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Assessment of characteristics and severity of giraffe skin disease in Tarangire, Manyara ecosystem

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ASSESSMENT OF CHARACTERISTICS AND SEVERITY OF GIRAFFE SKIN DISEASE IN TARANGIRE, MANYARA ECOSYSTEM

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the degree of Master's in Life Sciences at Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Giraffe Skin Disease (GSD) is a recently observed illness, mainly affecting adult and sub-adult giraffes, causing gray or crusty lesions on giraffe body. The general objective of this study was to assess and characterize GSD and its severity in Tarangire-Manyara Ecosystem (TME). The study used road transects to gather field information on GSD. Eighty-four giraffes were sighted by systematic random sampling in the six study sites. Examination of giraffes involved body distribution of lesions, severity of the lesions and whether they were associated with age and sex of the affected giraffes. Five giraffes with GSD were immobilized in Tarangire National Park and Burunge Wildlife Management Area for tissue collection and histopathological analysis and blood for hematological and biochemical analysis. Overall GSD prevalence was (69%,), affected animals typically had 1-5 lesions which were mostly moderate and were predominantly observed on the forelegs. GSD positivity rate was higher among females 54% versus males, whereas males had a higher rate of severe lesions and generally had more lesions than females. Calves showed no lesions. All tissue sections stained routinely with Hematoxylin and Eosin (H-E) and then to the special Grocott Methenamine Silver (GMS) staining showed the presence of large quantities of fungal elements (hyphae and spores). However, haematological parameters examined and biochemical profile analysis showed changes associated with the presence of fungus infection. Our findings suggest the involvement of fungal infection in GSD pathogenesis. We recommend further characterization of the lesions using modern molecular techniques and culture to identify primary and secondary or opportunistic etiologies, and the order in which the pathogens occur in the lesions.

Keywords: Giraffe skin disease, Immobilization, Prevalances, Transect survey.

DECLARATION

I, Faraja Elia Kiula, do here by to declare to the senate of Nelson Mandela African Institution of Science and Technology that, this is my dissertation, my own original work it has not ever been neither submitted nor being presently submitted for degree award in any other institution.

……. **…….**

Faraja Elia Kiula **Date**

The above declaration is comfirmed

……. **…….**

Prof. Linus K. Munishi (Supervisor 1) **Date**

…… **……**

Prof. Jaffu O. Chilongola (Supervisor 2) **Date**

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CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entintled Assessment of Characteristics and Severity of Giraffe Skin Diseases in Tarangire–Manyara Ecosystem as a fulfillment of requirements for Master's in Life Sciences at Nelson Mandela African Institution of Science and Technology (NM-AIST).

……. **…….**

Prof. Linus K. Munishi (Supervisor 1) **Date**

……. **…….**

Prof. Jaffu O. Chilongola (Supervisor 2) **Date**

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DEDICATION

This dissertation is dedicated to my lovely husband Mr. Alexander Mango'ha, our children, my mother Mrs. Hertha Kiula and young sister Vumilia for their patience gorgeous love, moral support and endless encouragement during study period.

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LIST OF ABBREVIATION AND SYMBOLS

IUCN	International union for Conservation of nature
GCA	Game Controlled Area
GPS	Geographical position system
GSD	Giraffe Skin Disease
KCMUCo	Kilimanjaro Christian Medical University College
KCRI	Kilimanjaro Clinical Research Institute
n	Total number of individuals
N	Number of individuals sampled
NP	National Park
TANAPA	Tanzania National Park
TAWA	Tanzania Wildlife Authority
TAWIRI	Tanzania Wildlife Research Institute
TME	Tarangire-Manyara Ecosystem
TNP	Tarangire National Park
WMA	Wildlife Management Area

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

The giraffe is a charismatic large herbivore (Park *et al*., 2008) endemic to many ecosystems in Tanzania and a popular mammal attracting tourists to watch in the wild (Bercovitch & Deacon, 2015; Muller, 2016) and thus contributing to the national economy. Tanzania is unique among the African countries as it has managed to maintain an extraordinary diversity of large wild herbivores including the giraffes. Being one of the important ecosystems in both local and international tourism, Tarangire-Manyara and its surroundings support a relatively large number of giraffes and their conservation is of paramount importance.

Decreasing populations become more vulnerable to stochastic factors, especially outbreaks of parasitic, viral and bacterial diseases (Kaitho *et al*., 2013). Habitat loss and illegal hunting negatively impact the giraffe species and consequently adversely affect economic revenues accrued from tourism (Nyamasyo & Kihima, 2014). Currently, giraffes are affected by an uncharacterized skin condition, which has been named as Giraffe Skin Disease (GSD) (Karimuribo *et al*., 2011). Giraffe skin disease is an illness of the skin characterized by proliferative crusty lesions. Visible lesions in affected giraffes appear with shells, furrowed skin, hard and dry or discharge blood (Lee *et al.*, 2016a; Bond *et al*., 2016). The lesions are on the shoulders, neck, carpal joints, brisket and the inner thigh (Karimuribo *et al*., 2011). A skin disease known as giraffe skin disease (GSD) was first described in Ruaha National Park in 2000, in which about 80-85% of the giraffe population with 92% of adults affected (Epaphras *et al.*, 2012). Field observations, coupled with surveillance studies revealed GSD to spread from the south to the northern protected areas of Tarangire-Manyara and Serengeti ecosystems population. Surveys conducted in Tarangire alone had about 79% of the giraffe population showing skin lesions (Lee & Bond, 2016).

The etiological agent of GSD has not yet been identified. However, different studies have proposed a range of possible aetiological agents ranging from ticks, nematodes, fungi or bacteria (Karimuribo *et al.*, 2011; Mpanduji & Karimuribo, 2011), although these speculations have not been confirmed. Unpublished data from a study conducted in Ruaha National Park, southern Tanzania suspected a spirurid nematode worm as a potential causative agent (Mpanduji *et al*., 2011) although this was not conclusive.

To date, the causative agent of GSD is unknown, since GSD could have significant effects on the survival of giraffe populations as some severely affected giraffe exhibit a stiffness or lameness of gait which would possibly predisposed them to predation and poaching (Epaphras *et al.*, 2012) as well as poor reproduction. In the absence of conclusive findings regarding the causative agent of GSD, control interventions are at suspense. There have been rare studies conducted on the pathogenesis of GSD, and information in the literature about the serum biochemical findings of infected giraffes is also missing.

The only previous study that attempted to identify the causative agent of GSD had inconclusively reported the involvement of a spirurid nematode as a potential etiology of GSD. Although bacterial and fungal elements were observed in the study, they were both considered as secondary invaders (Mpanduji *et al*., 2011). Nonetheless, none of these etiologies has however been confirmed as the primary cause of GSD. In the absence of conclusive results on the causative agent of GSD, we hypothesize that, in addition to what is known regarding the gross features of GSD, understanding the histopathological features of GSD will provide better insights into the identity of a potential etiology of GSD.

1.2 Statement of the problem

Declining number of giraffes in different ranges and their extinction in some regions of Africa, created worries in different ranges (Bercovitch *et al*., 2017). The drop-down report of giraffes is associated with a combination of factors, including disease like papillomavirus (Dyk *et al.*, 2011), anthrax, ear skin disease (Karimuribo *et al*., 2011), giraffe skin disease (GSD) (Epaphras *et al.*, 2012; Bond *et al.*, 2016; Muneza *et al.*, 2016) anthropogenic activities reported by Packer., (2015) and climate change (Hendry *et al.*, 2011). However, GSD prevalence in Ruaha National Park was 86% higher than anywhere else (Epaphras *et al.*, 2012), followed by Tarangire national Park with 79% (Lee & Bond, 2015). Despite the shocking records on GSD in different ranges little has been done and documented (Muneza *et al*., 2016). In East Africa, Tanzania is among the country which harbor huge number of mammals (Shorrocks, 2016; Connor *et al.*, 2019), including the Maasai giraffe (*Giraffa camelopardilis tippelskirchi*) in Tarangire-Manyara Ecosystem (Lee *et al.*, 2016). Despite, being the most promising environment for large mammals in Tanzania, Maasai giraffes still experience threats like diseases (Lee & Bond, 2015) land use changes and poaching (Nyamasyo & Kihima, 2014). Little is known on nature and extent of GSD, the distribution by locations, disease status and extent of the lesions in relation with age class, sex and part of

the body affected in TME. However, there are no studies that conducted histological analysis for GSD in TME and no any study reported on hematological analysis for GSD. This study focus on providing detailed information on histopathology, hematological and biochemical changes associated with GSD in TME.

1.3 Rationale of the study

This study provides necessary information on assessment and characterization of giraffe skin disease and its severity in Tarangire-Manyara Ecosystem. The study will also provide comprehensive information of GSD on stages of lesion, anatomical location of lesion, number of lesions per giraffe, severity by location in TME and between female and male, pathogen attacking giraffe skin, and changes observed to the GSD blood. This information will assist wildlife veterinarians and administrators to understand GSD and have new directions for temporary and long term mitigation strategies.

