CHARACTERIZATION OF GIRAFFE EAR DISEASE IN MIKUMI - SELOUS ECOSYSTEM

BY

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ABSTRACT

A study on GED was conducted in the Mikumi - Selous ecosystem with an overall objective of finding the cause and predisposing factors. Giraffes were examined for change in conformation, discharges, and lesions on the pinna. Samples were collected from immobilized giraffes. Water and browsing materials in giraffe habitat were collected for micro-organisms isolation. Tick and oxpecker surveys were conducted. GED overall magnitude was 11.7% (dry) and 11.1% (wet) seasons (1999 to 2006). 3.1% (dry) and 8.1% (wet) seasons (2007 to March 2010). Matambwe in Selous Game Reserve 1.2% (dry) 2.4% (wet), MINAPA 0.8% (dry) and 1.2% (wet) seasons (2007 to March 2010). The difference mean sick giraffe examined in each ecozone seasonally was not statistically significant ($P > 0.05$). Immobilized giraffes revealed thickening of the ridges of antihelix, and distal part of the scapha, superficial erosion, pus, and foul smelling of the pinna concave surface. GED harboured environmental bacteria *Pseudomonas auregenosa* and *Bacillus firmus*. *Rhipicephalus appendiculatus* and *Amblyoma variegutum* were common ticks associated with giraffes. Ticks and oxpeckers played no role on GED occurrence. Histologically GED biopsies had epidermal nematode larvae and interepidermal necrosis. Molecular investigation concluded the nematode was a *Spirurid*. GED is dermatitis caused by *Spirurid* nematode. The DNA sequence lies between *Onchocerca* and *Dirofilaria species* which are parasitic to human and animals. It is therefore a species that is new and specific to this ecosystem. Furthermore, possibly it is a species that have evolved from older, non pathogenic and common environmental agent. It is concluded that for the first time this work has been able to

demonstrate and isolate a *Spirurid* nematode which is the primary cause for GED with bacteria and fungi working together. The mechanism by which the nematodes and bacteria cause the damage to the pinna is of interest. Also the trend of the disease magnitude was downwards. It is recommended GED be known as Giraffe Pinna Dermatitis. Mechanisms by which the nematodes cause lesions and role of vectors need further study. Establishment of national nematode bank, reference archive with identification keys, micro weather stations, electronic geomaps, and monitoring programme.

DECLARATION

I, Vitalis Hippolyte Lyaruu, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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DEDICATION

This work is dedicated to my late parents who laid the foundation of my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CPE	Cytopathic effect
DNA	Deoxyribo nucleic acid
FCS	Fetal Calf Serum
GED	Giraffe Ear Disease
IUCN	International Union for Conservation of Nature
MINAPA	Mikumi National Park
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffered Saline
rDNA	Ribosomal Deoxy ribonucleic acid
Rpm	Revolutions per minute
SGR	Selous Game Reserve
TANAPA	Tanzania National Parks

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

In early 1999 two adult giraffes were sited near the Mikumi National Park (MINAPA) headquarters being seriously sick with an unknown disease that mainly affected their ears (Mlengeya *et al.,* 2002). Later the disease spread to affect about 90% of the giraffe population in the entire park (Mlengeya *et al.,* 2002). Affected animals had swollen ears that were hanging downwards and shaking their heads from time to time. With time the ears sloughed off leaving raw wounds that attracted swarms of flies.

The *El Nino* rains of 1997/1998 period caused many environmental and physical changes in different parts of the country including the Mikumi-Selous ecosystem. One of these changes reported is high tick and fly density (Mlengeya *et al.,* 2002). The tick and fly dynamics were considered as a risk factor in the causation of the emerging disease that affected giraffes (Kagaruki *et al.,* 2005). These were thought to cause damages to the ear that paved way to invasion by the actual causative agent(s) considered to be parasites, bacteria or viruses. In fact, scientists who were following up the disease in Mikumi-Selous ecosystem were also suspecting that feeding behavior of giraffes had changed from predominantly browsing to a combination of browsing and grazing (Kagaruki *et al.,* 2005). This change was thought to facilitate the rate of ticks attack to the head region and thence the ears.

Apart from the suspicion related to *El Nino*, alluded to earlier, the disease was thought to have originated from cattle in the neighboring Mkata Ranch and surrounding villages with high cattle population but also affected by Bovine parasitic otitis (Msolla *et al.,* 1993). This contention was based on the fact that giraffes, like cattle are ruminants and that there is a clear wildlife-livestock interphase that allows for contact between wild animals and domestic animals Wambwa (2002); MINAPA annual protection report (2009). The disease in cattle had been earlier proven to be caused by *Rhabditis bovis* (Msolla *et al.,* 1993).

There was paucity of information as regards to infectious ear disease in wildlife. It was also the first report of ear disease thought to be infectious otitis in free ranging wildlife species in Tanzania. The disease was confined to giraffes. Also there was lack of information on the cause, pathogenesis, epidemiology, and features of ear disease in giraffes. The disease process was thought to involve bacteria, ticks, insect vectors, parasitic nematodes and environmental factors such as weather changes (Mlengeya *et al.,* 2002).

Preliminary investigation indicated that, the disease could be associated with ticks, biting flies and bacterial infection. Environmental factors such as high giraffe population, decrease in browsing material, animal stress, decreased predator population and high tick challenge were considered to be contributing to the spread of the disease (Kagaruki *et al.,* 2003). However no concrete studies had been carried out to clearly identify the causative agents and link them to the problem. There were

no records of major wildlife disease problems in Tanzania and the wildlife industry was faced up with a unique problem seen for the first time.

Preliminary treatment trials also indicated that the disease responded to combination of antibiotics and antiparasitic drugs. However due to technical problems treatment of all affected giraffes in Mikumi-.Selous ecosystem was expensive and unfeasible. However, it was necessary to establish the exact causative agents and environmental factors perpetuating the disease to be able to attempt disease management in a more holistic and sustainable way. One such attempt might be manipulation of the environment and try to minimize the predisposing factors (Kagaruki *et al.,* 2005). This study was therefore planned to fill the knowledge gap regarding the giraffe ear disease (GED).

1.2 Problem statement and Justification

The giraffe is a National symbol and of Mikumi – Selous ecosystem that attracts tourists. Sick giraffes were an eye sore to tourists and other communities within the ecosystem. The disease was viewed as threatening to reduce the attractive giraffe to an endangered animal species status. This might have resulted in serious conservation and economical implications such as drop of wildlife populations, and fall of number of tourists who visit our National parks. This would have affected the National economy. There was also a fear that this unknown disease could spread to other wild animal species and that it might be zoonotic thus posing danger to man and non-human primates. In view of the potential threats, giraffe might be viewed as an indicator species in conservation of this ecosystem. Moreover West African

giraffe (Nigeria giraffe – *G. c. peralta*) have been reported by Fennessy and Brown (2008) to have been listed by IUCN as an endangered species. Based on this development Tanzania cannot allow her giraffe to assume endangered species status let alone face the prospect of extinction. It is therefore imperative to conserve the giraffe gene pool. The present study therefore aimed at shedding light on the causes, features and impact of the disease as contribution to the conservation efforts.

1.3 Objectives

1.3.1 Overall Objective

To investigate the cause, associated factors and characterize GED in Mikumi–Selous Ecosystem

1.3.2 Specific objectives

(i) To fully characterize GED as a means towards facilitating its recognition

(ii) To investigate factors that predisposes giraffes in the Mikumi – Selous Ecosystem to GED.

(iii) To investigate the etiology of GED.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Giraffe characteristics and its habitat

The giraffe (*Giraffa camelopardalis)* is an even-toed ungulate, the tallest of all land-living animal species and the largest ruminant. It is related to deer and cattle, but is placed in a separate family, the *Giraffidae,* consisting of only the giraffe and its closest relative, the okapi (Wilson and Reeder, 2005). Its range extends from Chad in Central Africa to South Africa (Estes, 1991).

All extant giraffes (*Giraffa camelopardalis*) are currently considered to represent a single species classified into multiple subspecies of *Giraffa camelopardalis peralta, G. c. rothschildi, G. c. reticulata, G. c. tippelskirchi, G. c. giraffa* and *G. c. angolensis* (Figure 1). However, geographic variation in traits such as pelage pattern is clearly evident across the range in sub-Saharan Africa and abrupt transition zones between different pelage types are typically not associated with extrinsic barriers to gene flow, suggesting reproductive isolation (Brown *et al.,* 2007). Further, our results indicate that neighboring subspecies as well as those that are geographically separated are essentially reproductively isolated, suggesting that some might represent distinct species rather than a single polytypic form (Brown *et al.,* 2007).

Giraffes usually inhabit savannas, grasslands, or open woodlands especially where *Acacia* species, *Commiphora* species and *Terminalia* species dominate (Fennessy *et*

al., 2001). Also they are found in semi-arid, open woodlands with scattered trees and bushes. They are not present in deserts, dense forests and mountains (Fennessy *et al.,* 2001). However, when food is scarce they will venture into areas with denser vegetation. They drink large quantities of water when available which enables them to live for extended periods in dry, arid areas (Skinner and Smithers, 1990).

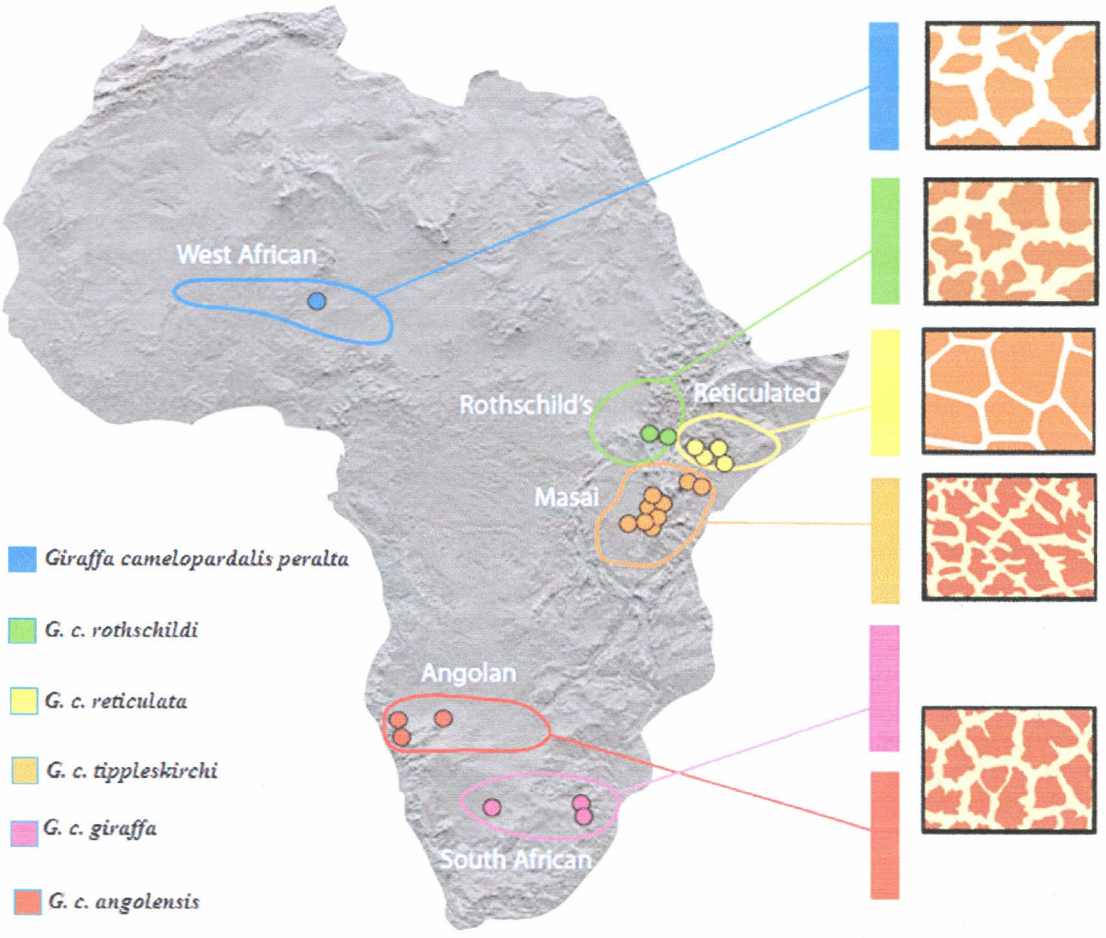


Figure 1: Giraffe distribution and genetic diversity in Africa (Brown *et al.,* 2007)

In the Mikumi-Selous ecosystem, giraffes are among the predominant species of animals which are most sighted on the Mkata flood plain (Mikumi, 2000).

Internationally, giraffe have been categorized under the group called Least Concern (LC) which is an International Union for Conservation of Nature (IUCN) category assigned to extant species or lower taxa which have been evaluated but do not qualify for any other category (Fennessy and Brown, 2008).

The giraffe is a protected species in most of its range. The total African giraffe population has been estimated to range from 110 000 to 150 000. Kenya (45 000), Tanzania (30 000) and Botswana (12 000) have the largest national populations (East, 1998).

2.2 Diseases

Generally giraffe (*Giraffa camelopardalis*) have no unique diseases but are susceptible to most contiguous diseases of domestic ruminant (Raphael *et al.,* 1986). The giraffe is susceptible to infectious and non infectious diseases that are seen in domestic ruminants and common on its relative the okapi (Raphael *et al.,* 1986). In 1975 49 giraffes were slaughtered in Czechoslovakia Socialist Republic due to outbreak of a rare form of SAT 2 strain of Foot and mouth disease virus (Raphael *et al.,* 1986). Morever it is also susceptible to viral diseases among them being rinderpest, malignant catarrhal fever, cutaneous viral papillomas and lumpy skin disease (Jolly, 2003). The Okapi, its closest relative can also be infected by viral diseases such as okapi pox virus and rotavirus (Raphael *et al.,* 1986).

2.2.1 Bacterial and viral diseases

The giraffe is susceptible to different kinds of bacterial and viral diseases. These include Clostridial diseases, Leptospirosis, anthrax, Pasterolosis, Johnes disease and

tuberculosis (Jensen, 1999). Brucellosis has occurred in giraffes in Uganda (Jerry and Innocent, 2003). In Tanzania *Pseudomonas aeruginosa*, *Corynebacterium haemalyticus* and *Staphylococcus aureus* have been isolated from giraffes (Mlengeya *et al.,* 2002).

Fatal meningoencephalitis was diagnosed in reticulated giraffe (*Giraffa camelopardalis reticulata*) and equine Herpes virus -1 (EHV-1) was isolated after death following a history of stumbling, incoordination and abdominal pain (Hoenerhoff *et al.,* 2006). Furthermore, pest virus infection has been reported in giraffe and phylogenetic studies suggested that it was different from that of cattle, sheep and goats. It was from a different taxon (Harasawa *et al.,* 2000).

Malignant catarrhal fever which is caused by two separate Herpes viruses that are identical and are fatal to cattle, deer and bison has been reported in giraffe by Khan (2005). Apart from the Czechoslovakia incident in 1975, sera obtained from giraffe in FMD survey in Tanzania have also tested positive to the virus (Bronsvoort *et al.,* 2008).

Pest viruses especially Bovine Viral Diarrhea virus (BVDV) are wildlife and domestic ruminant pathogens of major economic importance and were discussed by Knipe and Howley (2007). Virus detection, vaccination of pest viruses and its implications in giraffes has been discussed by Ridpath (2003), Schirrmeier *et al.* (2004) and Vilcek .and Nettleton (2006).

