.85			
17th	18	31.2	24.6	20.3	32.5	26.4			
18th	14.9	29.8	22.35	19	33.9	26.45			
19th	15.9	30.6	23.25	18.9	33.7	26.3			
20th	15.6	31.4	23.5						
21st	17.5	30	23.75						
22nd	18.9	33.4	26.15						
23th	19.1	32.7	25.9						
24th	17	29.9	23.45	18.5	35.2	26.85	16.1	36.2	26.15
25th	18.3	31.3	24.8	19.1	34	26.55	21	36.6	28.8
26th	16.1	28.4	22.25	20.9	35.1	28	22.6	36.2	29.4
27th	14.3	30.1	22.2	18.7	33.8	26.25	22.6	36.4	29.5
28th	18	31.1	24.55	20	33.8	26.9			
29th	18.1	30.6	24.35	19.1	34.3	26.7			
30th	17.4	30.4	23.9	20.6	34.3	27.45			
31th	17.532	30.8	24.166	21.8	34.8	28.3			

	Dec. 07	Dec. 07	Dec. 07	Jan. 08	Jan. 08	Jan. 08	Feb. 08	Feb. 08	Feb. 08
Dates	Min Tem	Max Temp	Av Temp	Min Tem	Max Temp	Av Temp	Min Tem	Max Temp	Av Temp
1st									
2nd									
3rd							20.7	36.4	28.55
4th				23.6	38.9	31.25			
5th									
6th				22.1	39	30.55	19.9	28.8	24.35
7th				19.7	39.1	29.4	19.8	34.2	27
8th				22	37	29.5	20.7	36.8	28.75
9th	20.7	36.2	28.45	19.8	32.8	26.3	19.6	36.1	27.85
10th	19.3	35.4	27.35						
11th	21.9	35.8	28.85	20.9	34.6	27.75	18.7	32.7	25.7
12th				19.7	34.4	27.05	19.6	34.1	26.85
13th				19.7	36.9	28.3	19.2	31.3	25.25
14th	22.4	38.7	30.55						
15th	20.6	37.2	28.9						
16th									
17th	22.1	36.9	29.5	22.2	33.4	27.8	17.8	35.7	26.75
18th	19.6	37.1	28.35	23.4	37	30.2	21	36.9	28.95
19th				15.6	34.3	24.95	21.2	37	29.1
20th				20.3	35.1	27.7	22.4	37.2	29.8
21st				20	36.1	28.05	22.6	37.5	30.05
22nd	21.1	35.4	28.25	20.2	35.8	28	21.7	36.8	29.25
23th	22.5	36.9	29.7	20.2	33.2	26.7	21.1	33.9	27.5
24th				20.1	35.6	27.85	21.8	37.6	29.7
25th				20.5	35.8	28.15	20.1	37.2	28.65
26th				20.3	34.4	27.35	18.9	36.3	27.6
27th				20.1	29.6	24.85	18.3	34.8	26.55
28th	22.9	38.2	30.55	21.2	36.7	28.95			
29th	21.2	38.7	29.95	21.2	37.5	29.35			
30th									
31th									

	Mar. 08	Mar. 08	Mar. 08
Dates	Min Tem	Max Temp	Av Temp
2nd			1st
3rd	18.7	37.8	28.25
4th	20.1	36.4	28.25
5th	22.1	37.4	29.75
6th	20.4	36.5	28.45
7th	21.2	37.9	29.55
8th	22.6	38.3	30.45
9th	21.3	35.9	28.6
10th	21.6	34.7	28.15
11th			
12th	20.2	37.4	28.8
13th	21.9	36.3	29.1
14th			
15th			
16th			
17th	21.3	39.3	30.3
18th	22.3	37.4	29.85
19th	21.4	38.1	29.75
20th	21.9	36.3	29.1
21st	22.5	35	28.75
22nd	23	37.2	30.1
23th	23.3	38.7	31
24th	22.9	34.8	28.85
25th	20.8	37	28.9
26th	20.2	35	27.6
27th			
28th			
29th			
30th	21.3	30.9	26.1
31th	20.7	31.2	25.95

Appendix 7: Procedure used for isolation and identification of bacteria

Identification of *Bacillus subtilis*
The organism grew aerobically on Blood agar after 24 hours of incubation at 37°C. The colonies were flat, gray non haemolytic and sticky. Also it is large with roughened edge or crenate margins and irregular. Morphologically the organism is Gram positive, cylindrical rods, straight or slightly curved, singly or in chains. They are actively motile.

Identification of *Staphylococcus epidermidis*
The organisms grow aerobically on Blood agar after 24 hours of incubation at 37°C. The colonies are round, smooth, opaque, low convex, edge entire, and whitish in colour. Also they are non haemolytic. No growths on McConkey agar morphologically the organism are Gram positive, spherical or ovoid. Cells were arranged in grape like clusters. They are non motile.

Identification of *Pasteurella multocida*
The organism grows aerobically on Blood agar after 24 hours of incubation at 37°C. The colonies are round, smooth, mucoid, and whitish in colour. Also they are non haemolytic. No growth on McConkey agar. Morphologically the organisms are Gram negative, coccoid and non motile.

Identification of *Escherichia coli*
The organisms grow aerobically on Blood agar after 24 hours of incubation at 37°C. On Blood agar the colonies are moist, glistening, opaque and circular with an entire edge. Also they are non haemolytic and whitish in colour. On McConkey agar they are lactose fermenting colonies. Morphologically the organism are Gram negative, coccoid. They are motile.

Identification of *Proteus*
The organisms grow aerobically on Blood agar after 24 hours of incubation at 37°C. On Blood agar the organism grow with swarming feature, whereby the organism spread rapidly as a film over the surface of media. The swarming phenomenon occurred in waves so that after inoculation of the culture onto an agar plate, the resultant growth develop as series of rings. Also they are non haemolytic and whitish in colour. On McConkey agar they are non lactose fermenting colonies. Morphologically the organisms are Gram negative, coccoid.

Identification of *Klebsiella*
The organisms grow aerobically on Blood agar after 24 hours of incubation at 37°C. On Blood agar the colonies are large, raised and round with an entire edge. Also they are non haemolytic and whitish in colour. On McConkey agar they are lactose fermenting colonies. Morphologically the organism are Gram negative, coccoid. They are non motile.

Identification of *Citrobacter*
The organisms grow aerobically on Blood agar after 24 hours of incubation at 37°C. On Blood agar the colonies are moist, opaque and circular with an entire edge. Also they are non haemolytic and whitish in colour. On McConkey agar they are late lactose fermenting colonies. Morphologically the organisms are Gram negative, coccoid.

Results of biochemical tests of different bacterial isolates

	B. subtilis	S. epidermidis	P.multocida	E.coli	Proteus	Klebsiella	Citrobacter
Indole	–	–	+	+	+	–	–
MR	–	+		+	+	–	+
VP	+	+		–	–	+	–
Citrate				+	+	+	+
Nitrate red.	+	+		+	+	+	+
H_2S	+/-	–	–	–	+	–	+
Urea			–	–	+	+	–
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	+	+/-	+/-	+	+/-
Maltose	+	+	–	+			
Mannitol		–	+	+			
Lactose			–	+	–	+	+
Salicin				–			
Raffinose			+	–			
Inulin			–				
Galactose			+	+			
Sorbitol			+				
Fructose			+	+			
Xylose			+	+			
Arabinose			+	+			