1.4 Research objectives

1.4.1 General objective

To assess and characterize giraffe skin disease and its severity in Tarangire-Manyara Ecosystem in Northern Tanzania

1.4.2 Specific objectives

(i) To assess nature and extent of giraffe skin disease in Tarangire-Manyara Ecosystem.

(ii) To conduct histological characterization of giraffe skin disease (GSD) to determine its degree of severity in Tarangire-Manyara Ecosystem.

(iii) To conduct hematological and biochemical profiles for (GSD) in Tarangire-Manyara Ecosystem

1.5 Research questions

(i) What is the nature and extent of GSD in Tarangire-Manyara Ecosystems?

(ii) What are the histological characteristics of giraffe skin disease in Tarangire-Manyara Ecosystem?

(iii) What are the hematological and biochemical changes associated with GSD?

1.6 Significance of the study

The findings of this study will provide necessary information on causes of GSD through analyzing tissue biopsies (histopathology) and examined blood from giraffe with skin disease. Furthermore, the study will expose the distribution of GSD by locations, disease status and grades of the lesions related with age class, sex and part of the body affected by GSD in Tarangire-Manyara ecosystems. This information will assist wildlife veterinarians and administrators to understand the nature and extent of t GSD. However, the research findings will enable managers in developing better management protocols toward controlling the disease in protected areas.

1.7 Delineation of the study

The general purpose of the study is to assess and characterize giraffe skin disease and its severity in Tarangire-Manyara Ecosystem. The parameters considered are size of the group, sign of skin condition, geographical location, location of the lesion on the body, age and sex of the affected individual. However, the study immobilize giraffe with skin disease for biopsy taking intended for histological analysis and blood taking for hematological analysis for GSD.

CHAPTER TWO

LITERATURE REVIEW

2.1 Different giraffe species and their distributions

Giraffes were widely distributed through the semi-arid savannah of Africa and South Eurasia between 15 and 1 million of years ago. Giraffe may possibly symbolize one of the earliest artiodactyls, thought derived 20 million of years ago (Kingdon, 1997). The name giraffe originates from ancient Greece, it was called camelopard (camel with leopard coat), which contributed to the giraffe scientific name *Giraffa camelopardilis*. Giraffe is found in the Kingdom Animalia, phylum Chordata, class Mammalia, Order Cetartiodactyla, family Giraffidae with two genera called Giraffa and Okapia (Kingdon, 1997; Shorrocks, 2016; Agaba *et al.*, 2017). Giraffe belong to Okapi, an ungulate mammal with an average height of 175 cm, the weight of about 250 kg and life expectancy of 15-20 years. The female Okapi is hornless with chocolate to dark brown body color while black color and white lines radiated to the thigh. Okapi males have the same color of the stripes but they are thin with purplish body color, and possess horns covered with skin like giraffe. Okapi youth have mane, that decreased or diminished at the adult stage (Jolly, 2003; Shorrocks, 2016; Agaba *et al.*, 2017).

Formally Okapi were found in North East Zaire, Bwamba and Uganda. Fossil evidence indicates that Okapi were widely spread but environmental stressors has led to extinction. Okapi preferred habitat of dense vegetation and on hills during wet season. Browse on undergrowth plant obtained in shades, home range for females is about 5 km², males are suspected to have a territorial wider range. The gestation period is about 14-15 months, calf is hidden by mother after birth and suckled when called. Okapi produce high-pitched sound when there is a danger or trouble. Males fight with their necks and knock each other when charging (Kingdon, 1997; Shorrocks, 2016).

A relative animal to Okapi is a giraffe in which adult giraffe has an average height of 5.5 meters tall, weighing between 500 kg and 1900 kg (Jolly, 2003). Identification of giraffe subspecies considers geographical range location, coat pattern and color. There are nine subspecies of giraffe that differ based on their localities; West Africa giraffe (*Giraffa camelopardilis peralta*) Namibia (*Giraffa camelopardilis angolensis*), South Africa, Botswana, Mozambique, Swaziland and Senegal (*Giraffa camelopardilis giraffa*), Senegal, Sudan (*Giraffa camelopardilis antiquorum*), Zambia (*Giraffa camelopardilis thornicrofti*),

Sudan, Ethiopia (*Giraffa camelopardilis camelopardilis*) Somali arid, Ethiopia and Kenya (*Giraffa camelopardilis reticulate*), Kenya, Uganda (*Giraffa camelopardilis rothschildi*), Zambia (*Giraffa camelopardilis thorncroft*) Rwanda, Kenya and Tanzania (*Giraffa camelopardilis tippelskirchi*) (Shorrocks, 2016; Muller, 2016; Bercovitch *et al*., 2017).

2.2 Population status of giraffe

Giraffes' life span is between 20 and 30 years, they are now contained in the conservation protected areas in most regions. Over 30 years now data indicate that giraffe population has dropped in their localities by 40% and reports from more than five countries of Africa shows that giraffes were in extinction (Muller, 2016). *Giraffa camelopardilis reticulate* was categorized as endanger species whereas, *Giraffa camelopardilis giraffa* (Nubian giraffe) and *Giraffa camelopardilis antiquorum* are critically endangered. further categorized *Giraffa camelopardilis peralta* and *Giraffa camelopardilis rothschildi* as vulnerable, *Giraffa camelopardilis thorncroft* near threaten, *Giraffa camelopardilis angolensis* categorized in least concerned while *Giraffa camelopardilis giraffa* were not assessed.

The Maasai giraffe (*Giraffa camelopardilis tippelskirchi*) are dominant in East Africa but mainly in Tanzania, they have big, sharp dark brown spots of vine-leaf structure, differently with other giraffe subspecies (Shorrocks, 2016). There was a significant drop of the Maasai giraffe by 50% from 66 000 to 31 000 between 1980 and 2015 respectively (Lee & Bolger, 2017). However, in Tanzania Maasai giraffe population is estimated to drop by a range of 37% to 43%, they are categorized from the list of lowest favored species (Bolger *et al.*, 2019).

2.3 Giraffe habitat

Giraffes are found in savanna woodland where acacia, Commiphora and Terminalia trees are plenty, in seasonal thicket on flood grasslands, nearby elephant forage, along the river and open areas (Bercovitch & Berry, 2015). Giraffes are savanna large mammal, stay in pairs, individually or in a group made up of different sexes and age classes (Jolly, 2003). Giraffe take most of the time foraging particularly early in the morning and evening time (Jolly, 2003). An adult giraffe has a long tongue of more than 40cm, which enable them to select and feed leaves from thorns and other vegetation. Giraffe Feeds more than 100 plants, acacia is the frequently preferred diet (Kingdon, 1997; Lee & Strauss, 2016; Deacon & Smit, 2017).

Female change habitat every time when pregnant or rearing calf depending on the availability of the resources needed and cover from predators. At birth the calf weigh about 50kg-70kg and try to browse within seven days of their life (Jolly, 2003). One-month old, calf use most of its time standing or lying down while mother foraging around 40 meter nearby calf (Jolly, 2003; Bercovitch & Berry, 2015). Maasai giraffes dwell in forests, shrub land and savanna. *The Giraffa camelopardilis peralta* found in West Africa and are exposed to semi-arid habitat whereas, subspecies *Giraffa camelopardilis camelopardilis* found in Northern East Africa resides in areas with trees.

The *Giraffa camelopardilis rothschildi* is found along the river, open areas, grassland open areas and woodland habitat commonly *Colophospermum mopane*, acacia, combretum, Terminalia, *Acacia drepanolobium, Acacia tortilis, Acacia hockii* and *Acacia nigrescens* in thicket area, (Anyango & Adhiambo, 2013; Bercovitch & Berry, 2015; Muller, 2019). *Giraffa camelopardilis giraffa* dwells in bush vegetation, grassland, open savanna, woodland species like *Acacia erioloba*, *Acacia melifera* and dense thicket (Deacon & Smit, 2017a). *Giraffa camelopardilis angolensis* inhabit in miombo ecosystems, mopane, vegetation found on riverine and on deciduous tree during wet season. *Giraffa camelopardilis giraffa* resides on savanna woodland and in riverine (Gandiwa & Munyaka, 2018) whereas, the Maasai giraffe (*Giraffa camelopardilis tippelskirchi*) resides on acacia, miombo woodland, palm woodland, Combretum Acacia bush vegetation, Commiphora, riverine vegetation and Vachellia acacia (Prins *et al.*, 2008; Bolger *et al*., 2019).

2.4 Giraffe home range

Home ranges for giraffe vary with the quality and accessibility of the needs, seasonal changes, sex category and predation (Fennessy, 2009). In most of the ranges giraffes are either few or absent and declined in number for more than three decades (Connor *et al.*, 2019). Giraffes have small home range during rainy season where forage is plenty and big home ranges during dry season, competing for leaves with other browsers. Seasonal variations encourage wildlife movements while home range size is determined by the presence of different preferred vegetation (Deacon & Smit, 2017). Giraffes have 5 km^2 - 80 km^2 home range (Jeugd & Prins, 2000) and yearly movement range to over 650 km^2. Female home range differ during oestrous, gestation and after giving birth on the first three weeks (Kingdon, 1997; Bolger *et al.*, 2017; Lee *et al.*, 2017). Female with calve forage in an open area to protect calf and evade enemies, while pregnant ones feed on dense thicket to acquire

quality and quantity nutrients. Giraffe feeding is not effective in low height reduced by competition of other browser members (Bercovitch *et al*., 2005).