2.2.2 Diseases caused by *Mycoplasma*

A case of *Mycoplasma*-associated polyarthritis was diagnosed in a giraffe (*Giraffa camelopardalis*) which was characterized by lameness and temporary response to antimicrobial therapy (Hammond *et al.,* 2003). The disease was also detected by presence of nucleic acid in the synovial fluid by using polymerase chain reaction. This was the first report of Mycoplasma associated polyarthritis in giraffe (Hammond *et al.,* 2003).

2.2.3 Parasitic diseases

The giraffe is susceptible to external and internal parasites similar to other ungulates (Jensen, 1999). The giraffe has a long tail that can switch away flies, can rub against objects, and tolerate oxpeckers which prey on the ticks attached to the animal (Moore *et al.,* 2000). All giraffe species can switch away flies from the hind quarters, but unlike other ungulates, the giraffes cannot scratch their heads with their hind hooves, and do not oral groom but instead use other strategies (Moore *et al.,* 2000). They are susceptible to common intestinal parasites that are seen in domestic hoof stock. Cytauxzoonosis occurred and was diagnosed in a giraffe which died of anemia and haemaglobinuria (McCully *et al.,* 1970). Similarly, Peter *et al.* (1998) demonstrated the presence of hematological parasite *Cowdria ruminantium* in wild animals from Africa including the giraffe. Diagnosis of *Lawsnia intracellularis*, an obligate intracellular bacterium which causes prolifarative intracellular enteropathy was diagnosed in a giraffe Hebst *et al.* (2003).

Helminthes (*Parabronema skrjabin*, *Skrjanema species*, *Hemonchus mitchelli*, and *Echinococcus species*) were isolated from giraffes in Etosha National Park, Namibia (Krecel *et al.,* 1990); (Chowdhury, 2001). *Trichuris species* (whip worm) was reported to have been collected from the caecum of a giraffe (Ryodi, 1955).

2.2.4 Mycoses

Fungi have been isolated from the pinna and aural canal of healthy okapi (*Bovidae*, a relative of giraffe) and their environments. Many fungal pathogens are reported to be found in the okapi ear canal (Matthew *et al.,* 2008). It has been suspected that fungal load and environmental conditions such as air humidity, and temperature may be the predisposing factors that lead to ear infections in this species (Matthew *et al.,* 2008). There were no reports of fungal infections to giraffe; however, it is more probable that they are susceptible as well.

2.2.5 Other ailments

Diet and nutritional problems have been encountered due to giraffe's selective browsing habits (Claus *et al.,* 2001). Hoof problems have been encountered in the wild and that over grown hooves impair movement which lead to complication such as sprained tendons and arthritis (Veasey *et al.,* 1996). Research on giraffe population has shown that inbreeding has had a significant effect on survival of calves. Thus it results to high infant mortality rate and if not managed and avoided within a population; the 30 day and one year infant mortality rates continue to rise (Jolly, 2003).

A giraffe with dystocia underwent a caesarian operation but the survival of the dam was not reported (Wilson and Reeder, 2005). Sick giraffes which defecated frequently, feces had a pulp like consistency, shiny, grey to greenish and gaseous were diagnosed to be suffering from exocrine pancreatic insufficient like syndrome (Roman *et al.,* 1991).

2.2.6 Factors associated with giraffe health

2.2.6.1 Environmental factor on disease occurrence

Poor body condition and changeable weather state has been a contributing factor to the causation of death of giraffes (Jolly, 2003). Giraffes are known to be vulnerable to a drop in temperature as it occurs when there are rains and strong winds. The large body surface makes it difficult for adult giraffe to find shelter from wind. Giraffes are very susceptible to cold and their body temperature is likely to fall when ambient temperatures become low (Claus *et al.,* 1999). Factors such as high giraffe population, decrease in browsing material, animal stress, low predation population and high tick challenge contribute to the causation and spread of disease in giraffes (Kagaruki *et al.,* 2003).

2.2.6.2 The effect of vectors

Presence and increase of tsetse flies in and around conservation areas increase the risks of transmission of trypanosomiasis in wildlife animals and humans (Magai *et al.,* 2002). Two types of trypanosomes: *Trypanosoma rhodensiance* and *Trypanosoma brucei brucei* which cause human trypanosomiasis was also detected in humans and wild animals including the giraffe (Magai *et al.,* 2002). On the other

hand, high tick challenge has been considered to be contributing to the spread of diseases in giraffes and other wild animals (Kagaruki *et al.,* 2005).

Scavengers such as non biting flies (*Musca domestica*, *Musca sorbens*, *Musca domestica vicinia* and *Drosophilia melanogaster*) transmit diseases to humans, wild and domestic animals Greenberg (1971), (Nmorsi *et al.,* 2007). Also they are globally known to be capable of carrying bacterial species such as *Klebsiella*, *Salmonella*, *Escherichia coli*, *Staphylococcus*, *Pseudomonas*, *and Streptococcus* in dry and wet seasons. Viral diseases causing pathogens have also been isolated from these arthropods (Nmorsi *et al.,* 2007).

Face fly (*Musca sorbens*) studied at Kambala; a pastoral village close to Mikumi-Selous ecosystem revealed that the fly was attracted to wounds, sores, and skin lesions (Mbilu *et al.,* 2007). Although not a biting species, the fly transmits diseases to human, domestic and wild animals as they carry pathogens on their feet, faeces and digestive juices they regurgitate (Curtis *et al.,* 2001).

2.2.6.3 Feeding habits

Changes of feeding habits of giraffes in Mikumi- Selous ecosystem were suspected to have contributed to the occurrence of disease in giraffes. It was suspected that giraffes shifted from browsing to grazing as result of low availability of browsing material. Because of grazing the head region was predisposed to ticks and enhanced rate of tick climb and bites to the ears followed by secondary bacterial infection (Kagaruki *et al.,* 2005). Studies have shown that grazing and browsing behavior is

influenced by weather such as ambient temperature, relative humidity and wind direction; animal physiological state as well as day-to-day changes in plant community (Andres *et al.,* 2009).

2.2.6.4 The influence of seasonal changes

The *El Nino* rains of 1997/1998 resulted in severe flooding, infrastructure damage with loss of human, livestock and wild animal lives. The after effects of these heavy rains were later followed by upsurge of vectors that transmit diseases that might have resulted in emerging of new diseases. In addition to the direct loss of life and property there was an increase in vector-borne diseases (Chester, 1999) and (Jonathan *et al.,* 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The present study was conducted in the Mikumi-Selous ecosystem which covered Mikumi National Park (MINAPA) and Matambwe in the North Western part of Selous Game Reserve in Tanzania (Fig. 2 and Fig. 3).

3.1.1 Site description (MINAPA)

3.1.1.1 Location and History

MINAPA is the 4th largest National Park in Tanzania. It covers an area of 3,230 km^2 and is located in Morogoro region (Figure 2). It lies between latitude 7°00' and 7°50' S, 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 (Kagaruki *et al.*, 2003). The Park shares one ecosystem with Selous Game reserve, thus allowing animals such as giraffes, elephants, buffaloes and zebras to move between these two protected areas (Keyyu *et al.,* 2003; Collett *et al.,* 2007).

MINAPA is known for its diverse habitats, fauna and flora. Historically, it was gazetted in 1964 as a National Park with initial area of 1,070 km^2 and named "MIKUMI" after the village just beyond its border on Dar es Salaam – Zambia highway (Anonym, 2000; Mikumi, 2000; MINAPA, 2007). In 1975 MINAPA was extended north by 140 km^2 and south by 2,020 km^2. The extension to the south

covered the area between the Park and Selous Game Reserve. This was done to achieve ecological balance of diverse habitats to satisfy the needs of wide range of species requirements and ensure movement access between the Park, the Selous Game reserve and the adjacent protected areas in the ecosystem (Mikumi, 2000)

Figure 2: Map of Tanzania showing location of Mikumi – Selous ecosystem

3.1.1.2 Climate

MINAPA receive an average amount of annual rainfall of 508 mm 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 month, although the rainfall pattern is unpredictable (Mikumi, 2000).

The rain pattern varies in different zones of the park. The Park headquarters receives average rainfall of 635 mm per-annum but along the hills is as high as 1067 mm per

annum (Mikumi, 2000). Although there is a definite dry period in January and February, the wet months are associated with 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 temperatures range is 25.5° C (Anonym, 2000; MINAPA, 2007).

3.1.1.3 Vegetation and land form

MINAPA lies within a horseshoe of mountains formed by the Uluguru Mountains (east) and forest foothills of Vidunda (southwest), that have different vegetation and land form (Figure 3). These have been described by Kagaruki *et al.* (2005) as follows:

(i) The Mkata river flood plain grass land 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 and Matambwe the Northern part in Selous Game Reserve.

(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. Ikoya area is included.

(v) Acacia-Dalbegia woodlands, is found in the northern part of the Park, where it also occurs in 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 in the park, forming part of the eastern arc montane forest and covers the peak of Malundwe and Ngolwe mountains.

(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 (Figure 3)

This study was restricted to four sites covered by three ecosystems where giraffe inhabit. These were the Mkata river flood plain, the miombo woodland and the mixed woodland (Figure 4).

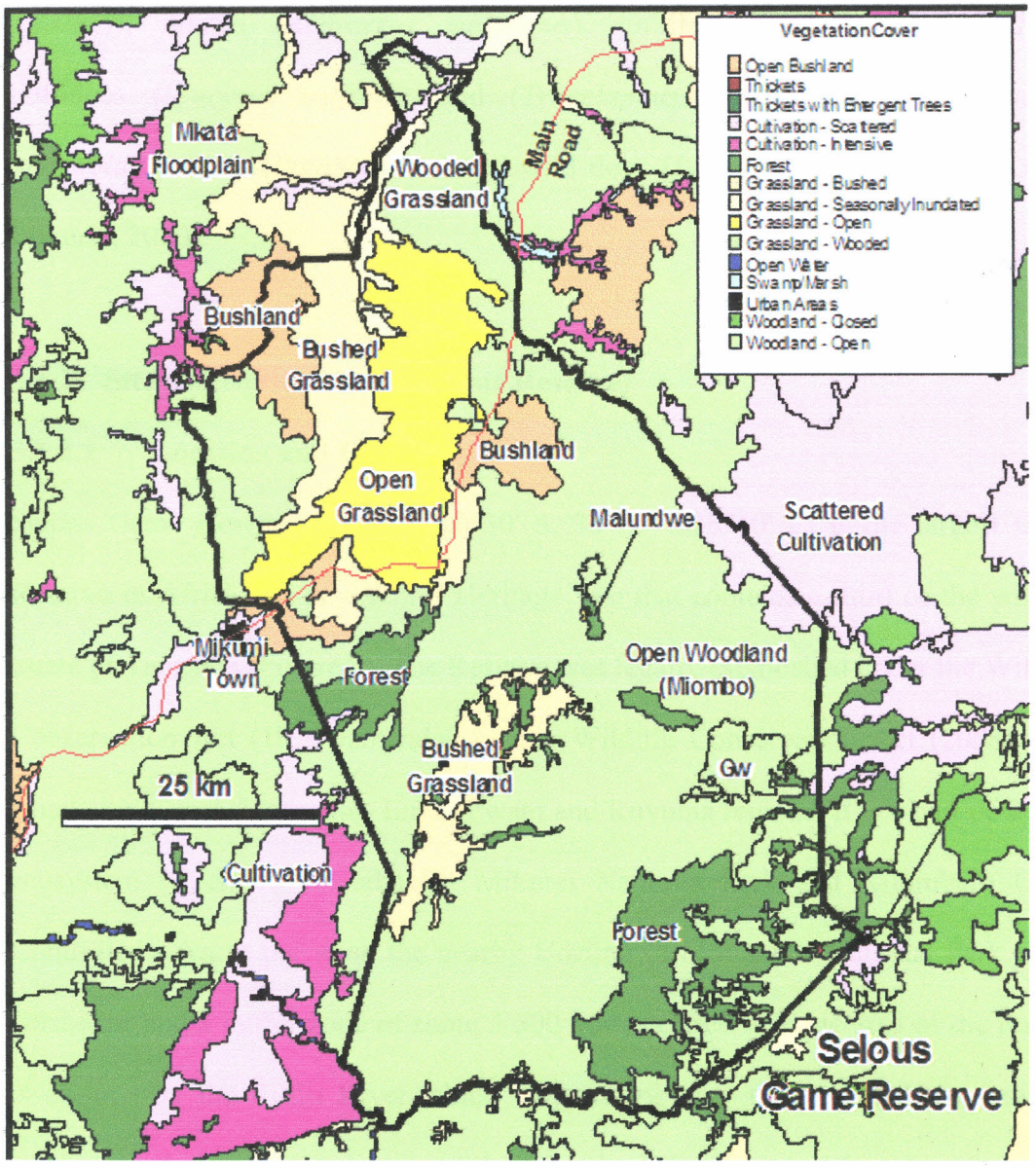


Figure 3: MINAPA and part of Selous game reserve map showing vegetation and its distribution. Source: Animal Behavior Unit (ABRU) - MINAPA

3.1.1.4 Animals

MINAPA have a diversity and numbers of wildlife animals which include mammals, reptiles, birds, and amphibians (Kagaruki *et al.,* 2003; Collett *et al.,* 2007). The popular animals include zebras (*Equus burchellii*), wildebeest (*Conochaetes*

taurinus), impala (*Aepyceros melampus*), giraffes (*Giraffa camelopardalis*), buffaloes (*Syncyrus caffer*), eland (*Tragelaphus oryx*) hippo (*Hippopotamus amphibious*), lions (*Panthera leo*) and wild dogs (*Lycaon pictus*) (Anonym, 2000, Mikumi, 2000).

3.1.2 Site description (Selous Game Reserve)

3.1.2.1 Location and History

Selous Game Reserve (7°20' to 10°30' S, 36°00' to38°40' E) is the largest Game Reserve in Africa and is a World Heritage Site that contains a third of the wildlife estate of Tanzania (Figure 2). The Reserve was legally established under the Wildlife Conservation Act (1974) amended by the Wildlife Conservation Act (2008). It is situated in Coast, Morogoro, Lindi, Pwani and Ruvuma regions. It is a part of Selous ecosystem which covers adjacent Mikumi National Park and Kilombero Game Controlled Area to the west, the nearby Udzungwa Mountains National Park to the northwest and a buffer zone of some 3 500 000 hectares. A large area of the reserve is drained by the Rufiji River which with its tributary the Ruaha drains most of south-central Tanzania and is formed where the Kilombero and Luwegu rivers join above the Shughuli Falls. Large numbers of elephants, buffaloes, giraffes, hippopotamuses, ungulates, crocodiles and about 350 species of birds live in this immense sanctuary, which measures almost 50 000 km^2 (Cleveland, 2008).

3.1.2.2 Climate

The Reserve has a dry sub-humid climate influenced by the prevailing southeasterly winds which bring rainfall to the Eastern Arc Mountains along its western border. The annual rainfall ranges from 750 mm in the east to 1 300 mm in the west, falling

mainly between mid-November and mid-May. The six months of winter are very dry. The average annual range of maximum and minimum temperatures at Kingupira Research Station on the hotter eastern edge is between 17.9° C and 37.3° C but for the whole Reserve range from 13° C to 41° C, depending on elevation (Cleveland, 2008).

3.1.2.3 Vegetation

The Reserve is between the Somalia-Maasai and Zambezian regional centers of endemism, mostly within the latter. Two main vegetation types dominate the reserve: the sector north of the Ruaha-Rufiji rivers (17%) is mainly open wooded grassland underlain by poorly drained alkaline sandy clay dominated by the flat-topped tagalala *Terminalia spinosa* and dotted with doum palm *Hyphaene thebaica*, with swamps along the rivers covered by tracts of borassus palm woodland *Borassus aethiopium*. The remainder of the Reserve (about 75%) is deciduous miombo woodland which provides the chief elephant habitat and much of which is maintained by fire.

Its dominant species are *Brachystegia spiciformis* and *Muyombo B. boehmii* with *Julbernardia globiflora*, bloodwood *Pterocarpus angolensis*, Blackwood *Dalbergia melanoxylon* and *Isoberlinia spp.* with a shrub layer of *Diplorhyncus condylocarpus* and species of Leadwood *Combretum*. This occurs as closed woodland and dense thickets in the center and south, in open woodland in the west, and in the east in scattered tree grassland. But there is a great diversity of other vegetation: areas of rocky acacia-clad hills, gallery and groundwater forests characterized by the wild date palm *Pheonix reclinata*, seasonally flooded sand rivers, swamps and lowland

rain forest. 2,149 plant species have been recorded, but it is thought that even more might be found in the remote forests of the south (Cleveland, 2008).

3.2 Study design and Data collection

This work had two components namely the retrospective and the prospective parts. The retrospective aspect covered the period 1999 to 2006 while the prospective study was undertaken from 2007 to 2010. The work was done on four ecozones namely mixed woodland ecosystem, Mkata flood plain ecosystem, *Acacia Dalbegia* wood land ecosystem, and mixed woodland (Matambwe-Selous Game Reserve) (Fig. 4). It also, constituted two different but complementing studies (field and laboratory work) which were designed to meet study specific objectives. These were characterization of the disease, establishing the predisposing factors, and finding the etiology for GED.

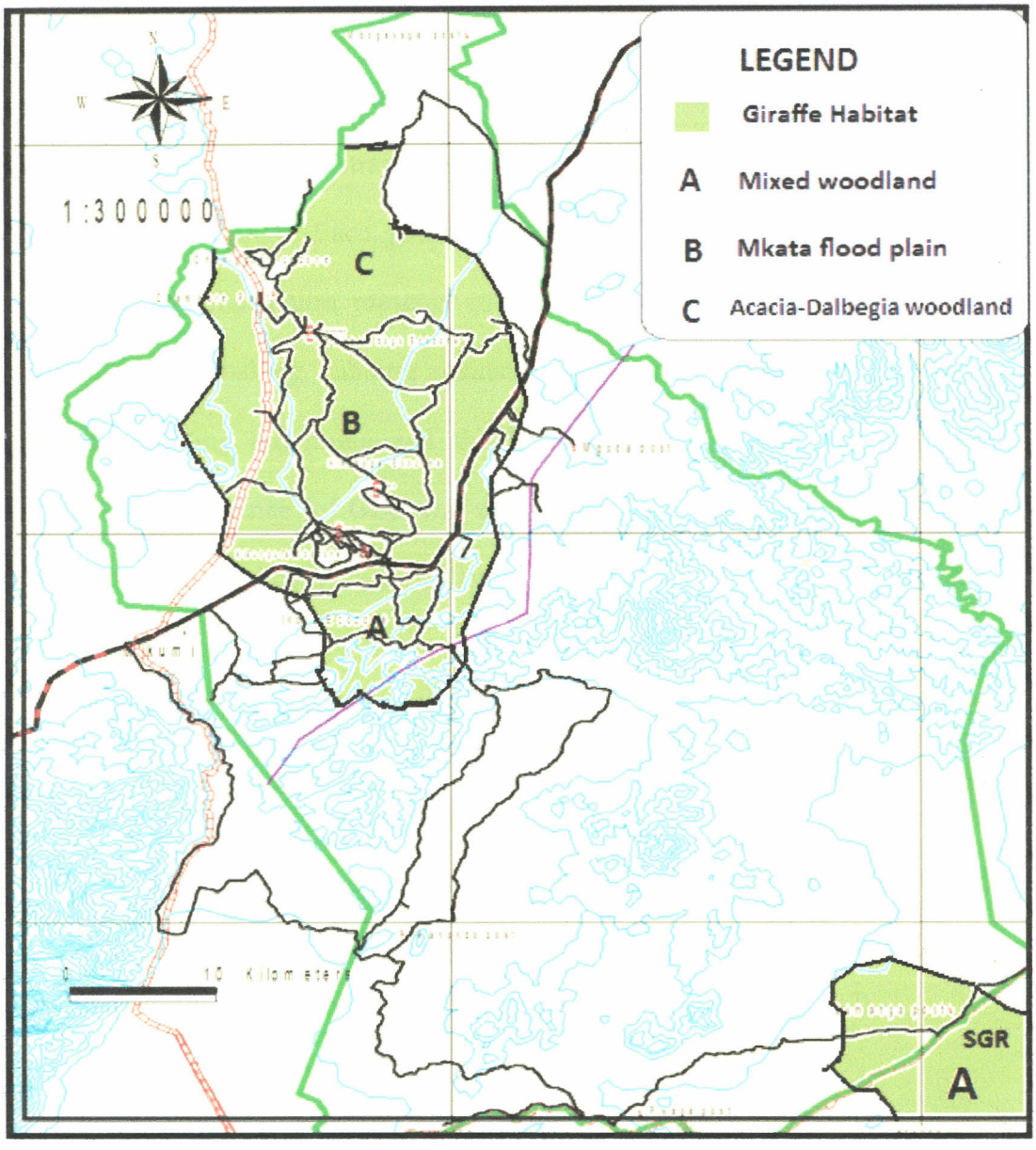


Figure 4: The map of MINAPA indicating the study area (the ecological zones and giraffe habitats

3.2.1 Characterization of GED

3.2.1.1 Quantification of the magnitude of GED in the Mikumi –Selous ecosystem

This involved both field and laboratory work, whereby retrospective and prospective data were collected. Retrospective data on GED covered a period from 1999 to 2006.

Prospective data covered a period between 2007 and March 2010. Giraffes with and without GED were sighted and recorded in their habitat in different parts of the ecosystem by using line transects method on large quadrants as described by Buckland *et al.* (2004). Each giraffe was assessed from a distance for shape of the ears (pinna), conformation, presence or absence of discharge, presence or absence of signs like head shaking, rubbing against hind region and trees. Also, other unusual features affecting the pinna and the head region in general. Quantification of magnitude (prevalence) of GED was determined by using standard procedure and a formula as described by Thrusfield (1995).

$$\text{Magnitude} = \frac{\text{Number of giraffes with GED}}{\text{Total number of Giraffes examined}} \times 100$$

3.2.1.2 Characterization of disease in immobilized animals

The number of giraffes for immobilization for sample collection was determined based on giraffe population and by formula described by Wyne (1987),

$$n = \frac{N}{1 + N(e^2)}$$

Whereby;

n = sample size

N = Population size

e = Sampling error ranging from 5 to 10%

3.2.2 Investigation of predisposing factors

3.2.2.1 Tick and Flies

Ticks were collected from the rangelands, browsing and as well as grazing areas by use blanket drag method as described by Norval *et al.* (1992, 1994). A piece of cloth of a size of a square meter fixed on metal bar was used as a tool for sampling ticks from grass. The cloth was thrown at random by an assistant in each location within an area of 100 m^2 and ticks attached in the cloth were collected and stored in 10 ml silicon tubes containing 70%ples were collected ethanol as preservative. The tubes were identified using the names of the location from where samples were collected to avoid ambiguity during counting and identification. In the laboratory ticks from each tube were poured off into a Petri dish and with help of dissecting microscope (Olympus VMT® 233195), the tick species were identified. Also flies were trapped from vegetation on which the giraffes browsed by using a hand sweep net. Some of these flies were mounted in insect boxes for identification in the laboratory using identification key as described by Walker *et al.* (2003) and Soulsby (1983) and the rest were washed in sterile nutrient broth for bacterial culturing using standard procedure described by Barrow and Fethalm (2004) and Cater and Lemma (1998). Also another set of flies was incubated in nutrient broth and physiological saline at room temperature for possible presence of nematodes on the body surface of the flies as described by Solismaa *et al.* (2008).

3.2.2.2 Browsing materials and watering points

Browsing materials from *Acacia* and *Lonchcarpus* species from browsing areas of giraffes were collected and cut into small pieces. The pieces were put in sterile

universal bottles. Also water was collected from permanent and temporary watering points in the areas habited by giraffes. All samples collected were stored at 4^0 C until processed at Sokoine University of Agriculture (SUA) laboratories. These were cultured for the presence of bacteria and fungi as described by Cater and Lemma (1998).

3.2.3 Causes of GED; Field and Laboratory work

3.2.3.1 Giraffe Immobilization and handling

Giraffes were immobilized if they were at a reachable distance for the dart, good position for darting and on the periphery of the herd. A total of 31 giraffes of both sexes were thoroughly examined for their health. Twenty nine (29) giraffes were conveniently immobilized using Etorphine Hydrochloride (M99®) and a dart gun (Captur® 32mm Gauge, NE 220581) as described by Michael and David (2006). Two (2) were opportunistically sampled after road accident. On close observation special attention was given to the external ear, which was evaluated for lesions, changes in conformation and other unusual/abnormal features on both surfaces of the pinna, the external ear canal and the rest of the head region. All immobilized giraffes were ear tagged for future identification and to avoid recapture.

3.2.3.2 Sample collection and processing

(a) Bacteriological examination

Sterile cotton swabs were applied into the concave and convex side of the pinna of all immobilized giraffes. Thereafter the swabs were preserved in nutrient transport media and transported to SUA laboratories in an ice packed cool box for storage at

4° C. Isolation and identification of bacteria were done as described by Monica (1984), Carter and Lema (1998) and Barrow and Feltham (2004). Pure cultures of interest were taken aseptically and inoculated in special media for biochemical test. The mixture was incubated at 37° C in the incubator (Memmert ®). The color change of the specific medium in specified patterns gave an indication on the type of bacteria which was identified as described by Monica (1984), Carter and Lema (1998) and Barrow and Feltham (2004). On bacterial cultures, all swabs that revealed different types of bacteria were counted and recorded. The percentage bacterial species isolations from the swabs were calculated and recorded. Results were expresses into histograms.

(b) Mycological examination

Skin scrapings for mycological investigation were collected by scrapping the skin of the concave side of the pinna using a scalpel blade. The scrapings were stored and transported in dry envelop until processed as described by Monica (1984). The scrapings were inoculated on Sabouraud dextrose agar and incubated at room temperature for 24 up 72 hours. Morphological identification was done by the method described by Carter and Lema (1998).

(c) Histopathological examination

Three sets of skin biopsies of an approximate size of 2 cm by 2 cm were taken from the concave and convex sides of the pinna and the ear canal by use of scalpel blade. One set was preserved with 10% formalin in sterile plastic bottles until processed for

histological examination as described by Bancroft and Steven (1990) and John and Alan (1990), McGavin and Zacharia (2007).

(d) Virological examination

The second set was collected and preserved in ice parks for virological investigation as described by Carter and Lema (1998) and Barrow and Feltham (2004). Virological investigation was done using a technique as described by Parthiban *et al.* (2005) and Govindarajan *et al.* (2008). Whole bovine fetal kidney with a capsule and fascia was collected in sterile phosphate buffered saline (PBS), pH 7.6 containing antibiotics. Then primary cell culture was prepared. The fascia and capsule were removed and the kidney was washed thrice in PBS with antibiotics. Cortex tissues were removed and minced to small pieces and washed thrice in PBS with antibiotics. Then the cortex tissue was trypsinised (0.25% trypsin in PBS with antibiotics). Trypsinisation was done for 20 minutes at 37°C. The cells were harvested with basal medium (MEM) and centrifuged at 700 rpm for 15 min followed by pelleting of cells. Then the cells were reconstituted to a final volume at the ratio of 0.5 ml of pelleted cells in 250 ml of growth medium (GMEM) with 10% fetal calf serum (FCS) and antibiotics. Antibiotics for PBS and GMEM were same (Penicillin 100 IU, Streptomycin 100 IU, Kanamycin 25 IU/ml). The cell suspension was sieved through sterile gauze and seeded into cell culture flasks, at the rate of 10 ml and incubated at 37° C until monolayer was formed.

The second set of biopsies was prepared for tissue culture inoculation. The biopsies were ground using a motor and pestle with sterile sand. PBS with antibiotic was

added and the mixture was stirred. There after the mixture was filtered and the filtrate was collected in sterile tubes ready for inoculation into tissue cultures after the growth media had been poured off. The cultures were inoculated with 2ml of inoculums and incubated for 30 minutes at 37^0 C. The inoculums were poured off and growth media was added. The cultures was incubated at 37^0 C for 24 hours and observed for cytopathic effect (CPE) there after. Since there was no CPE observed in first inoculation, three subsequent inoculations (passages) were carried out to confirm the presence or absence of virulent viruses in the samples.

(e) Parasitological investigation

Third set was further cut into pieces of approximately 1 cm^2 in size and washed in fresh water then incubated in physiological saline for 24 hours at room temperature (21-25^o C) for worm recovery. This process followed the method described by Solismaa *et al.* (2008). Skin scrapings for mite investigation were collected by scrapping the skin of the concave side of the pinna using a scalpel blade. The scrapings were stored and transported in dry envelop until processed as described by Soulsby (1983).

Ticks were hand picked from the concave side of the pinna and whole body. Also in dry and wet seasons ticks were collected from giraffe habitats by using a blanket drag method as described by Norval *et al.* (1992). The collected ticks were preserved in 10% alcohol in plastic bottles for laboratory identification. Also flies were captured from browsing material on giraffe habitats by using a hand swipe net and preserved in 10% alcohol as described by Kagaruki *et al.* (2003) and (2005).

Identification of ticks and flies was done under stereo microscope as described by Norval *et al.* (1992) and Walker *et al.* (2003).

(f) Hematological investigation

Whole blood and blood smears were collected from sick and healthy giraffes for hematological investigation as described by Jain (1986) and McGavin and Zacharia (2007). Whole blood in EDTA was collected in a vacutainer for study on blood picture as described by Nemi (1986). Blood slides were prepared for examination for blood parasites as described by Collins *et al.* (1995).

(g) Physiological parameters

Physiological parameters (temperature, heart rate and respiration rate) were recorded during the entire period of sample collection. The immobilized giraffes were ear tagged for future identification and to avoid recapture. They were then revived at the end of sample collection by using Diprenorphine Hydrochloride (M5050) as described by Michael and David (2006).

(h) Molecular biological investigation

(i) **DNA extraction, amplification and sequencing**

Molecular analyses were done on nematodes from which crude DNA preparations were obtained by proteinase-K treatment as described by *Bandi et al.* (1998). Then after cox1 and 12S rDNA gene amplification and sequences were respectively generated as described by Casiraghi *et al.* (2001, 2004).

(ii) Molecular phylogenic reconstruction

Obtained cox1 and 12S rDNA genes sequences where aligned with available sequences. The alignment generated was analysed using distance matrix method by using a neighbor joining method as described by Tamura *et al.* (2007). Also phylogenic analysis was performed by using MEGA 4.0 as described by Tamura *et al.* (2007).

3.3 Statistical analysis

Data was entered and stored in Microsoft excel® spread sheet. The data from field work was statistically (descriptively and quantitatively) analyzed by using Microsoft excel®. Graphical processing and presentation of the analysed data was done using Microsoft excel® program. The results were presented as graphs, tables, and chart (Pie charts and histograms). P- Values were generated using ANOVA and Chi-square test (Microsoft excel®).