The current data on giraffe subspecies ranges show changes throughout their ranges. Maasai giraffe are widely distributed in Tanzania both Northern and Southern Highland National Parks. Serengeti, Tarangire, Kilimanjaro, Manyara, Arusha, Ruaha, Mikumi, Katavi. In Kenya most of giraffe are located in Kajiado, Mara ranches, Laikipia National Park and Tana river district (Bolger *et al*., 2019).

2.5 Giraffe social behavior

Maasai giraffes are sexually dimorphic animals, they are always very worried animals (Marealle *et al*., 2010) they stay in detached groups, territorial less, and males are roaming much than females. They can move up to 5 kilometers per day, and normally interchange the sites frequently (Jeugd & Prins, 2000). Giraffe herd made up of more relatives created for long term association resulted to kinship (Bercovitch & Berry, 2013; Carter *et al*., 2013). The giraffe social system is pairwise for the female (Shorrocks, 2016). Male giraffes feed in thicket while females, calves together with youths browse on open environment where they can increase vigilance to escape predators. Their main natural enemies are African lions, leopards, poachers and diseases (Strauss & Packer, 2013).

Social units in giraffes are transitory, adult male giraffes are vestigial defensive, budget time for feeding and checking the reproductive status of females through sniffing and nuzzling the female's genitalia (Kingdon, 1997; Jolly, 2003; Lee & Strauss, 2016). Males mate with many females, oestrous female giraffes attract many males but mostly mate with giant bulls. Oestrous cycle lasts for two weeks. Females start reproducing at 4-5 years**,** produce five to ten offspring when they reach 30 years (Jolly, 2003). Male fertility peaks at the age of 6-14years and continue up to 24 years (Jolly, 2003). Once a male notes an oestrous female, tries to form a bond, and chases away competitors to monopolize mating. Gestation takes about 427-450 days and lactation lasts between six months and a year. Giraffe may lactate a calf while pregnant six months after giving birth (Kingdon, 1997; Jolly, 2003; Lee & Strauss, 2016). Giraffes like many other mammals have no specific season within a year for reproduction, giving birth and rearing calves on specific sight.

Giraffes will not prefer to rear calf in a site where death occurred previously. Calves double their body height within a year that enables them to sustain predation from lions and leopards

(Bercovitch *et al.,* 2005). They have two unique impressive gait, ambling by walk with the left limbs then the right limbs and the neck support the motion of the body. Gallop walk when front limb and hind limb are structured in sets. Giraffes may sleep for a short time while standing still or when lying down (Jolly, 2003). Both matured sexes develop three horns overhead (Janis, 2008). Necking happen to the calves and juvenile males, need for creating authority but not for mating access (Muller, 2016).

2.6 Giraffe threats

Giraffe (*Giraffa camelopardilis*) in various ranges showed shrinks in number (Fennessy, 2009). Giraffes are subjected to many threats that contributed to the population decline over most of the ranges. Ecological stressors such as fire and climate change which result to drought enlarge the vulnerability to the species (Epaphras *et al*., 2012). Female giraffe with pregnancy may be in high risk of predator attack few weeks before giving birth and after parturition as they isolate themselves from the herds to feed on condensed vegetation to meet nutritious forage (Strauss, 2014). In addition, only quarter of the calves survive up to one year due to predation from lion, crocodile, leopard and hyena (Lee *et al.*, 2017).

Poaching particularly adjacent to protected areas is seen as a significant primary cause for the falling of giraffe population outside protected areas, through the snares encountered on the park boarders (Packer, 2015). Habitat loss resulting from anthropogenic activities including agriculture around protected areas exert pressure to giraffes' survival. In addition, massive housing developments, mining, industries and building fences also block the seasonal movement through wildlife corridors (Lee *et al.,* 2017; Connor *et al.,* 2019). Furthermore, inbreeding causes high mortality rate for the calves with one month to one year of age (Jolly, 2003).

2.7 Disease challenges

Giraffes are believed to be more or less favorably affected by ectoparasite on and in skin influenced by season. Lice, ticks on manes and flies affect the giraffe on the skin surface while mites penetrate to the skin causing mange and scab lesion on shoulders, carpel joint and round hooves (Sachs & Debbie, 1969). Giraffe internal parasites include, Thelazia eye worms, tape worms in shoulder's muscles, nematodes and subcutaneous filarial that infest musculature and connective tissues. Setaria, round worms and liver flukes infest digestive

system likewise blood and blood vessel are also affected by parasites (Sachs & Debbie, 1969).

Previous studies indicate that giraffes are affected by outbreaks of rinderpest, papillomavirus disease and lumpy skin diseases and currently giraffe are affected by newly emerged disease that has been observed in different countries namely giraffe skin disease (GSD). Giraffe skin disease (GSD) is a disorder of undetermined etiology that causes lesions on giraffe's body. GSD is readily distinguishable from giraffe ear disease or lumpy skin disease (Karimuribo *et al*., 2011; Epaphras *et al.*, 2012; Lee & Bond, 2015). GSD mostly attack adult and sub-adult giraffes, causes gray lesions on limbs, neck, brisket and other parts of the body. Data from an unpublished study conducted in Ruaha National Park and northern Tanzania suspected a spirurid nematode worm as a putative aetiological agent (Mpanduji & Karimuribo, 2011).

The survey conducted in Northern National Parks of Tanzania indicated that Maasai giraffes were assessed and found with the signs of GSD (Lee *et al.*, 2016). The study was restricted to adult giraffe only and did not assess the GSD prevalence within the sites of Tarangire Manyara ecosystem. The study conducted in Tarangire Manyara ecosystem and other Northern protected areas employed capture recapture method, assess the sign of GSD to adult giraffe only on limbs (Bond *et al*., 2016a). While the survey conducted in Ruaha National Park shows that GSD lesions can be observed in different parts of giraffe's body (Epaphras *et al*., 2012). The disease is prevailing in different ranges of Tanzania (Muneza *et al*., 2017) although the crude estimates of GSD in different National Park, prevalence, the severity of GSD by location and its variation overtime are not known.

The samples of GSD collected from different National parks and zoos for histopathological analysis shows unlike results. In Paignton zoo samples collected for pathology analysis show only the growth of *Aggregatibacter aphrophilus.* While in B. Bryan Preserve, USA the results did not indicated any presence of bacteria, fungal or worms (Muneza *et al*., 2016b). The histopathology results from 12 giraffes of Ruaha National Park showed spirurid nematode worms and test on fungal isolates shown the presence of fungal spores (Mpanduji & Karimuribo, 2011). The sample's results of seven free ranging giraffe from Murchison Falls National Park-Uganda, reported that the GSD lesions had microfilaria, nematodes and bacteria (Cortes-rodriguez *et al.,* 2020). According to the study done on parasitic infestation of the wild animals, the scab lesion observed to the giraffe and buffalo are suspected to be caused by fungus and roundworms very common on carpel joints, face, neck and shoulders

(Sachs & Debbie, 1969).Therefore, pathology results from both studies conducted previously differ, Ruaha NP, Murchison Falls National Park and Paignton zoo showed presence of bacteria which are not similar. However, even the worms observed in those National parks are not alike.

In the absence of conclusive findings regarding the causative agent of GSD, control interventions are at suspense. Since there have been rare studies conducted on the pathogenesis of GSD, information in the literature about hematological and serum biochemical findings of giraffes with GSD is also missing. Giraffe skin disease still needs more comparable histopathology studies on different ecosystems so as to understand the etiology and rescue these important species which are endangered. Haematological and biochemical serum profile are very vital assessments in determining possible effects associated with diseases for domesticated and wild animals (Jenni-eiermann *et al*., 2006). Haematological parameters analyze the count for red blood cells, white blood cells, platelets and the volume percentage of red blood cell in blood (Hematocrit) while biochemical serum profile shows the level of protein, albumin and creatinine.

However, inflammation response may comprise a wide variety of change to acute, chronic and granulomatous inflammation (Wilson & Procop, 2001). Inflammation may suggest a certain type of fungi, parasites, bacteria, or virus. Histopathological examination needs the use of staining techniques to identify related pathogens which assist to identify the disease and treatment. Hematoxylin-eosine stain is used in histopathological examination of tissue sections to detect pathogens like virus that produce cytopathic effect, parasites like worms, arthropods and protozoa (Wilson & Procop, 2001). On the other hand, there are organisms like fungus that can only be detected in special staining such as Grocott-Gomori methenamine silver staining (Grocott, 1955). The successful morphological description of biopsy significantly contributes to diagnosis of infectious disease (Elarabany, 2018).

Therefore, the key objective of this study is to assess the nature and extent of GSD, specifically, to know how geographical locations, disease status and grades of the lesions relate with age class, sex and part of the body affected by GSD in Tarangire-Manyara ecosystems. To conduct histological characterization of giraffe skin disease to determine its degree of severity of the disease through observation of the whole cellular makeup of the specimen. And to conduct haematological and biochemical indices of giraffes with skin disease to investigate the changes in serum biochemical values of giraffes with GSD.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out in Tarangire-Manyara Ecosystem (TME) which is found within Maasai steppe, Northern Tanzania. Six sites were established, comprised of Tarangire National Park (TNP) which covers 2800 sq.km, Lake Manyara National Park that covers 330 sq.km and protected areas such as Burunge Wildlife Management Area (WMA), Lolksale Game Controlled Area (GCA), Nou Forest Reserve and Simanjiro Game Reserve (SGR) (Fig. 1). Tarangire-Manyara ecosystem is essential and vital ecosystem as it harbors large wild herbivores of different species like giraffes, African elephants (*Loxodonta africana*), buffaloes (*Syncerus caffer*), oryx, (*Oryx beisa*), zebra (*Equus burchelli*),wildebeest (*Connochaetes taurinus*) as well as carnivore species including lions (*Panthera leo*), leopards (*Panthera pardus*) and good number of birds such as ostriches, flamingoes and flora of different species that make the ecosystem be endowed with suitable habitats for diversity of fauna species.

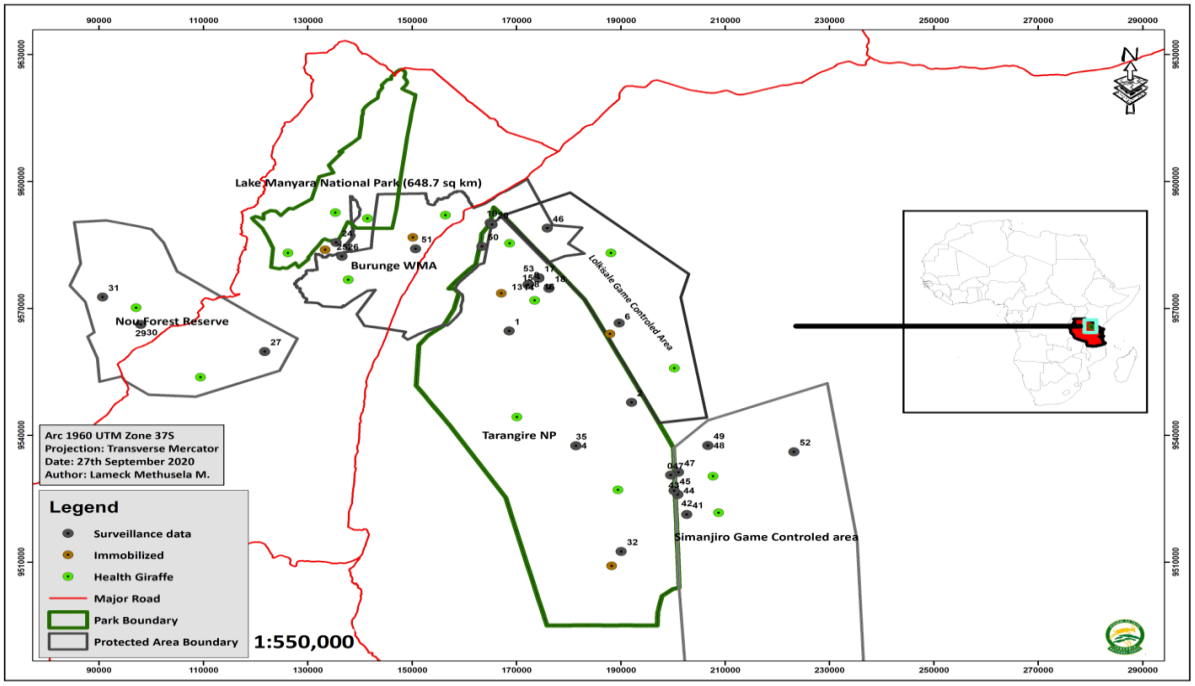

Figure 1: Map of Tarangire-Manyara ecosystem showing study transects of giraffes

3.2 Field Assessment of Giraffe Skin Disease

Giraffe observation survey was carried out from February to April 2019 by using road transects. The transects were laid down based on road network. During field assessment of GSD, eighty-four giraffes were clearly observed out of 204 giraffes sighted in 16 groups from six sites established of TME (Fig. 1). Two researchers were equipped with binoculars and hand held global positioning system (GPS) units, sitting on the middle sit of the car observing each side of the transect. The car was driven on maximum speed of 20 km/h and any individual or group of giraffes sighted the car stopped for observation of each individual for the skin lesion sign. Observation of giraffes was done during the morning and evening time or when the weather was cool. In order to minimize potential bias of double sighting of giraffes, a systematic random selection of study animals was adopted. On each day, out of the first 10 giraffes encountered, every 5^{th} giraffe was randomly selected for examination. In cases the 5^{th} giraffe was not clearly visible, the 6^{th} giraffe was examined. Binoculars were used for the animals sighted at a distant.

Parameters taken into consideration during observation included size of the group, sign of skin condition, location of the lesion on the body, age and sex of the affected individual. By using handheld GPS, the location of individual encountered were also recorded. The observation lasted within 15 minutes and when a giraffe disappeared before the first 15 minutes of observation was achieved, the stop watch was stopped and that was the end of observation for that particular giraffe.

3.3 Definition of GSD lesion severity and age categories

Giraffe skin disease lesions were categorized into four categories based on severity. These categories were grouped as: (a) asymptomatic (b) mild lesion (initial stages of nodules of < 5 cm diameter, (c) moderate lesion (between 5 and 10 cm of diameter, and (d) severe calculi lesions with a diameter ≥11 cm (Epaphras *et al.*, 2012). Age classes were categorized as calves (<12 months), sub adult (12 months to < 4 years), or adult (4+ years). Calves which stay with their mother's show folded or wrinkled skins, large eyes and ears relative to the face. Sub adults have smoother skins with small ossicones having black hair at their tips. While the tall adult giraffes have tight skin on the face and jaw areas, darkening coat color with mane waving.

3.4 Giraffe immobilization procedures

Cross sectional survey was carried out from 23rd - 30th August 2019 through the accessibility of park roads network, which were used as transects. Five giraffes were immobilized in Tarangire National Park (TNP) and Burunge Wildlife Management Area (WMA) in Tarangire-Manyara Ecosystem. Immobilization was done in the morning at 7:30-11:00 am before they drunk water, in scarce vegetation and appropriate terrain (Fig. 3, 4 and 6), excluding all pregnant giraffes which were many after wet season. Three animals were selected for immobilization from Tarangire National Park (TNP) at S3º 50' E 36º 0'. Two males, one with moderate scar lesion on hind limb and another one with severe lesion on fore limb and one female with mild lesion on fore limb. Also in Burunge Wildlife Management Area (WMA) giraffes were studied and selected for immobilization two males were selected, one with severe lesion on brisket and another with moderate scar lesion on fore limb at S 34º 90' 7.25" E35º 54' 17.28. They were immobilized at a distance of about 30 m to 40 m, Etorphine hydrochloride (M99) (Wildlife Pharmaceuticals, SA) was used at total dose of 20-25 mg per animal using a dart gun (Cap-chur®, Palmer Company, USA).

Figure 2: Dart gun used to administer the immobilizing drug remotely

3.5 Monitoring of immobilization

Once darted, animals were followed cautiously until they showed typical signs of immobilization characterized by trotting, swaying movement, star gazing posture (Hackney gait), and leaning on bushes and small trees and finally roped down. Sometime, if the animal is within the herd, the entire herd run and leave alone the darted animal undergoing the induction process. Immobilization was terminated by intravenous injection of a reversing agent (antidote) Diprinophine (M5050) (Wildlife Pharmaceuticals, SA) at 72 mg/kg. To boost the cardio-pulmonary performances, inotropes and respiratory stimulants were given at the time of recovery.

Figure 3: Adult male GSD at Burunge Wildlife Management Area immobilized

3.6 Histopathological processing of skin biopsies

One skin biopsy was taken from GSD lesions of each of the five immobilized giraffes by using the punch method. Briefly, the most affected area or abnormal-appearing sites or the edge of an actively growing lesion of GSD were selected for biopsy. The area to be biopsied was cleansed with povidone-iodine solution (Fig. 4). The punch biopsy instrument with a

diameter of 2 mm was held perpendicular to the surface of the lesion. The instrument was pressed down into the lesion while it was being rotated clockwise and counter-clockwise, cutting down into the subcutaneous fat. The punch biopsy instrument was removed and the biopsy specimen gently lifted with a needle to avoid crush artifact. Scissors were used to cut the specimen free at a level below the dermis. Since the punch biopsy defects were small, no suturing was done post biopsy.

Biopsies were immersed into 10% neutral buffered formalin to prevent decomposition and stored in biohazard bags filled with dry ice at –20°C until transported to the laboratory for histopathological analysis. By using a microtome (Fig. 5), tissues were sectioned in thin sections of 3-5 µm thick, then placed in microscopic glass slides ready for staining. Frozen biopsies stained by Hematoxylin and Eosin (HE) as described previously by Feldman and Wolfe (2014) while Grocott Methenamine Silver (GMS) (Agilent, Santa Clara, CA, USA) staining was performed according to Ma and colleagues (Ma *et al.*, 2013). The tissue sections were oxidized in 0.5% periodic acid solution for 15 min at room temperature, rinsed three times in distilled water, and incubated in methenamine silver working solution for 30 min to 1 h at 60 °C.