CHAPTER FOUR

4.0 RESULTS

4.1 Magnitude of GED in Mikumi – Selous ecosystem

When the giraffe ear disease (GED) was first recorded in the year 1999 the prevalence (p, ±CI) in that year was 20% ± 4% (mean ± SE) and this triggered an alarm to conservation authorities and stakeholders. The general trend shows that the magnitude of the disease decreased over the years to 1.5% ± 1.5% ((mean ± SE) in the year 2009/10 as shown in Fig. 5.

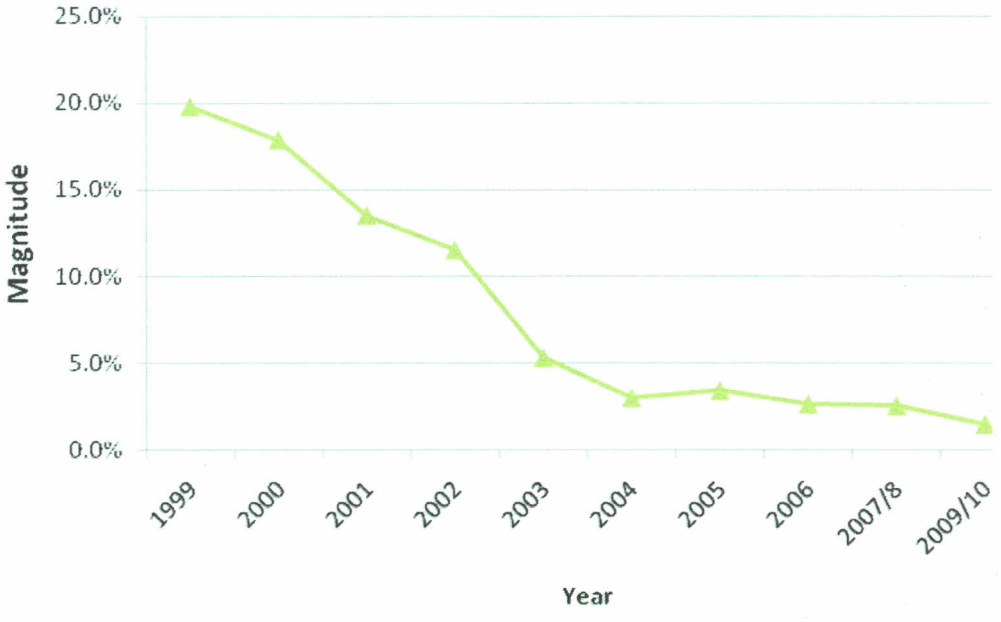


Figure 5: General trend for GED from 1999 to 2010

4.2 Seasonal trend and magnitude of GED in Mikumi – Selous ecosystem.

From 1999 to 2006 in dry seasons 285 giraffes out of 2749 examined had severe GED and in wet season 145 giraffes out of 1517 assessed had severe GED. The overall magnitude of the disease was 10.55% (12.27%, 8.84%) during dry season while in wet season it was 10.22% (11.74%, 8.69%) (Table 1).

Table 1: GED in Mikumi – Selous ecosystem in dry and wet seasons for period between 1999 and 2009/10.

Dry season				Wet season			
Year	Total examined	Number sick	Magnitude %	Year	Total examined	Number sick	Magnitude %
1999	210	38	18.5	1999	209	45	21.5
2000	197	32	16.7	2000	200	39	19.5
2001	190	30	16	2001	194	22	11.5
2002	106	16	15	2002	180	17	9.5
2003	129	4	4.1	2003	210	14	7.1
2004	144	6	4.16	2004	223	5	5.5
2005	123	2	1.7	2005	170	8	4.9
2006	133	2	1.5	2006	131	5	4.1
2007/8	196	4	2.04	2007/8	158	5	3.1
2009/10	190	2	1.05	2009/10	145	3	2.06

The trend over the years for GED showed that the disease was decreasing in both seasons simultaneously (Fig. 6). The differences in magnitude was not significant ($\chi 2 = 0.33$, 3df, $p > 0.05$) although there was a slightly higher prevalence in the wet season than in the dry season.

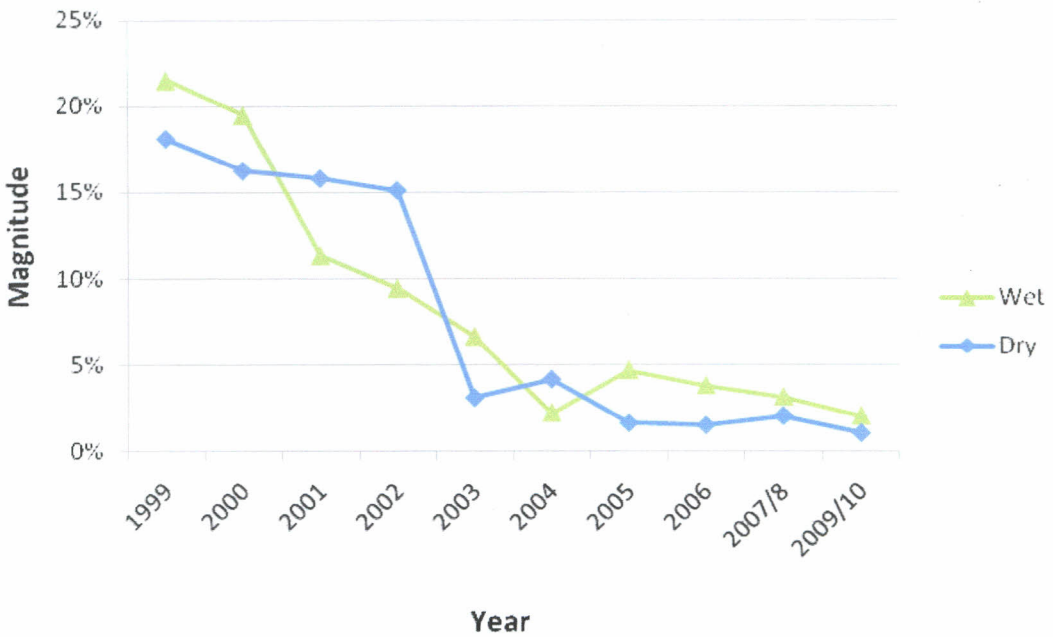


Figure 6: The seasonal trend of GED in Mikumi - Selous Ecosystem 1999-2010

In dry season during the period 2007 to 2010 a total of 386 giraffes were examined. Out of these six (6) were found to have GED, the overall prevalence was 1.55% (2.79%, 0.32%). In wet season a total of 303 giraffes were examine out of which eight (8) giraffes were found to have GED with a overall prevalence of 2.64% (4.45%, 0.83%)

4.3 Magnitude of GED in different ecozones.

In the Acacia-Dalbegia woodland ecozone a total of 72 giraffes were examined and there was no case of GED recorded for the entire prospective study period (2007 – 2010).

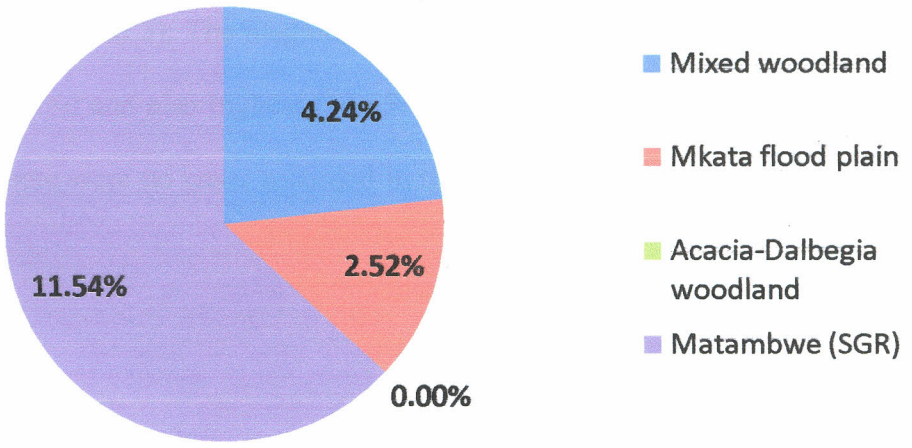


Figure 7: The magnitude of GED in giraffe habitats

Matambwe (Selous Game Reserve) an extension of the southern tip of MINAPA which falls under the mixed woodland ecozone had the highest magnitude of GED of 11.54% (17.68%, 5.40%). This was followed by the mixed woodland ecozone to the north in MINAPA which had 4.24% (7.87%, 0.60%) and has the same vegetation as Matambwe. Lastly was the Mkata flood plain with 2.52% (Fig. 7). The overall results indicated that there was a significant difference (χ^2 = 11.32, 3*df*) $p< 0.05$ in the distribution of GED among the different ecozones.

4.4 Seasonal pattern of GED in different ecozones of Mikumi – Selous ecosystem.

In wet season a total of 208 giraffes were examined of which 13 had GED. The overall magnitude was 6.02% and there was a significant difference (χ^2 = 9.63, 3*df*)

$p< 0.05$ with the distribution of disease among the ecozones. The magnitude of GED each ecozones during the dry and wet seasons was also revealed and varied from each other (Table 2 and 3). The magnitude in Matambwe ecozone in dry season was the highest (8.16%) and also highest (14.55%) in wet season (Fig. 8 and 9, Table 2 and 3) while there were no cases recorded in the Acacia-Dalbegia woodland in both seasons.

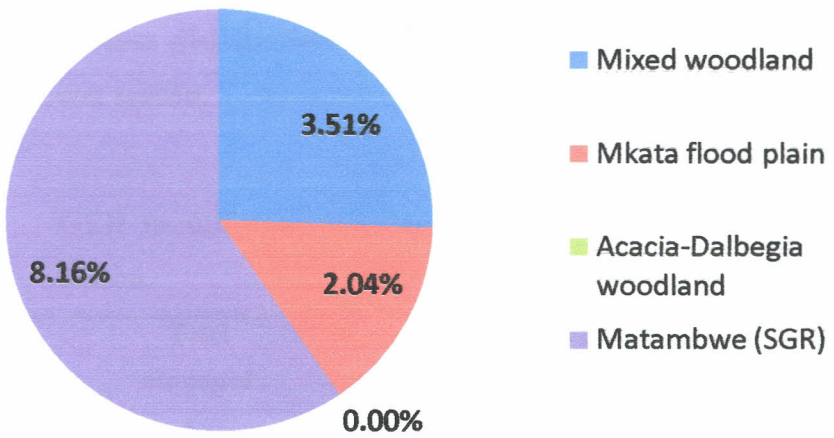


Figure 8: Disease distribution across ecozones in MINAPA and Selous in the dry season

There was a significant difference between cases among the different ecozones in the dry and wet season ($\chi 2 = 11.32$, 3df, $p < 0.05$) with distribution of disease among the ecozones.

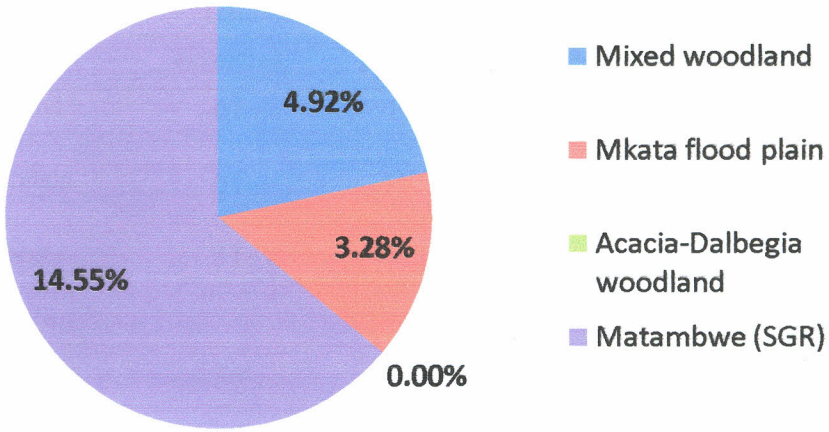


Figure 9: Disease distribution across ecozones in MINAPA and Selous in the wet season

Table 2: GED in different ecozones of Mikumi – Selous ecosystem - Dry season.

Ecozones	Total examined	Mean examined per herd	Total number of sick giraffe	Mean Sick giraffes
Mixed woodland	57	6.33	2	0.2
Mkata flood plain	98	7.2	2	1.6
Acacia-Dalbegia woodland	41	8.2	0	0
Matambwe (SGR)	49	9. 8	4	0.8
Grand Total	**255**	**7.8**	**8**	

Table 3: GED in different ecozones Mikumi – Selous ecosystem - wet season

Ecozones	Total examined	Mean examined per herd	Total number of sick giraffe	Mean Sick giraffes
Mixed woodland	61	8.7	3	0.42
Mkata flood plain	61	16.7	2	2
Acacia-Dalbegia woodland	31	6.2	0	0
Matambwe (SGR)	55	7.8	8	1.12
Total	**208**	**16.0**	**13**	

4.5 Factors that predispose giraffes to GED in Mikumi – Selous ecosystem

4.5.1 The GED in relation to tick burdens

Three-host ticks belonging to the Phylum *Arthropoda* and Family *Ixodidae* (*Rhipicephalus appendiculatus* and *Amblyoma variegutum*) were found in the three ecozones of the Mikumi-Selous ecosystem. Overall picture indicate that tick burden and GED do not correspond over both seasons. In the dry season, the mixed woodland ecozone had high tick burden but few sick giraffes. Mkata flood plain had high tick count and many sick giraffes. On the other hand in the Acacia Dalbegia ecozone the high tick burden did not correspond with the number of sick giraffes since there were no sick giraffes in the ecozone in question. Details are shown in Fig. 10.

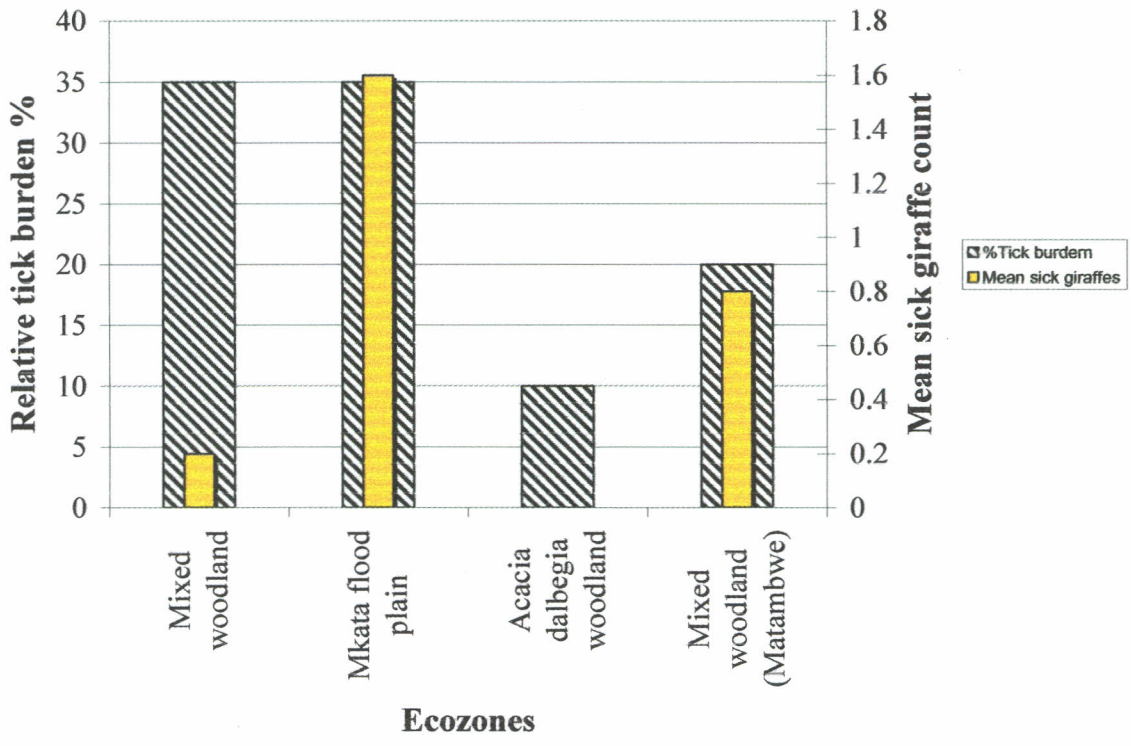


Figure 10: Effect of tick burdens on magnitude of GED in dry season

Effect of tick burdens on magnitude of GED in wet season showed that in mixed woodland (Matambwe) there was high mean count of sick giraffes (1.12) and low tick burden (0.81) (Fig.11) and again there was no sick giraffes observed in Acacia Dalbegia woodland ecozone (MINAPA) tick burden was around 20% of tick burden in this ecozone. These results were different from Mkata flood plain whereby there was a high tick burden and a correspondingly high mean sick giraffe count. Details are shown in Fig. 11.