The processes taken for biopsies are shown in (Table 1). Sections of the tissue were rinsed in hot distilled water, checked microscopically, and then rinsed in distilled water at room temperature and toned in gold chloride solution for 1 min, rinsed in distilled water, treated with sodium thiosulfate solution for 2 min, and then washed in running tap water for 10 min. The sections were counterstained in nuclear light green for 5 min, and then subjected to dehydration, clearing in xylene, and mounting with a coverslip.

Table 1: Protocol for tissue processing of giraffe skin specimens

Process	Solution	Time
Dehydration	70% alcohol	60 minutes
Dehydration	90%alcohol	45 minutes
Dehydration	Absolute alcohol	45 minutes
Dehydration	Absolute alcohol	45 minutes
Dehydration	Absolute alcohol	60 minutes
Clearing	Xylene	60 minutes
Clearing	Xylene	60 minutes
Clearing	Xylene	60 minutes
Infiltration	Paraffin Wax	30 minutes
Infiltration	Paraffin Wax	60 minutes
Infiltration	Paraffin Wax	90 minutes
Blocking Out	Paraffin Wax	n/a

Figure 4: Female giraffe (GSD) of Tarangire National Park immobilized for sampling

3.7 Histopathological examination of giraffe skin specimens

Tissue specimens were submitted to the Pathology Department of Kilimanjaro Christian Medical Center for processing and examination. The specimens were observed, examined, dissected and recorded the gross dimensions, described lesions and placed in the designated areas including putting the specimens into tissue cassettes. Then the processing of the specimen was done by an automated machine (SLEE MTP Tissue Processor, Germany).

Figure 5: Microtome machine used for slicing tissue biopsies

3.8 Collection of blood samples and processing for haematology

Blood samples were collected from five immobilized giraffes. Venous blood was aspirated from the jugular vein (Fig. 6) with 20-gauge needle into commercial vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ). Blood was collected into K3 EDTA tubes with a concentration of 1.27 mg A/K3 per ml of blood for hematologic evaluation. Mixing was done gently by carefully inverting the tube several times. Samples were then stored in a cool box at-4°C and immediately transported to the laboratory for analyses. The Cell-Dyn 3500 haematology system (Abbott Laboratories, Abbot Park, IL, USA) was used to determine the red blood cell (RBC) count, total White blood cells count, and the volume percentage of red blood cell in blood (Hematocrit). Blood for the biochemical evaluation was collected into red-top serum tubes that did not comprise anticoagulant. The blood was allowed to clot for 30–60 min at room temperature and then centrifuged at 3000 rpm for 10–15 min. Serum was

removed from the clot and administered with a Cobas Mira chemistry analyzer (Roche, CA, USA) to understand the blood serum chemistry.

Figure 6: Blood sample of GSD collected from jugular vein

3.9 Data processing and analysis

Field and laboratory data was analyzed using Statistical Product and Service Solutions (IBM SPSS Armonk, NY, and USA) software version 22. Descriptive data of categorical variables from six sites established in Tarangire Manyara ecosystem were presented in the form of numbers and percentages organized into tables. The relationship between gender, disease status and part of the body affected were considered. Chi square test (χ^2) was used to determine associations between variables using a P-value of 0.05 as the statistical cut-off point.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Nature and extent of giraffe skin disease in Tarangire-Manyara Ecosystem

A total of 84 giraffes in 16 groups were sighted and observed in 6 different locations over a span of 3 months (February to April, 2019). The overall GSD prevalence in Tarangire-Manyara ecosystems was 69% (58/84) (Table 2), with symptomatic animals almost entirely adults plus one sub-adult, and no calves showing lesions (Table 3). Prevalence among adults was 79%. Affected animals 55.2% (32/58) typically had 1 to 5 lesions on the body (Table 4), had mostly moderate lesions, and lesions were mostly observed on the forelegs (Table 1). GSD positivity rate was higher among females 59% (34/84) versus males (Table 3), but males had a higher rate of severe lesions and generally had more lesions than females (Table 4).

Table 2: Descriptive statistics for prevalence and distribution of Giraffe Skin Disease in studied giraffes

Variable (n)		Number	Percent
Positivity for GSD (n=84)	Negative	26	31.0
	Positive	58	69.0
Sex (n=84)	Female	44	52.4
	Male	40	47.6
Lesion Severity (n=58)	Mild	21	36.2
	Moderate	25	43.1
	Severe	12	20.7
Partly affected (n=58)	Front Leg	42	72.4
	Brisket	3	5.2
	Hind Leg	13	22.4
Number of Lesions (n=58)	1 lesion	16	27.6
	1 to 5 lesions	32	55.2
	> 5 lesions	10	17.2

Table 3: Association between Sex and Age of giraffes with GSD positivity (n=84)

Variable	Category	GSD positivity		Total (n=84)	χ^2, p value
		[a]Affected; n (%)	[b]Non-affected ; n (%)		
Sex	Female	34 (59%)	10 (39%)	44 (52%)	χ^2 =2.93,
	Male	24 (41%)	16 (61%)	40 (48%)	p=0.08
	Total	58 (100%)	26 (100%)	84 (100%)	
Age Group	Calves	0 (0%)	4 (15%)	4 (5%)	χ^2 = 24.342,
	Sub adults	1 (2%)	7 (27%)	8 (9%)	p=0.000
	Adults	57 (98%)	15 (58%)	72 (86%)	
	Total	58 (100%)	26 (100%)	84 (100%)	

(Legend): [a]Giraffes with GSD (n=58); [b] Giraffes without GSD (n=26); Positive; Positivity for GSD was strongly associated with adult giraffes (χ^2 = 24.342, p=0.000).

Table 4: Distribution of GSD lesions by severity, affected parts and number of lesions across sex and age groups of giraffes

Lesion severity		Category	Mild	Moderate	Severe	Totals
	Age group	Sub-adult	1 (100%)	0 (0%)	0 (0%)	1
		Adults	20 (35%)	25 (44%)	12 (21%)	57
		Total	21 (36%)	25 (43%)	12 (21%)	58
	Sex	Female	11 (32%)	17 (50%)	6 (18%)	34
		Male	10 (42%)	8 (33%)	6 (25%)	24
		Total	21 (36%)	25 (43%)	12 (21%)	58
Affected Parts		**Category**	**Front Leg**	**Brisket**	**Hind Leg**	**Total**
	Age group	Sub Adults	1 (100%)	0 (0%)	0 (0%)	1
		Adults	41 (72%)	3 (5%)	13 (23%)	57
		Total	42 (72%)	3 (5%)	13 (23%)	58
	Sex	Female	27 (79%)	0 (0%)	7 (21%)	34
		Male	15 (63%)	3 (13%)	6 (24%)	24
		Total	42 (72%)	3 (5%)	13 (23%)	58
No. of Lesions		**Category**	**1 Lesion**	**1 - 5 Lesions**	**> 5 Lesions**	**Total**
	Age group	Sub-adult	1 (100%)	0 (0%)	0 (0%)	1
		Adults	15 (26%)	32 (56%)	10 (18%)	57
		Total	16 (28%)	32 (55%)	10 (17%)	58
	Sex	Female	9 (26%)	20 (59%)	5 (15%)	34
		Male	7 (29%)	12 (50%)	5 (21%)	24
		Total	16 (28%)	32 (55%)	10 (17%)	58

In study sites, GSD prevalence was 31% in Tarangire National Park 14.2% in Burunge Wildlife Management Area and other sites of Lolksale Game Controlled Area, Lake Manyara National Park, Nou Forest Reserve and Simanjiro Game Reserve had 23.8%. The only subadult with GSD was found to have a single lesion in the forelimb (Tables 2, 3 and 4). Generally, the gross lesions observed included scabs, wrinkled skin, encrustations while dried or fresh oozing blood was observed on some GSD lesions. However the severity of the lesion is shown in Fig. 7, as: (a)mild lesion on fore limb, (b) moderate lesion with lumpy appearance and sores, (c) moderate lesion on brisket characterized by inflammation (d) severe lesion, severe wrinkling and inflammation**.** Other lesions noticed include skin flaps, swollen carpal joint, pendulous skin and cracking of the skin with exudates due to presumed secondary infection.

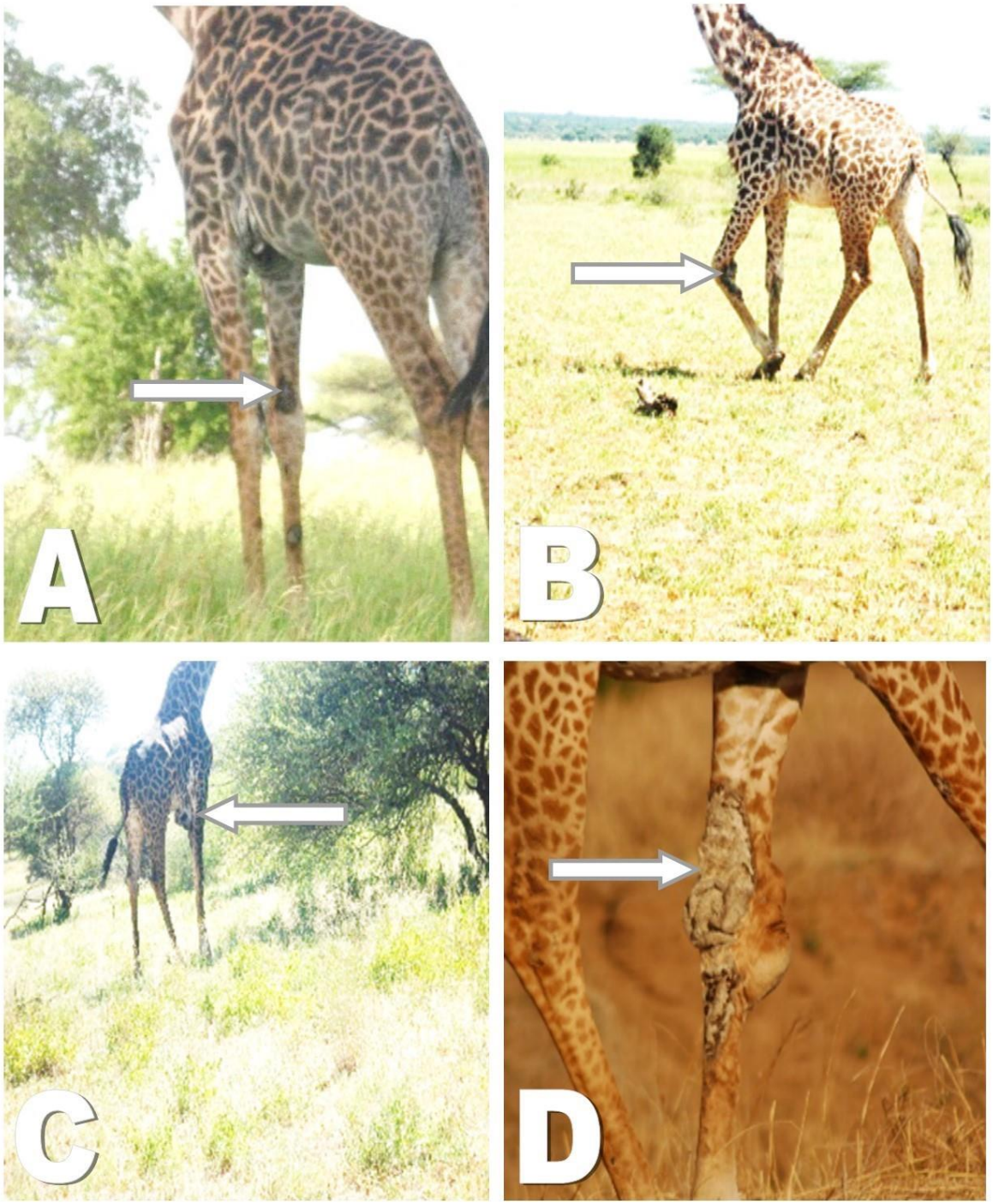

Figure 7: Characterization of GSD lesions based on degree of severity.

4.1.2 Histopathological analysis of GSD tissues biopsies

Biopsies from giraffes with severe GSD lesions were subjected to histopathological staining. All tissue sections from five affected giraffes showed the presence of large quantities of fungal elements (hyphae and spores) that involved hair shafts and sub-cutaneous tissue as revealed by photo microscopy (Fig. 8 and 9). Grocott's methenamine silver stain (Fig. 10) revealed numerous round spores with thick double walls, occurring singly or in chains

connected by tubular projections. Fungi were seen as prominent black filaments of varying length with two parallel borders. Septae and branching fungal filaments were clearly identified in all 5 tissue sections. GMS special stain is also used for staining some bacteria such as *Nocardia spp.*, *Mycobacterium spp.*, and non-filamentous bacteria with polysaccharide capsules, such as *Klebsiella pneumoniae* and *Streptococcus pneumoniae*. Our histopathological results have revealed none of these bacterial species. The GMS technique is not used to detect nematodes. We were therefore unable to identify any types of previously reported nematodes by this technique.

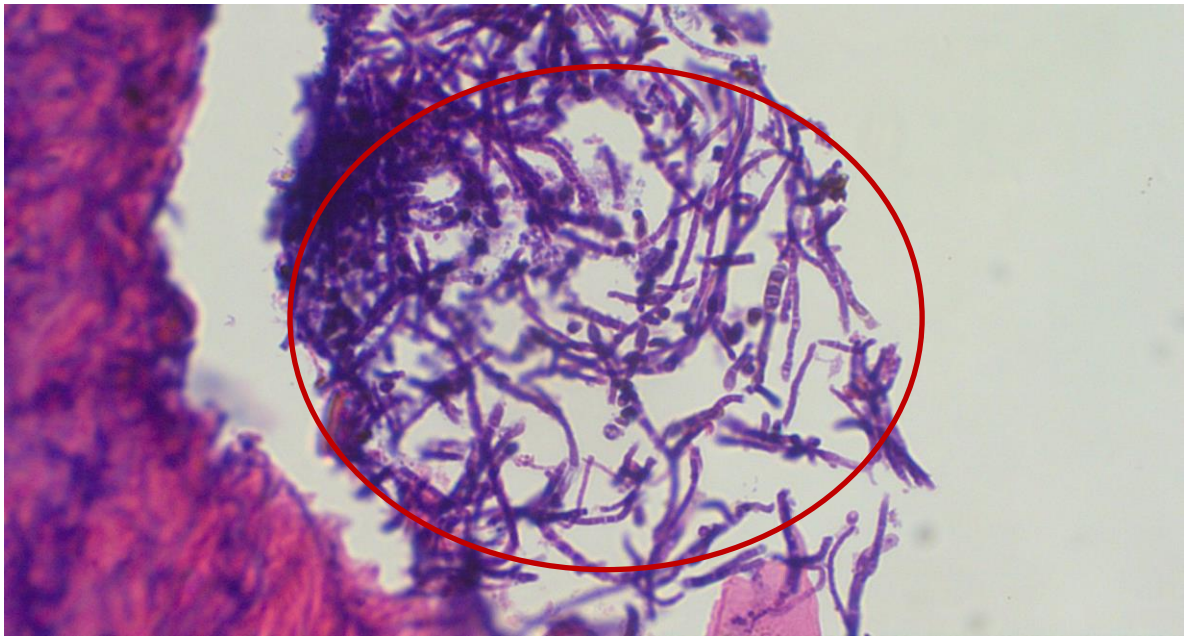

Figure 8: Giraffe skin disease section by HE staining showing a complex of fungal hyphae (circle). x100magnification

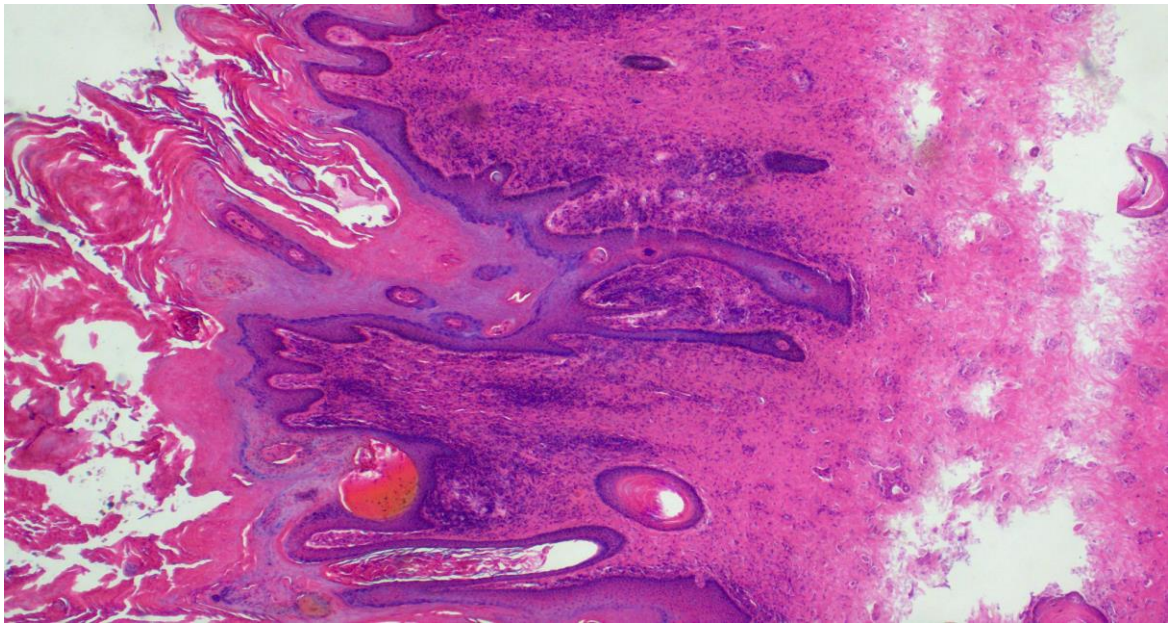

Figure 9: Giraffe skin section stained with HE showing chronic inflammation and extravasation of red blood cells (x40-magnification)