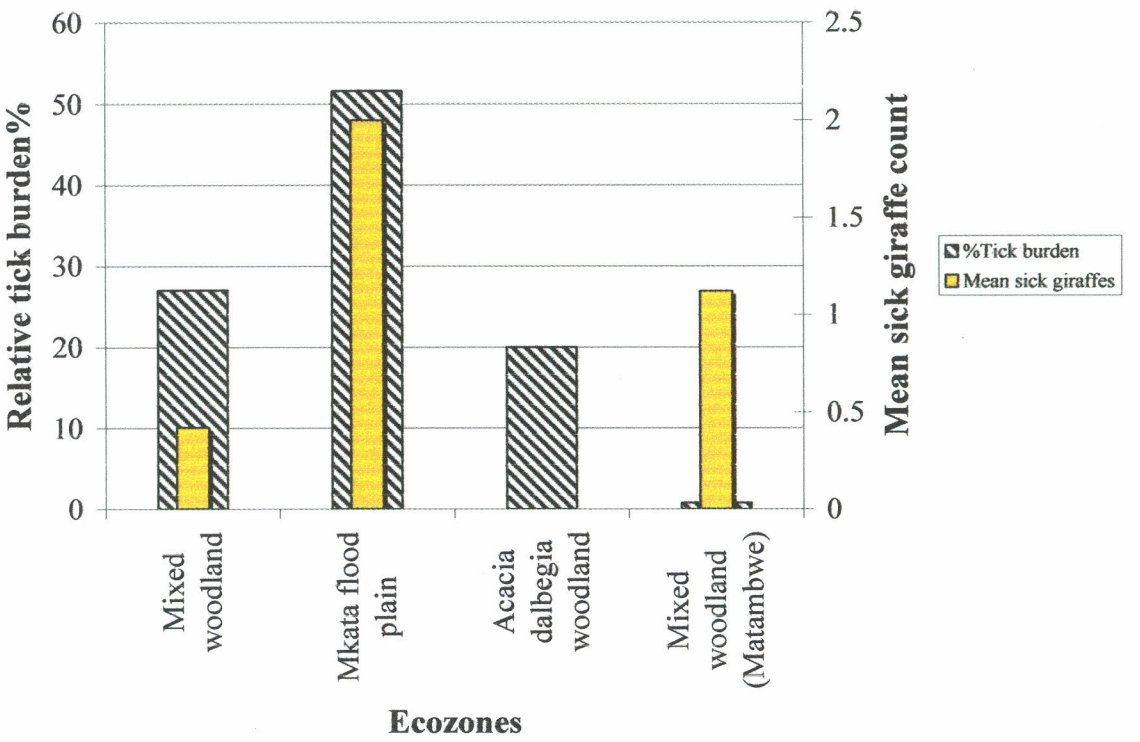


Figure 11: Effect of tick burdens on magnitude of GED in wet season

4.5.2 Influence of Oxpeckers on magnitude of the disease in dry season

Fig.12 and Fig.13 show the relationship between oxpeckers and GED in dry and wet season in the study area. In the wet season in Mkata flood plain there was high mean count of sick giraffes and low percentage of oxpecker per herd. In the mixed woodland ecozone there was high percentage oxpecker per herd and low mean sick count. There were neither sick giraffes observed in *Acacia Dalbegia* woodland ecozone nor oxpecker per herd in this ecozone.

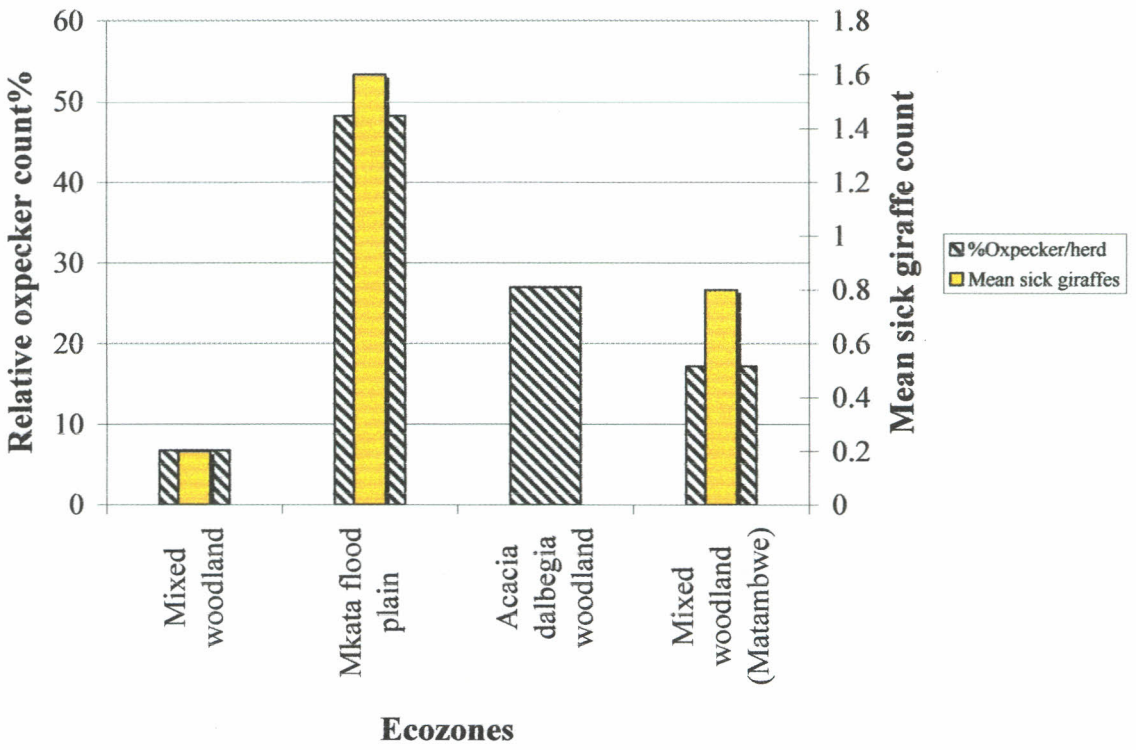


Figure 12: Influence of oxpecker on magnitude of GED in dry season

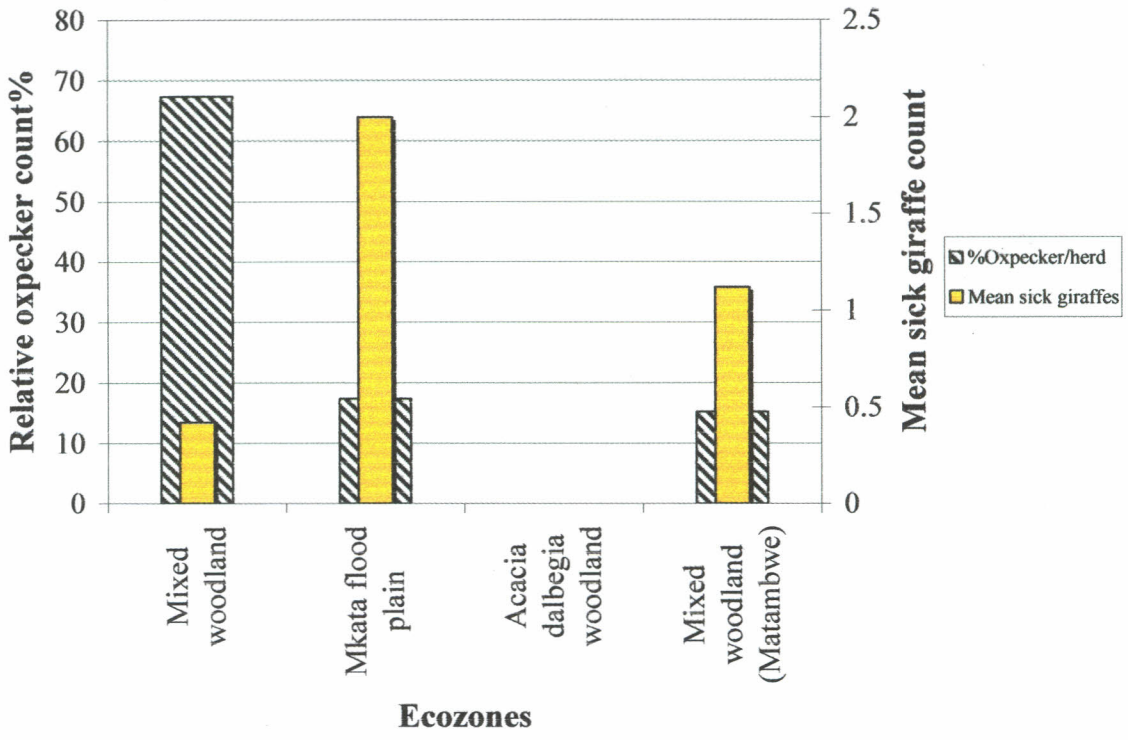


Figure 13: Influence of oxpecker on magnitude of GED in wet season

4.5.3 Association of flies, water, and browsing materials with the disease

Flies collected from giraffe habitats were *Musca sorbens*, *Musca domestica*, and tsetse flies (*Glossina morsitans* and *Glossina pallidipes*). Bacteria isolated from different types of flies were *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Citrobacter freundi*, *Pseudomonas aeruginosa* and *Bacillus firmus*.

Water collected from different watering points was found to contain different types of bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundi*, *Pseudomonas aeruginosa* and *Bacillus firmus*.

Furthermore browsing materials from the same habitats revealed the presence of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundi* and *Bacillus firmus*. There were no nematodes on body surfaces of all flies sampled. Fig.14 shows the relationship between environmental bacteria isolates and GED.

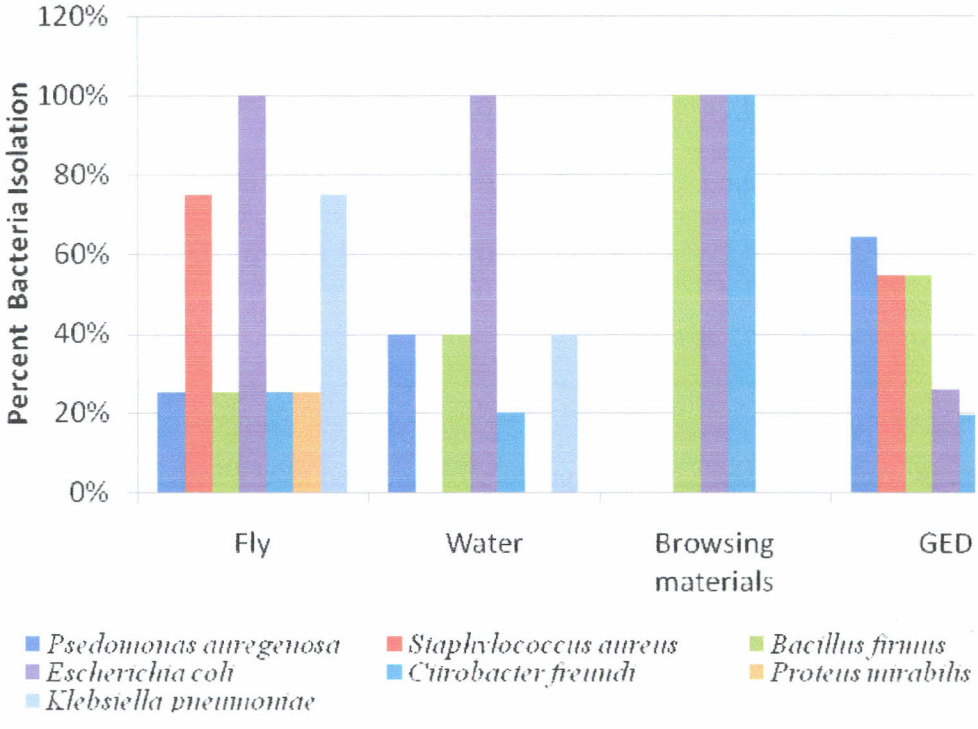


Figure 14: Relationship between bacteria in the habitat and GED

4.6 Clinical features

4.6.1 Blood picture

Table 4: Recorded and obtained value of blood from the darted giraffe.

	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	PCV (%)
Normal value	65	2.5	0.5	30.5	1.5	43
Samples value (Average)	45.4	3.8	0.03	44.81	5.97	39.77

The results indicate that there was an increase of lymphocytes, and monocytes while PCV was lower than normal values (Table 4). Blood slides revealed no parasites and bacteria.

Table 5: Summary of physiological parameters of immobilized of giraffe

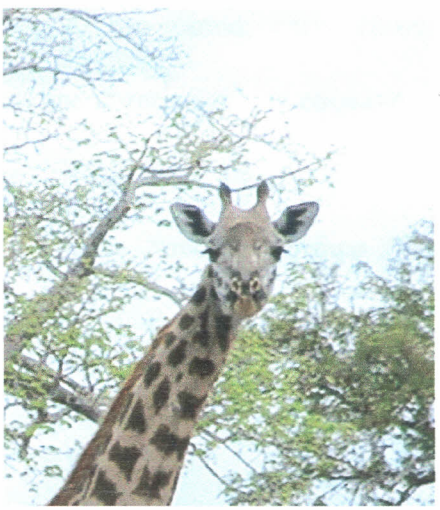
	Mean	Std Dev	Max	Variance	Range	Median	Mode
Dose	2.51	0.81	3.0	0.65	3.0	3.0	3.0
Temper.	35.93	9.75	41.0	95.10	41.0	38.7	
Heart rate	55.93	15.18	65.0	230.66	65.0	60.0	62.0
Respiration. rate	59.16	16.59	72.0	275.40	72.0	62.0	68.0

There were correlations between dose of capture drug and pulse rate, whereby χ^2 was 27.994, and $P<0.0001$, dose and respiration rate as χ^2 was 21.237, $P<0.0001$, and correlation between dose and combine pulse rate and respiration rate was χ^2 43.016, $P<0.0001$. The correlations were very highly significant (Table 5).