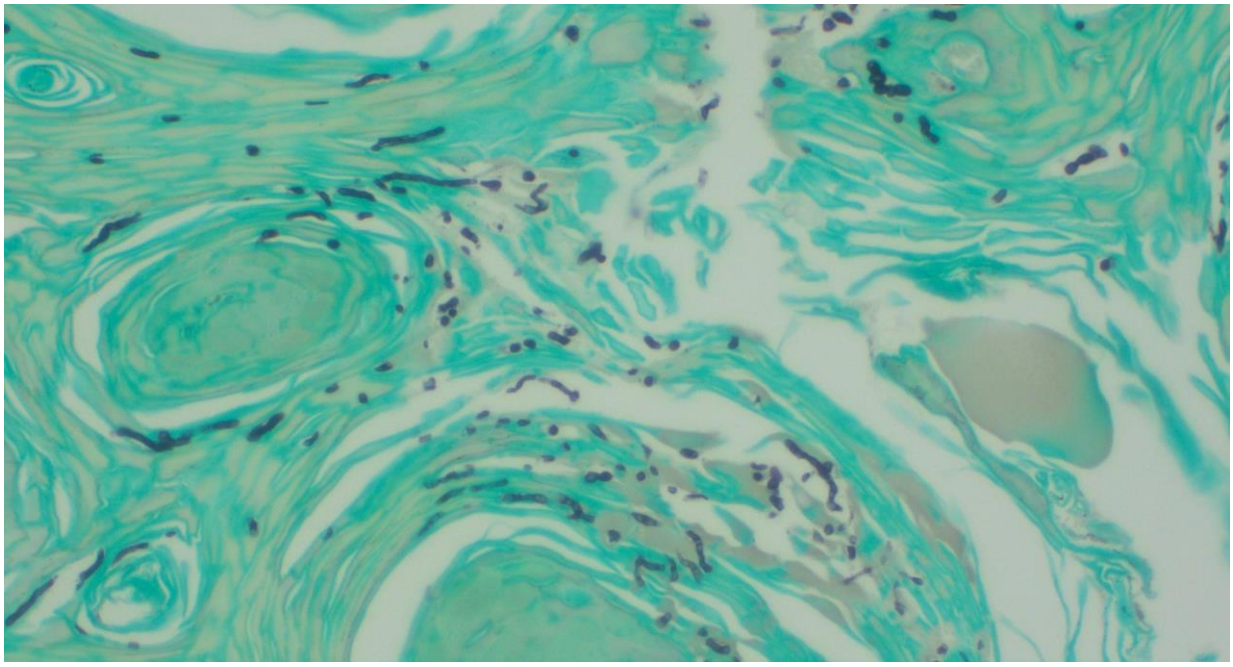

Figure 10: Photomicroscopy of the giraffe showing fungal spores and hyphae (arrows) which are positive for Grocott Methenamine-Silver (GMS) special stain (x20 magnification)

4.1.3 Hematological and biochemical profiles for (GSD)

The blood samples of five adult GSD collected from two sites of TME, were analyzed for different haematological parameters (Table 5), in which showed rise in lymphocytes, eosinophil and neutrophil. Likewise, serum biochemical profile exposed rise in creatinine and slight rise in albumin.

Table 5: Haematological Indices of giraffes with Giraffe Skin Disease (GSD) compared to normal values, in Tarangire-Manyara Ecosystems

	Normal Reference range		**GSD**		**Mean Percent Change #**
Index	Mean	SD	Mean	SD	
Hemoglobin	11.3	1.8	11.81	1.84	4.3
Red blood cell	10.02	2.48	11.78	2.2	14.9
Hematocrit	31.5	6	36.54	5.82	13.8
Lymphocyte**	0.719	1.488	3.338	1.376	78.5
Eosinophil**	0.368	0.381	6.554	0.435	94.4
Corpuscular volume	31.9	9.3	30.34	3.66	-5.1
Mean corpuscular V	9.4	3	10.13	1.03	7.2
Monocyte	0.395	0.371	0.321	0.241	-23.1
Neutrophil**	6.807	4.434	34.759	2.915	80.4
Basophil	0.253	0.239	0.236	0.129	-7.2
Mean corpuscular volume conc.	32.5	3.5	34.29	2.5	5.2
White blood cell	10.34	4.997	11.99	3.4	13.8

4.2 Discussion

Across Africa where giraffes are endemic, GSD varies in severity and occurrences depending on subspicies (Muller *et al.,* 2016). Most studies revealed that giraffes in different ranges including Tanzania are declined in number, among the factors that contributed to the decrease, is giraffe skin diseases (Muneza *et al.*, 2016). The disease was first observed in Ruaha National Park, Southern Tanzania (Epaphras *et al.*, 2012) fifteen years later, GSD was also observed in Northern National Parks and other protected areas (Lee *et al.*, 2015). This study confirms that the six sites established for survey in Tarangire-Manyara ecosystem

(TME) were affected by GSD. This study assessed the nature and extent of GSD, performed histopathological and haematological characterization GSD and its severity.

Findings from this study showed that the overall GSD prevalence in Tarangire-Manyara ecosystem was 69% (58/84) smaller than the previous study reported (Lee & Bond, 2016; Lee *et al*., 2016). This happened due to the fact that the study was done once, in one season with a small sample size encountered opportunistically through the available park road network. The severity, distribution and number of the lesions found in the TME vary by locations, disease status and extent of the lesions related to age class, sex and part of the body affected by GSD. More than three-quarters of GSD severity lies between mild to moderate and 21% giraffes had severe lesions. Elsewhere in Tanzania, where Maasai giraffes are found like Ruaha National Park the prevalence of GSD was reported to be 80% (87/109), (Epaphras *et al*., 2012). Such variations were also reported in other countries such as Uganda where GSD was reported to be 19%.

Generally in Africa variations ranged from 2% to 80% (Muneza *et al.*, 2016a). The wider range observed may be attributed to differences in subspecies, sample size of the study populations, locations, and seasonal variations (Deacon & Smit, 2017; Epaphras *et al.*, 2012). This observation was contrary to what was previously reported in Ruaha national park, more than half of the giraffes studied were severely affected.This study also confirms that Tarangire National Park is highly affected by GSD than other sites, as reported by Lee and Bond (2016). During our survey, we were able to study a number of giraffes encountered in TNP than in any sites because most of the giraffes found in TNP were not much worried as compared to other sites nearby communities in which giraffes were observed to be more worried and running as they experience hunted outsides the protected areas (Marealle *et al*., 2010). Though there is high GSD prevalences in Tanzania still there is no evidence of mortalities that has been directly linked to GSD (Epaphras *et al*., 2012; Lee & Bond, 2016; Lee *et al*., 2016).

This survey exposed that, the majority of the observed giraffes with skin lesions were adults, and few subadults and none for the calves. Females were sighted more frequently than males this is because the study done during off-breading season in which most of the adult males have dispersed. This study also found that most of the giraffes with skin disease were adults, (Lee & Bond, 2016; Epaphras *et al*., 2012), the same findings were also reported in Uganda, Kenya and other countries (Lee & Bond, 2015a; Muneza *et al*., 2016b; Muneza, 2016). For

the disease to be common in adult giraffes, may be caused by necking behavior when males create authority or courtship which is common to adults and less to the juveniles. In this study female giraffes are the most affected animals than males, however, males are the only giraffes affected on brisket. Probably lession on the brisket is influenced by the behavior of males mating many oestrous female giraffes, in which some may be affected by skin disease (Jolly, 2003).

During this survey, it was revealed that most of the giraffes with skin lesions were affected on the fore limbs, and hind limbs and few giraffes had lesions on brisket areas, contrary to the survey conducted in Northern Tanzania indicated that the lesion was on forelimbs only (Lee & Bond, 2016). This may be probably the disease is new to TME after fifteen years since it has been reported from Ruaha. It seems that as time goes the lesion will appear in the same pattern as that of Ruaha National Park on forelimbs, hind limbs, hindquarters, vulva area, coffin and brisket area (Mpanduji & Karimuribo, 2011; Epaphras *et al*., 2012). However, studies conducted in other countries within and outside Africa indicated that giraffes are mostly affected by limbs, upper body, entire body, head, testicles and the inner thigh (Muneza *et al.*, 2016). Yet, all these studies showed that GSD lesions are similar, having a defined pattern of infection on the giraffe's body (Muneza *et al*., 2016a).