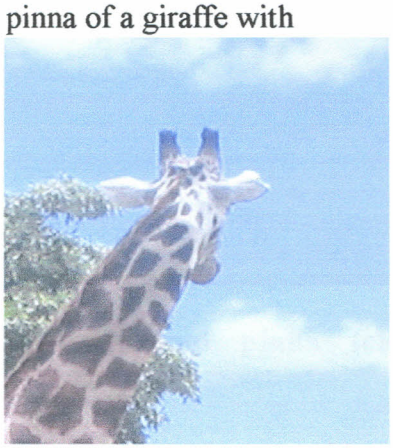
4.6.2 Gross pathological features

On distant observations severely affected pinna appeared enlarged and some of these had obviously distorted ear shape including pendulous, twisted, or areas of thickening, alternating with constricted parts. In very advanced cases the pinna sloughed off. Pinnas of normal giraffe were sharp, erect and pointed upwards (Fig. 15 A-F).

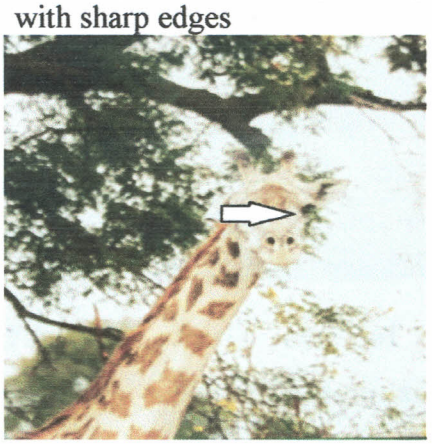
A: Swollen and pendulous pinna of a giraffe with



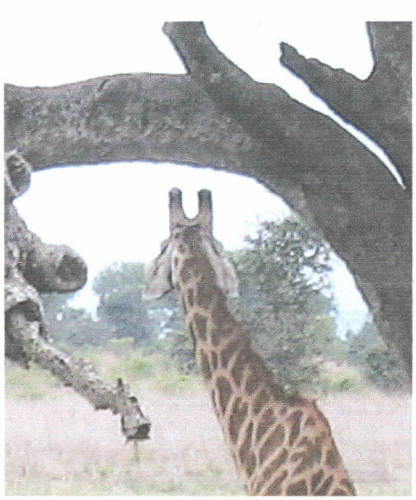
B: Normal pinna upwards pointing with sharp edges



C: Normal ears (back view)



D: Constricted pinna pointing down



E: Back side. Both pinnas pointing downwards.

F: The right pinna sloughed off in severe case of GED

Figure 15: Clinical signs of giraffe with GED (A to F).

On close observation of the concave surface of the pinna, early cases were characterized by hyperkeratosis, ulceration and discoloration of the concave surface of the pinna. Furthermore thickening of ridges of antihelix and distal part of the scapha, and superficial erosion was evident on the concave surface (Fig. 17) compared to normal pinna (Fig. 16). There were no lesions in the entrance and initial part of the ear canal.

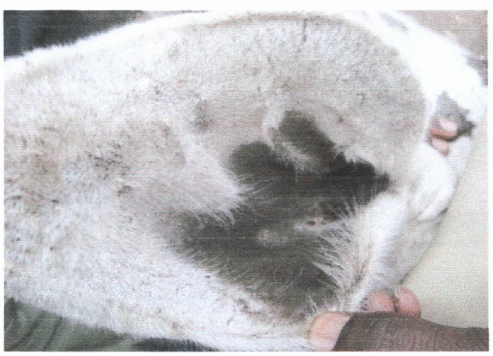


Figure 16: Normal pinna (close-up)

Figure 17: Early case of GED: Note the desquamation and erosion of the skin surface on the concave side of the pinna.

In advanced stage and severely affected cases, the pinna appeared enlarged, had deposits of black grey material that were almost solid in consistence but with fissures and this material was variably moist. In some cases the material had live or dead parasitic larvae (Fig. 18) and emitted foul smell. In other cases the cavity of the external ear was compacted with this material to the extent that the opening to the external ear meatus was completely blocked (Fig. 19). Only the superficial part of the deposited material could be removed, the rest being firmly attached to the skin. The

ridges on the concave surface of the pinna were swollen closing the space between them, a factor that contributed to inaccessibility of the opening to the external ear meatus. In some of the giraffes both ears were almost equally affected or at different stages of the disease process. In other giraffes only one ear was affected.

Figure 18: Severe case of GED

Figure 19: Flies on necrotic case

4.6.3 Histopathological features

The main microscopic features were a necrotizing suppurative inflammatory reaction characterized by marked epidermal cell desquamation or out right loss of the epidermal layer, Neutrophils cell infiltration in the epidermis and dermis, parakeratotic hyperkeratosis and epidermal hyperplasia with prominent rete ridge formations (Fig. 20).

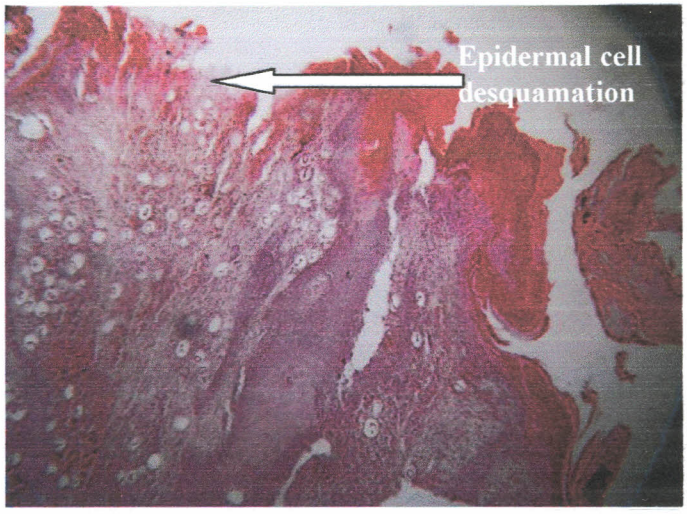


Figure 20: Histological picture of severe case. Note the necrosis of dermis and epidermis, desquamation of the epidermal cell layer. Hair follicles and glands are not visible. Also nematode larvae are evident in the section (HE X 150).

In some specimens mononuclear cells (lymphocytes, macrophages and a few plasma cells) were more prominent than neutrophils in the lower dermis but the reverse was true in the upper dermis. Overlying the epidermis and in some hair follicle lumens was an exudate of dead neutrophils, desquamated epidermal cells, bacterial colonies (mostly cocci) and other detritus. In other specimens, sections of parasites which appeared to be nematodes larvae probably in eggs were in the dermis between hair follicles and rete ridges. (Fig. 20 and Fig. 21) Parasites were also evident inside hair follicles. Around the parasites in the dermis there was a mononuclear cellular reaction with a scattering of eosinophils and neutrophils. The parasites were found at all levels of the dermis.

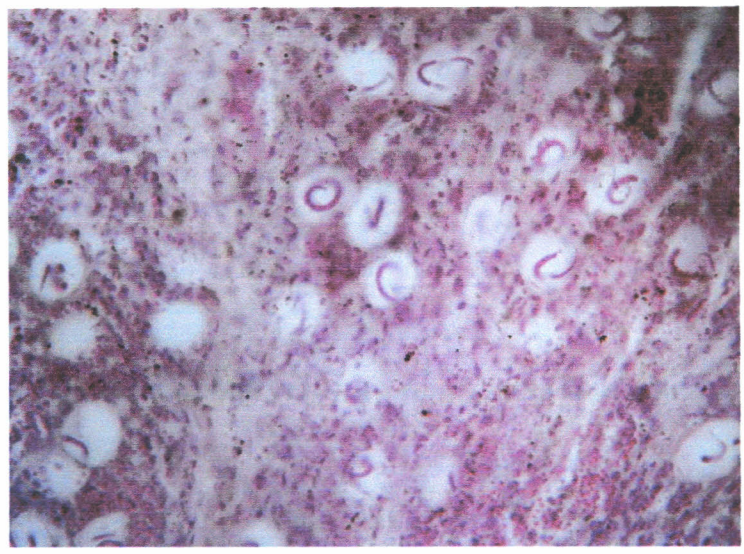


Figure 21: Histological section of severe case. It is showing nematode larvae and inflammatory cells infiltration (HE X 250).

4.7 Aetiology of GED.

4.7.1 Bacteria and fungi

Bacteria and one fungus were isolated from giraffe samples (Table 6). *Pseudomonas aeruginosa* was the most prevalent bacteria isolated from giraffes. There was no significant difference (P>0.05) in isolation of bacteria for male and female giraffes. Only one species of fungi (*Blastomycetes dermatitidis*) was isolated.

Table 6: Bacteria and fungi species isolated from GED.

Isolates	Affected giraffes	GED(+VE) bacteria isolates %	Male		Female	
			Total	%	Total	%
Pseudomonas aeruginosa	20	64.5	10	32.3	10	32.3
Bacillus firmus	17	54.83	10	32.3	7	22.3
Staphylococcus aureus	17	54.83	10	32.3	7	22.3

When results were combined with cases of GED and analyzed, it was observed that; 64.51% with GED harboured *Pseudomonas aeruginosa* compared to 25.08% of animals without GED. It was also noted that some animals with GED harboured *Bacillus firmus*.

4.7.2 Bacteriological and mycological findings in relation to giraffe health status

As shown in Table 6 *Pseudomonas aeruginosa, Bacillus firmus* and *Staphylococcus aureus* were most isolated (over 30%). When bacterial and mycological data were analyzed together with data on the health status, it was noted that 20 out of the 21 with GED harboured *Psedomonas aeruginosa.* It was also noted that 17 out of the 21 with GED harboured *Bacillus firmus.* The same trend was seen with *Staphylococcus aureus,* but different for *Blastomycetes* fungi in which animals without GED were characterized by high isolation rates (16.5%) compared to 1% for animals with GED.

4.7.3 Viruses

Bovine fetal kidney cells monolayer infected with GED extract did not reveal any cytopathic effect after three passages. This was an indication that there were no specific virological activities leading into this effect. Due to infrastructural limitations there was no other tests carried out to determine the presence of viruses.

4.7.4 Nematodes

Histology of skin biopsies from affected concave side of pinna revealed presence of nematode larvae (Fig. 21, 22). This was seen in 20 out of the 21 giraffes with GED.

Eight (8) animals that were free of GED had no nematode larvae. Adult nematodes were also noted and were found to have a length of 2.7 mm to 4.3 mm (head to tail) as illustrated in Fig. 23. The anterior end was knob like, and blunt while the posterior end was blunt (Fig. 24a and 24b). The adult nematodes had no tail sheath (Fig. 24b) and the esophagus was filiform. The adult male had a spicule which was 240 μm long, and the gubaernaculum was 40 μm long (Fig. 24b). In Females the uterus had eggs. The eggs were oval and with a size of 25 μ – 26 μ x 30 – 31 μm (Fig. 25). The flies did not reveal presence of nematodes on their bodies after incubation in nutrient and physiological saline.

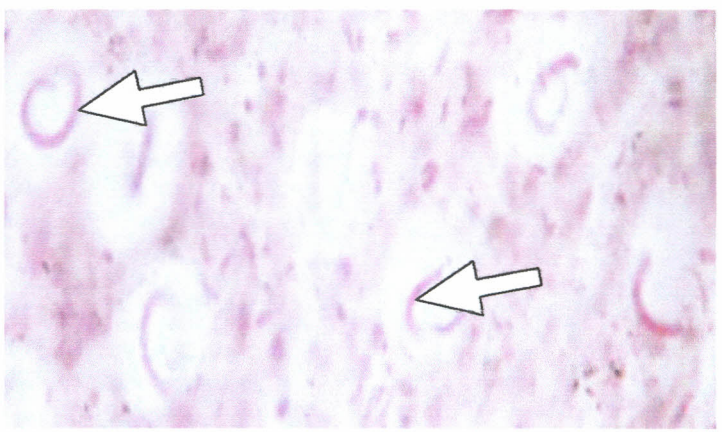


Figure 22: Histopathological section of a GED case showing Spirurid nematode larvae (arrows) (HE X 500).

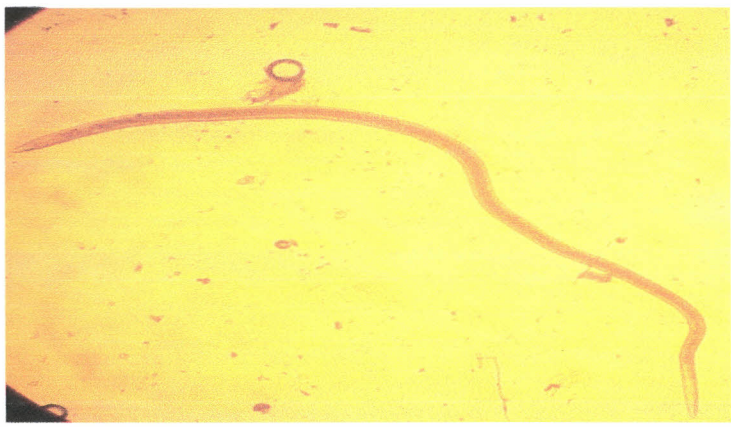
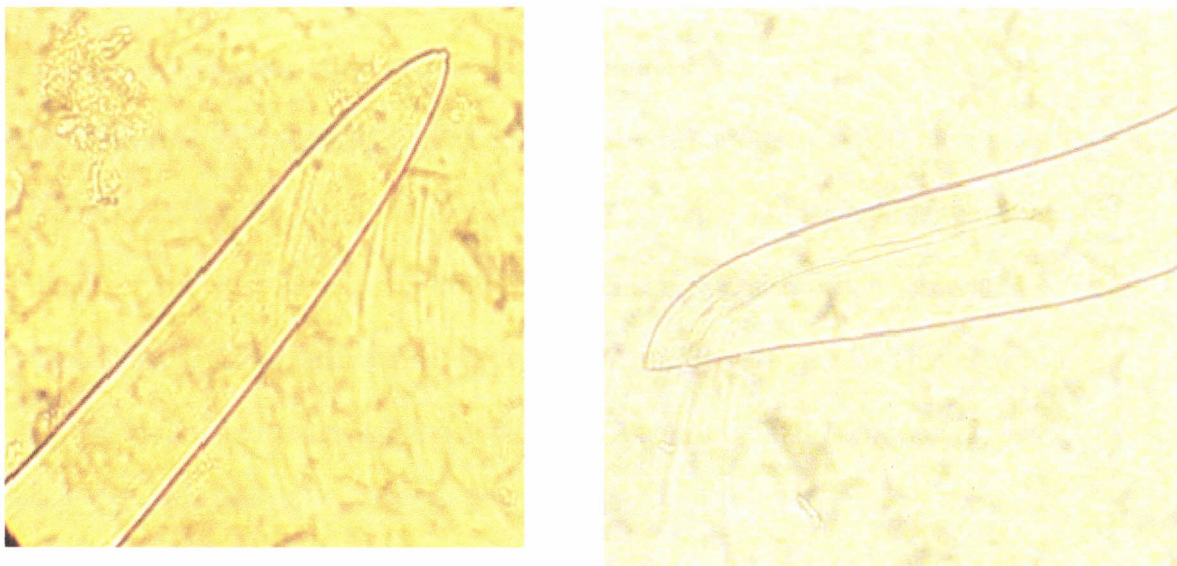


Figure 23: An adult Spirurid nematode isolated from pinna skin biopsies (GED) (X 49).



a B

Figure 24: Anterior (Fig. 24a) and Posterior (Fig. 24b) end of spirurid nematode isolated from pinna skin biopsies (GED) (X 125).

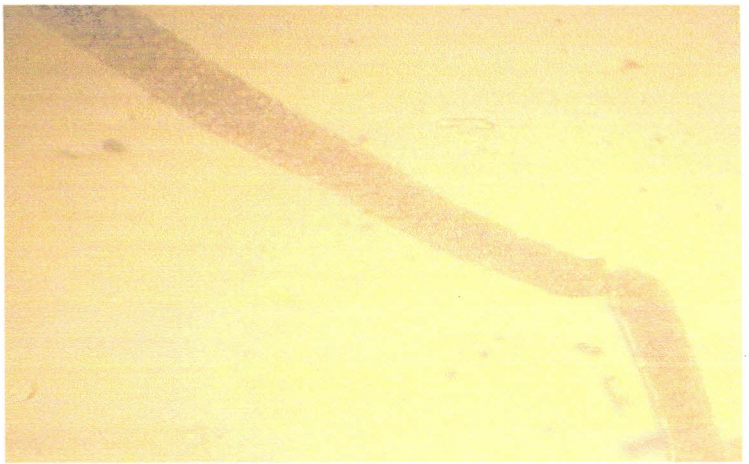


Figure 25: Section of a female Spirurid nematode full of eggs isolated from GED infected pinna (X 100).

4.7.4.1 Molecular characterization of nematodes

The amplified and sequenced cox1 gene of the nematode had 650 base pairs long while the 12S gene had 450 base pairs (Appendix 1). Using the concatenated alignment of these genes, the Neighbor Joining Reconstruction generated a tree which showed that the nematode was of a *Spiriruid* type (Fig. 26). This nematode on DNA sequence and analysis fell between the *Onchocerca* and *Dirofilaria species*.

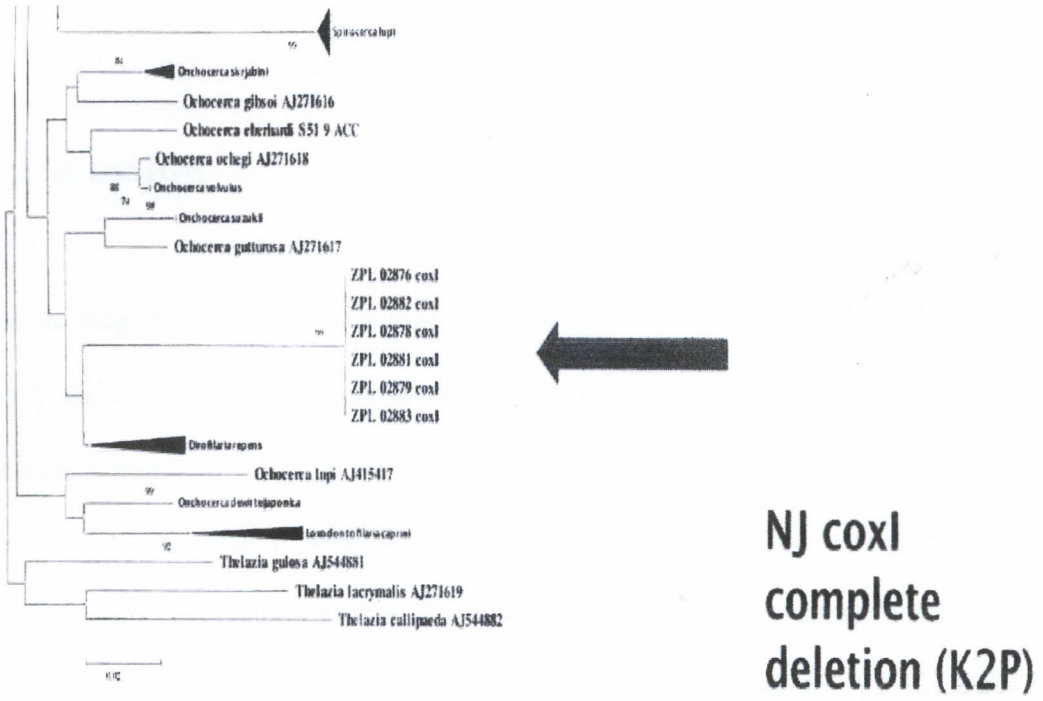


Figure 26: Phylogenic tree to show position of sequenced DNA from GED material

CHAPTER FIVE

5.0 DISCUSSION

The disease magnitude was initially high and this was attributed to the fact that there was heavy and abnormal *El Nino* rains which was more than 1000 mm in some areas in Tanzania, Uganda and Kenya and persisted through the years 1997/1998. Those heavy rains were associated with changes in weather patterns and emergence and spread of diseases especially vector and arthropod borne diseases such as Rift Valley Fever (RVF) and Malaria in animals and human as well Chester (1999). GED erupted for the first time during this period and in very high magnitude. The rapid spread of the disease among herds throughout the park could be attributed to abundant vector activity and surface rain water. Moreover, fluctuations of most of the weather elements including temperature and humidity as a result of the heavy rains within the ecosystem could have affected the balance in favor of vector survival and perpetuation. Such effects include accelerated vector life cycle and colonization of new areas within the ecosystem. This might have contributed to the increase of magnitude of GED within the giraffe population in the ecosystem. On the other hand, these extreme weather conditions also could have affected the behavior of giraffes and other wild animals. Animal displacements as a result of large areas being uninhabitable lead to congregation into small parts and hence overcrowding, including the giraffe populations.

The fact that the disease started during this period, when extreme weather conditions prevailed suggests that animals within the park were subjected to a considerable degree of stress that would have affected their ability to cope and withstand diseases. GED is one of the diseases that affected this species at that time. The primary etiological agent causing this disease therefore is most likely to be a free living opportunistic organism that flourishes during these favourable conditions or changing to virulent variant due to the favourable weather.

The general trend shows that the magnitude of the disease decreased over the years to low levels by the year 2009/10. These observations could mean that the factors that favored the disease were slowly being diminished over the years.

Analysis of seasonal and ecozone GED trend and magnitude suggests that they are intricately intertwined and therefore more reasonable to discuss the findings together. In all seasons, the disease was absent in the Acacia-Dalbegia ecozone. The main reason for this observation might be explained by the type of vegetation predominantly present in the zone coupled with the plant density and cover. That giraffes in Mikumi-Selous ecosystem prefer the *Lanchocarpus capassa* as the main browsing material which is very scarce in this ecozone might explain the observed trend. Also the vegetation cover is very dense to the extent of not providing the best habitat for giraffes on account of security; therefore, animals do not aggregate into herds. This minimizes the possibility of disease spread among animals. However there is a presence of free living opportunistic organisms but these will only infect already damaged skin of the giraffe pinna.

The difference in magnitude of GED between Mkata flood plain and mixed woodland ecozone might be attributed to season and vegetation type. The ecosystem is known to have a black cotton soil which becomes very sticky during wet season as reported by Mikumi (2000). During wet seasons wild animals including giraffes move and congregate on higher grounds whereby high numbers of giraffes in one area could have affected the rate of GED occurrence and transmission among individuals, groups, and herds. High number of animals in one area has been reported by Curtis *et al.* (2001) to increase the rate of disease occurrence and transmission in animals in question. There is a possibility that in wet seasons, giraffes could have moved to higher grounds such as the mixed woodland ecozone within the ecosystem resulting to a high magnitude of GED. Also this could have resulted to high numbers of giraffes in one area, close contact and increase rate on the transmission of GED. Hence this could have resulted in the increase of GED magnitude during wet season. Furthermore presence of insect vectors such as flies could contribute to the transmission and spread of GED even from one case of sick giraffe to health giraffes. In addition there is abundance of *Lanchocarpus capassa* giraffe browsing material as opposed to Mkata flood plain which is dominated by open grassland areas. This might be the reason for fewer cases since this ecozone is only used by giraffes in times of scarcity as is the case during the dry season.

Magnitude of GED progressively, with time, dropped in wet seasons but still there were more cases whereby the magnitude was higher than in dry season. There is a possibility that the magnitude of the disease was affected to dropping to lower levels

as the seasons stabilized. Also wet environment could have favored development of infections and infestation by micro and macro organisms as reported by Chester (1999). There is a possibility that during wet seasons these activities highly favored towards development of GED. Giraffe behavior could have an impact on the increase of magnitude of GED in wet seasons. Free mixing and necking behavior of giraffes as reported by Estes (1991) which led to contact between individuals could have contributed to the increase in magnitude of the disease within and between herds during the wet seasons when animals congregate into larger herds. Also as previously pointed, giraffes with GED have a habit of rubbing the sick ear onto the gluteal region. This leads to the smearing of pus from the infected pinna to the gluteal region. The pus with some necrotic material could contain the GED causing agent which could easily be picked by flies and other vectors. The GED agent may be carried to health giraffes and could get infected.

The GED distribution in both seasons and over years moved from the area of high magnitude in the South (Matambwe) to the North part of the Mikumi-Selous ecosystem. The main reason for this observation could be that the suitable giraffe habitat in that corridor is very small and borders a game reserve to the south. Hunting activities within the game reserve causes the animals to flee to a small part of Matambwe that falls under the National park for security, where hunting is strictly prohibited. This increases transmission owing to higher frequency of rate of contact which may have contributed to high number of GED cases in this ecozone.

The overall conclusion regarding GED distribution and magnitude is that the disease is present in all areas of the Mikumi-Selous ecosystem inhabited by giraffes for a considerable period of time during the year. This finding was in agreement with Mlengeya *et al.* (2002) who reported that all of MINAPA and the Northern Sector of Selous Game Reserve were affected by GED.

Earlier on, ticks, insect vectors and oxpecker were thought to be the major factors which contributed to causation of GED (Mlengeya *et al.,* 2002). What was not clearly explained was how these acarines and aves caused the lesions. There are three ways in which acarines could be involved; one through physical damage to the skin and predispose to opportunistic organisms; two, play a role as a vector of parasitic organisms with predilection to the ear and three, mechanically transfer agents from sick animals to healthy ones. In this study, none of the above has been proved to be involved, and further, circumstantial evidence from ticks dynamics and GED did not prove the association between the two.

Blood samples collected from GED cases did not reveal any parasite. This therefore overrules the possibility that ticks could be involved in the disease cycle as a biological vector. This investigation has also revealed that ticks that were present in giraffe and its habitats may have not contributed to the causation of GED. The absence of ticks in areas with many cases of GED and presence of cases during periods of low ticks density as well as absence of GED cases in times of high tick density do not suggest their role in this disease.

However, there are other factors that determined the distribution and density of ticks in animal habitats. These include availability of preferred hosts, ground vegetation cover and moisture. Therefore, abundance or lack of ticks in an area may not be attributed to presence or absence of giraffe alone, and this makes it difficult to associate GED and ticks given that few sick animals had tick infestation.

Previously it was suspected and reported by Mlengeya *et al.* (2002) and Kagaruki *et al.* (2003, 2005) that oxpeckers (*Buphagus africanus*) played a role on occurrence of GED. Again these birds could be involved in the disease process by three ways: one, causing physical trauma that predispose the giraffe to a range of opportunistic organisms; two, the birds being an intermediate host to a parasite that is capable of infecting giraffe in a variety of ways and three, involves the birds contaminating the ear with agents that are brought within their body parts as is the case with flies.

In this work, the role of oxpecker as a terminal or intermediate host of a parasite was not investigated because the etiological agent had not been identified. On the other hand, the dynamics of these birds in association with GED revealed no evidence of their involvement. A similar situation resembling the tick observations mentioned above was found. There was no conclusive evidence that oxpeckers density and distribution was related to the GED cases. There is, however, evidence that the entire relationship between these species is determined by other range of factors governed by the ecology of the ecosystem.

The findings of this study do not justify the assertion that ticks and oxpeckers have a major role in the GED causation and perpetuation. Attention could be focused on testing specific hypothesis within ecosystems to evaluate the species response to changes in ecosystems especially those that brings stress to animals and plants such as short term weather patterns changes to long term climate change.

Functional capability of all vital organs of immobilized giraffes were assessed through the measurement of cardinal parameters which gave values that were not what can be considered as normal. However, this was expected because in wild animals, the capture process stresses the animal and almost all the parameters are exaggerated.

Significant cellular changes in values of blood picture were observed in captured giraffes with GED. There was reduction in neutrophils while eosinophils, basophils, lymphocytes and monocytes were elevated. It is established that neutrophils are the first line cells of defense responsible for humoral response and formation of pus hence are the first to be killed during infection Nemi (1986). Therefore, their reduction in GED cases signifies a long lasting illness. Eosinophils, on the other hand, are commonly encountered in ruminants with helminthes infestations. The elevation of these cells in GED cases therefore, may suggest involvement of parasitic agents among other possibilities including allergies.

The elevation of lymphocytes and reduction of basophils which are migratory and form the second line of defense suggests advanced lesion in a process of formation

and increase in phagocytosis activity. Increase in monocytes also suggests persistence of the lesion and hence development of chronicity in the GED cases. Overall conclusion for blood picture findings suggests that the GED is a localized chronic disease that eventually brings in systemic response. A sequel to systemic involvement, irrespective of the cause is the reduction in the ability of the animal to defend itself against other diseases which might be the case with GED.

The clinical and pathological features of this disease point to an inflammatory process targeting the skin covering the pinna and sparing the external ear canal. Moreover ear diseases are normally considered dermatology cases until otherwise proven so the ear disease in giraffes basically might be a dermatological problem (Kristensen *et al.,* 1996). The disease starts with the epidermal layer of the skin and progresses to affect the dermis and may even destroy the cartilage as well as the convex part of the pinna as it progresses leading to sloughing of part or the entire pinna as result of inflammation followed by secondary bacteria infection and necrosis.

It has been shown in the present study that there is a possibility in some seriously affected giraffes to have undergone a suppurative inflammatory process extending to the subcutis and the underlying musculature (panniculus). This is probably an indication of a cellulitis, a reaction likely to lead to septicemia (Mlengeya *et al.,* 2002). These findings probably explains why few cases become very sick, off feed, emaciated, weak and eventually die from a generalized disease problem emanating from involvement of vital organs such as the lungs (Mlengeya *et al.,* 2002). Such

weak cases are also easily withdrawn from the ecosystem by predation as reported by Mlengeya (1994).

Pseudomonas aeruginosa, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus firmus*, and a fungi *Blastomycetes dermatitidis*, were isolated from GED cases, flies, water, and vegetation within the giraffe habitat. These are environmental microorganisms. *Blastomycetes dermatitidis* has been reported by Whitt and Salyers (2001) and Cornelis (2008) to be environmental fungi and it can be transmitted by insect vectors, water, soil and wind. Presence of this fungus in GED cases at very low levels could have been as a result of environmental contamination and also probably it does not grow or survive well on necrotic material as in case of GED cases. Presence of these microorganisms on healthy and sick giraffes could have been as a result of these factors acting singly or in combination within the giraffe environment. The presence of these microorganisms in the giraffe habitat or on GED is not coincidental. The microorganisms are readily carried by flies, fecal contamination, dead materials and water run offs within the ecosystem. They are also deposited by flies on vegetations on which giraffes browse.

Pseudomonas aeruginosa was the most frequent bacteria isolated from GED cases, flies, water and giraffe browsing materials. Also in preliminary investigation it was reported by Mlengeya *et al.* (2002) that *Pseudomonas aeruginosa* was associated with GED. *Pseudomonas aeruginosa* infect wounds, cause abscess formation, inner and external ear infections, and dermatitis Diekema *et al.,* (1999). *P.aeruginosa* is an opportunistic pathogen. It is "opportunistic" because it seldom infects healthy

individuals. There is a possibility that injuries caused by parasites such as nematodes and insect vectors on the skin of the pinna of giraffes were secondarily infected by *Pseudomonas aeruginosa* resulting into dermatitis. *Pseudomonas aeruginosa* could be pointed at as a perpetuating factor of GED. It is a gram-negative, rod-shaped, asporogenous, and mono flagellated bacterium that has an incredible nutritional versatility Lederberg and Joshua (2000). It is about 1-5 μm in length and about 0.5-1.0 μm in breadth and is an obligate aerobe, which means it requires oxygen and uses aerobic respiration as its choice of metabolism. *P. aeruginosa* is a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage and hospitals. In all aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients throughout, *P.aeruginosa* is the predominant inhabitant and this clearly makes it the most abundant organism Lederberg and Joshua (2000). Due to its abundance in the environment it can easily be picked by insect vectors, water and wind and be transmitted to injured skin. The pinna in giraffes that has been traumatized by nematodes, biting flies, or thorns of browsing material may be invaded by *P.aeruginosa*.

Staphylococcus aureus was also highly isolated from all GED cases and from giraffe habitat. It is a gram positive bacteria occurring in pairs, short chains and clusters. It is aerobic and facultative anaerobic, catalase and oxidase negative, non motile, non spore forming, and fermentative. It can survive on domesticated and wild animals and can survive for some hours on dry environmental surfaces. It can infect other tissues when normal barriers have been breached Whitt and Salyers (2001). It resides normally on the skin and mucous membrane of humans and animals. It is a

component of the normal microbial flora and it infects wounds Collins *et al.,* (1995). Hence injuries caused on the skin by parasites as nematodes and insect vectors will easily be infected by *Staphylococcus aureus* as in the case of GED. *Staphylococcus* infections can be spread through contact with pus from an infected wound, and skin-to-skin Curran, and Al-Salihi (1980). This point to the fact that giraffes necking behavior and over crowding in wet seasons could have facilitated the transmission of GED whereby besides this bacteria being present on the skin it can as well being picked by insect vectors and flies to a health or sick ear.

Insect vectors have been incriminated in transmission of bacteria in domestic and wild animal ecosystems as reported by Anderson (2000). Flies in the giraffe habitats could be suspected to have played a role as a perpetuating factor in GED by transmitting the bacteria and the parasitic nematode from sick to health giraffes. Soil, water, humans, animals, plants, sewage, and decay materials of plants and animals have been reported by Lederberg and Joshua (2000), and Cornelis (2008) to play a role as a source of microorganisms in the environment. Such sources of bacteria are readily available in the giraffe habitats and may act biologically or mechanically as source of contamination on already damaged skin of the giraffe pinna hence play a role as perpetuating factors of GED.

The presence of bacteria and fungi on wild animals with diseases such as in GED might be as a result of changes in environmental factors caused by the then prolonged heavy *El Nino* rains of 1997/98 Chester (1999). Since these are environmental microorganisms, they could also be environmental contaminants on

GED as previously suspected by Kagaruki *et al.* (2005). These also might have been transmitted by flies such as *M. sorbens* which were a common finding on giraffes and in their habitat.

Flies, especially *Musca sorbens,* were also found to harbor the same bacteria which were isolated from GED, watering points and browsing materials. These flies are scavengers by nature and the most prominent carrier of bacteria and other parasites as reported by Nmorsi *et al.* (2007). The fly is globally known to transmit diseases to humans, wild and domestic animals (Nmorsi *et al.,* 2007). Also it has been reported by Mbilu *et al.* (2007) that M. *sorbens* are attracted to wounds on animals. Probably there is a possibility that these flies might have contributed to the transmission of GED causative agent to the giraffe's skin of the pinna. These flies also carry pathogens on their feet, faeces and digestive juices they regurgitate (Curtis *et al.,* 2001). Hence when scavenging for food they might have deposited the bacteria on the already injured skin of the pinna thus perpetuating GED. This fly species has also been reported by Bech-Nielsen *et al.* (1982) and Nmorsi *et al.* (2007) that may contaminate a wound at any part of the body and to be a potential vector for carrying different pathogens. Therefore there is a possibility that the fly could have transmitted the bacteria and other parasites from the environment to the giraffes, between individual giraffes, and their herds. During and after the *El Nino* rains there was an abundance of decayed plant and animal materials which favored multiplication of different pathogens and disease vectors such as flies of the *Musca* species Kagaruki *et al.* (2005); Chester (1999). *Musca sorbens* may have played a role as transmitting agent for bacteria and possibly parasites to the already damaged

skin of the pinna of giraffes. The damage could have been caused by insect vectors, parasites such as nematodes within the giraffe habitat as reported by Kagaruki *et al.* (2003, 2005); Mlengeya *et al.* (2002).

For the first time this work has been able to demonstrate and isolate a *Spirurid* nematode which is most likely the primary causative agent for the GED. Specimens from the isolates were sent for identification and DNA typing to Milan University, Italy which has a world reference laboratory for identification of helminthes. The DNA sequence from the isolated *Spirurid* nematode lies between *Onchocerca* and *Dirofilaria species* which are both parasitic to human and wildlife. It is therefore likely that this could be a species that is new and specific to this ecosystem. Furthermore, there is a possibility that, it is a species that have evolved from older, non pathogenic and common environmental agent.

Various studies have incriminated the nematode to have caused ailments in various parts of the body of the hosts. *Spirurid* nematodes have been reported to infest domestic and wild animals through the skin Kock and Kock (1990); Wahl *et al.,* (1994); Pampinglion *et al.,* (2001) and Solsimaa, *et al.,* (2008). Also Solsimaa *et al.* (2008) reported that *Spirurid* nematodes usually inhabit the subcutaneous tissues, ligaments and aponeuroses of cattle and large mammals as predilection sites. Schmidt *et al.* (1982), Kock and Kock (1990) and Anderson (2000) reported that filarioid nematodes (*Onchocerca* spp., *Parafilaria* spp., and *Stephanofilaria* spp.) produce larvae into the skin. These nematodes cause parasitic infestations resulting in skin injury by penetration into the skin. The affected skin develops necrotic

dermatitis lesions as a result of *Spirurid* nematode damage followed by secondary bacteria infection. This also explains why there was a recovery after experimental treatment of GED cases with an antihelminthic (Ivomectin®) (Mlengeya *et al.*, 2002). Nevertheless, big questions still remain to be answered. These include the origin of the nematode, how does it reach the ear of a giraffe and why the ear and not any other part, how does it enter the tissue, multiply and proliferate itself. These will need to be answered for a better and effective management and conservation plans can be made.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

It was concluded that conclude that GED is a dermatological problem, whereby *Spirurid* nematodes act as a primary cause whereas the bacteria are secondary. The severe GED is superimposed by bacteria and fungi working together, possibly in stressing states as caused by climatic changes. However, the mechanism by which the nematodes and bacteria cause the damage to the pinna as was observed in this work is unknown

Also this work revealed that the trend of the disease magnitude was downwards as the years progressed. There was an indication that in future the number of disease cases will drop to a manageable level whereby the few that will be sighted within the ecosystem could probably be treated. Moreover there is a possibility that the next generation of giraffe offspring within the ecosystem would have developed immunity to the Spirurid nematode which causes GED and this might lead to fully or partially disappearance of the clinical disease.

6.2 Recommendations

From the present study it is recommended that:

(i) GED be known as Giraffe Pinna Dermatitis

(ii) The mechanisms by which the nematodes cause the primary pinna lesions need to be studied.

(iii) The role of arthropods and other vectors in the transmission of the disease also need further study.

(iv) Establishment of a national nematode bank and reference archive with identification keys for future reference.

(v) There is a need for establishment of micro weather stations at strategic points in each ecozone for ecological and climate monitoring

(vi) Establish electronic geomaps for every national park in the country to assist in future development of early warning systems

(vii) Establish GED monitoring programme and treat the few remaining cases in the ecosystem

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APPENDIX

Appendix 1: Sequencing of DNA from GED material

12S 475 bp

SEQUENCES PRODUCING SIGNIFICANT ALIGNMENTS

Accession	Description	Max Score	Total Score	Query coverage	E-Value	Max ident	Links
AJ544857.1	Thelazia gulosa mitochondrial partial 12S rRNA gene	374	374	86%	3e-100	83%	
EU182328.1	Dirofilaria immitis isolate Chongging 12S ribosomal RNA gene, specimen voucher Bain, 0.	333	333	100%	5e-85	79%	
AM77948.1	Setaria tundra mitochondrial partial 12S rRNA gene, specimen voucher Casiraghi	331	331	92%	2e-87	80%	
AM779828.1	Setaria tundra mitochondrial partial 12S rRNA gene, specimen voucher Casiraghi,	331	331	92%	2e-87	80%	
AF538716.1	Brugia malayi mitochondrion, complete genome	329	329	100%	7e-87	79%	G
EU182327.1	Dirofilaria immitis isolate Chengu 125 ribosomal RVA gene, partial sequence mit	327	327	100%	3e-86	79%	
EU169125.1	Dirofilaria immitis 125 ribosomal RNA gene, partial sequence, mitochondrial	327	327	100%	3e-86	79%	
AJ5375512.1	Dirofilaria immitis complete mito chondrial genome	327	327	100%	3e-86	79%	G
AY482913.1	Onchorerca gutturosa mitochondrial partial CO1 gene fr cytochrome oxidase subi	326	326	100%	9e-86	79%	
AY462912.1	Onchorca gibsoni 125 ribosoma RNA gene, partial sequence; mitochondrial	320	320	100%	4e-84	79%	
AJ544834.1	*Setaria tundra mitochondrial partial 125 rRNA gene*	*315*	*315*	*84%*	*2e-82*	*81%*	
GQ292761.1	Dirofilaria repens isolate freburg 12S ribosomal RNA gene, partial sequence; mitochondrial	307	307	100%	3e-80	79%	
AY462911.1	Litomosoides carini 125 ribosomal RNA gene, partial sequence; mitochondrial	305	305	100%	1e-79	78%	
AJ544836.1	Ochoterenella SD. 56 CV mitochondrial partial 12S rRNA gene	303	303	89%	4e-79	80%	
FM206484.1	Onchocerca ochengi mitochondrial partial 12S	302	302	100%	2e-78	78%	

Accession	Description	Max Score	Total Score	Query coverage	E-Value	Max ident	Links
AY462918.1	Onchocerca ochengi done 3c5 12S ribosomal RNA gene partial sequence; mitochondrial	302	302	100%	2e-78	78%	
AY462914.1	Onchocerca ochengi done 3cl 125 ribosomal RNA gene, partial sequence; mitochondrial	302	302	100%	2e-78	78%	
AM779845.1	Nematoda sp. MC-MOTU-5 mitochondrial partial 12S rRNA gene, specimen voucher	300	300	83%	6e-78	80%	
AM779835.1	Nematoda sp. MC.MOTU-1 mitochondrial partial 12S partial rRNA gene, specimen vouche	298	298	92%	2e-77	79%	
AF015193.1	Onchocerca volvulus mitochondrion, complete genome	298	298	100%	2e-77	78%	
AY462921.1	Onchocerca volvulus clone 9c3 12S ribosomal RNA gene, partial sequence, mitochondrial	296	296	100%	7e-77	78%	
AY462920.1	Onchocerca volvulus clone 9c2 12S ribosomal RNA ribosomal RNA gene, partial sequence; mitochondrial	296	296	100%	7e-77	78%	
AY462917.1	Onchocerca ochengi clone 3c4 12S ribosomal RNA, partial sequence; mitochondrial	296	296	100%	7e-77	78%	
AY462916.1	Onchocerca ochengi clone 3c3 12S ribosomal RNA gene, partial sequence; mitochondrial	296	296	100%	7e-77	78%	
AY462915.1	Onchocerca ochengi clone 3c2 125 12S ribosomal RNA gene, partial sequence; mitochondrial	296	296	100%	7e-77	79%	
AJ544841.1	Foleyela furcata mitochondrial 12S rRNA gene	294	294	88%	3e-76	80%	
AM779826.1	Setaria tundra mitochondrial partial 12S rRNA gene, specimen voucher Casiraghi,	292	292	84%	9e-76	78%	
AY462925.1	Onchocerca linealis clone 1c2 12S ribosomal RNA gene, p artial sequence; mitochondrial	291	291	100%	3e-75	78%	

Coxl 667 bp

SEQUENCES PRODUCING SIGNIFICANT ALIGNMENTS:

Accession	Description	Max Score	Total Score	Query coverage	E-Value	Max ident.
EU169124.1	Diroflaria immitis cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	640	640	100%	4e-180	84%
EU163945.1	Diroflaria immitis cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	640	640	100%	4e-180	84%
EU159111.1	Dirofilaria immitis cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochon	640	640	100%	4e-180	84%
AJ537512.1	Dirofilaria immits complete mitochondrial genome	640	640	100%	4e-180	84%
AF015193.1	Onchorerca volvulus mitochondrion, complete genome	623	623	97%	4e-175	83%
EF521410.1	Onchocerca lupi from Portugal cytochrome oxidase subunit 1 (CO1), gene, partial	621	621	94%	1e-174	84%
DQ358815.1	Dirofilaria immitis mitochondrial partial COI gene, partial cds; mitochondrial	609	609	97%	1e-170	84%
AJ271613.1	Dirofilaria immitis mitochondrial partial COI gene for cytochrome oxidase subunit	608	608	96%	1e-170	83%
AJ271616.1	Onchocerca gibsoni mitochondrial partial COI gene for cytochrome oxidase subunit	604	604	100%	1e-169	83%
D0097309.1	Setaria tundra NADH dehydrogenase subunit 4 and cytochrome oxidase subunit	601	601	95%	2e-169	83%
AJ544881.1	Thelazia gulosa mitochondrial partial COI gene for cytochrome oxidase subunit I	601	601	93%	2e-168	83%
AJ271617.1	Onchocerca gutturosa mitochondrial partial COI gene for cytochrome oxidase subunit	595	595	94%	9e-167	83%
DQ35884.1	Dirofilaria repens cytochrome oxidase subunit I gene, partial cds; mitochondrial	593	593	91%	3e-166	84%
AM749270.1	Onchocerca skrjabini mitochondrial partial coxl gene for cytochrome oxidase subunit	592	592	91%	1e-165	83%
AM749269.1	Onchocerca skrjabini mitochondrial partial coxl gene for cytochrome oxidase subunit	592	592	100%	1e-165	84%
EF394613.1	Spirocerca lupi isolate n.20 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	84%
EF394612.1	Spirocerca lupi isolate n.19 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394611.1	Spirocerca lupi isolate n.18 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%

Accession	Description	Max Score	Total Score	Query coverage	E-Value	Max ident.
EF394610.1	*Spirocerca lupi isolate n.17 cytochrome c oxidase subunit 1 (coxl) gene, partial c*	*590*	*590*	*100%*	*4e-165*	*82%*
EF394609.1	Spirocerca lupi isolate n.16 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394608.1	Spirocerca lupi isolate n.15 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394607.1	Spirocerca lupi isolate n.14 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394606.1	Spirocerca lupi isolate n.13 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394605.1	Spirocerca lupi isolate n.9 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394604.1	Spirocerca lupi isolate n.8 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394603.1	Spirocerca lupi isolate n.6 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%
EF394602.1	Spirocerca lupi isolate n.5 cytochrome c oxidase subunit 1 (coxl) gene, partial c	590	590	100%	4e-165	82%