From this study histopathological of GSD lesions were collected from five immobilized giraffes of Tarangire National Park and Burunge Wildlife Management Area. These two sites had a big number of giraffes studied and together they had 45.2% of GSD prevalence. The disease was categorized as non-affected, mild, moderate and severe lesion. Mild lesions starts from 1-3 cm coalescing to alopecic nodules and then widen in diameter. The percentages of giraffes with mild lesions in all sites of TME was 21% , perhaps because, it is the initial stage of GSD development, which need careful observation of the nodules raised with hairs to identify the abnormal skin condition. The moderate lesion size ranging from 5-10 cm, appears to be hardening, drying, and scaling of the skin with a gray appearance. The lesion appears raised, possibly with blackened areas and/or open sores on the edges of the lesions. The number of giraffes with moderate lesions in TME is higher perhaps because the lesions are wide they can be observed by naked eyes even at a distance.

However, during this study giraffes encountered with severe lesions are about 12% found in Nou forest reserve, which the lesion appears to be widen, with raw fissures and central collapse of the lesion, wrinkling and cracking. The GSD severe lesion was associated with

abnormalities such as swelling and stiffness in gait but other individuals with severe lesions had no noticeable lameness. In this study, giraffes with severe lesions were few probably because they were found in the adjacent protected area nearby the communities, hence easily hunted by poachers for the black market (Packer, 2015) as they experienced lameness and stood in one place for a long time as reported in Ruaha National Park (Epaphras *et al*., 2012).

This study attempted to characterize biopsy sections from GSD lesions using histopathological approaches. Giraffe skin disease was thought to be caused by multiple pathogens. It has been reported that the disease is probably caused by nematodes or fungi whereas, bacteria do contribute to the secondary infection in affected individuals (Bond *et al.*, 2016; Epaphras *et al.*, 2012). Giraffe skin disease is thought to be caused by multiple pathogens. Of the few studies done that characterized lesions from GSD in Ruaha National Park had implicated a nematode as the causative agent of GSD (Mpanduji & Karimuribo, 2011). Analysis of biological samples from seven affected giraffes collected from a skin disease with clinical manifestations similar to GSD from Uganda revealed a parasitic worm that was likely to originate from the genus *Stephanofilaria* (Cortes-rodriguez *et al*., 2020), transmitted among domestic cattle through biting flies. Other studies have previously reported a set of bacteria species as the likely primary cause of GSD, making it challenging to draw solid conclusions from these reports.

Although, our study attempted to investigated the etiology of GSD through the histopathological outlook. The characterized GSD biopsies from two sites of TME, subjected to Hematoxylin and Eosine staining (H-E) and Grocott's methenamine silver (GMS) staining technique found with consistent fungal infection. The studied samples tell us that fungal infection must be considered as one of the etiological agents for GSD in TME. Therefore, fungal infestation should be considered as an important etiological agent of GSD as also reported by Ruhnke1 *et al* (2012).

Efforts to control GSD will not be successful if the primary and secondary etiological agents and factors that enhance its severity and distribution still remain unknown. On the other hand, learning the pathology and epidemiology in free-ranging giraffes can be challenging in evaluating and finding both biotic and abiotic factors related to the GSD infection (Muneza *et al*., 2016) as giraffes used fission-fusion social system with home-based range varying up to 5 km^2 (Jeugd & Prins, 2000).

This study also conducted hematological and biochemical examination from five GSD in two sites of Tarangire-Manyara Ecosystem, Tarangire National Park and Burunge Wildlife Management Area to examine the changes associated with GSD. Hematological pictures are useful for describing blood parameters, an indicator of the health status of an animal while serum chemistry indices help in clinical assessment (Jenni-eiermann *et al*., 2006; Elarabany, 2018). This study points out the rising percentage of lymphocytes, eosinophils and neutrophils. Lymphocytes are bone marrow-derived cell (surface receptors), that enable the adaption of immune responses and create a memory section for future responses. A high level of lymphocytes may indicate fear or stress response that may occur with any chronic illness (Wilson & Procop, 2001; Poljicak *et al.*, 2009).

Therefore, probably the chronic lesion observed in the GSD are accelerated by a raised level of lymphocytes. The investigation also noted that the Eosinophils count was also high to GSD tested samples. Eosinophils are increased especially during hypersensitivity response by producing more new eosinophil to fight pathogens or when an animal skin reacts to something allergic to, as reported previously GSD may be transmitted through vegetation with pathogens spore (Epaphras *et al*., 2012). Neutrophils are the body first responder, and quickly act to an injury or infection. An increase in the number of neutrophils may suggest an inflammatory response observed in the giraffe with a skin lesion, which could be either a fungal infection (Guarner & Brandt, 2011). Likewise, neutrophils represent defensive cells that induce phagocytosis and secrete cytokines and chemokines against the fungal infection (Irimia *et al.,* 2021). Over-stimulation of neutrophils can cause severe tissue damage as a result of the release of toxic agents aimed at killing invading microbes (Ruhnke *et al.*, 2012). However, it can also accelerate the severe progression of the disease (Guarner & Brandt, 2011). Although sampling coverage was not based on the total individuals with GSD, this variation did differ significantly thus observation suggests a likely association between fungal infestation and GSD.

Giraffe skin disease blood samples examined showed high level of creatinine which was the waste product produced from muscles, which causes muscle cramps, but may also mean renal disease to an animal (Guarner & Brandt, 2011). However, serum profile indicated a slight increase in albumin, the role of albumin is to keep fluid moving and balancing throughout the body, and control leaking out of blood vessels, when the albumin rise points to dehydration (Schmidt *et al.,* 2011). All these changes to the GSD indicated the presence of the fungus (Ruhnke1 *et al.*, 2012). Therefore, one of the possible causes of GSD in TME is

likely fungal infection. Similar findings were also observed by Sachs and Debbie (1969). This study has provided valuable information for future population monitoring and suggests management actions for controlling and monitoring the disease.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This is a particularly important study on GSD and species conservation as it has shown GSD by locations, disease status and extent of the lesions related to age class, sex and part of the body affected. The study report for the first time that GSD lesions are now observed on brisket contrary to the previous study conducted in Northern protected areas of Tanzania. The study also found that GSD in Tarangire-Manyara ecosystem affect much of the limb's carpal joint and only males had more severe lesions on brisket. The histopathology study of GSD was conducted from five GSD in two sites of TME, which revealed the presence of heavy fungal infestations. However, the study analyses the changes in haematological parameters and biochemical serum profile, suggest a likely association of GSD and fungal infestation. To the available knowledge, hematological and biochemical analysis is the first study to be conducted and reported the findings in sites of TME and other areas affected by GSD. Our findings suggest the involvement of fungal infection in GSD pathogenesis.

5.2 Recommendations

Based on the findings of this study, the following may be recommended: The current data on giraffe subspecies ranges show changes throughout their ranges. Maasai giraffes are widely distributed in Tanzania both Northern and Southern Highland National Parks. Serengeti, Tarangire, Kilimanjaro, Manyara, Arusha, Ruaha, Mikumi, Katavi. In Kenya most of the giraffe are located in Kajiado, Mara ranches, Laikipia NP, Tana river district, introduced in Akagera NP Rwanda (Bolger *et al*., 2019).

(i) There is a need to do a further in-depth demographic assessment of giraffes in relation to the extent of infestation by GSD around the TME and other areas. This should be done to cover between seasons and across the ecosystem to establish the long term impact of GSD on the health and population dynamics of these animals.

(ii) Where possible some portable tracking devices can be mounted on individuals within the population to allow for long-term monitoring of the impact of the GSD on the population. This will also provide the opportunity for consistent observers of the giraffe population to understand the progression of the GSD.

(iii) With giraffe skin disease spreading throughout sub-Sahara countries, scientists should continue to investigate the cause and transmission determinants of the illness to halt its transmission.Also, culturing and characterization of the fungus by using molecular analysis technique would help understand better the colonies and types of fungi associated with GSD.

(iv) Further research should be done to identify the transmission ways of GSD from one individual to another and the habitat distribution of the disease. The study also recommends urgent characterisation of lesions using modern molecular techniques to identify primary and secondary or opportunistic etiologies and the order in which they occur in the lesions.

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OUTPUTS

Published paper

Kiula, F., Mjingo, E., Mremi, A., Chilongola, J., & Munishim, L. (2021). Prevalence and histopathological characterization of Masai Giraffe (*Giraffa camelopardalis tippelskirchi*) skin disease in Tarangire-Manyara ecosystem, Northern Tanzania, Veterinary Quarterly, 41:1, 242-249, DOI: 10.1080/01652176.2021.1970279 To link to this article: https://doi.org/10.1080/01652176.2021.1970279

Poster presentation

ASSESSMENT OF NATURE AND EXTENT OF GIRAFFE SKIN DISEASE IN TARANGIRE MANYARA ECOSYSTEM

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Introduction: Masai Giraffes have declined dramatically in recent decades due to loss of habitat , illegal hunting and diseases. Giraffe Skin Disease is a recently observed illness, mainly affect adult and sub-adult giraffes, causes gray or crusty lesions on giraffe body.

Methods: The study used road transects. Examination of giraffes involved body distribution of lesions, severity of the lesions and whether they were associated with age and sex of the affected giraffes.

Result and Discussion: : Prevalence among adults was 79%. Affected animals typically had 1-5 lesions which were mostly moderate and were predominantly observed on the forelegs. GSD positivity rate was higher among females versus males, whereas males had a higher rate of severe lesions and generally had more lesions than females. Calves showed no lesions.

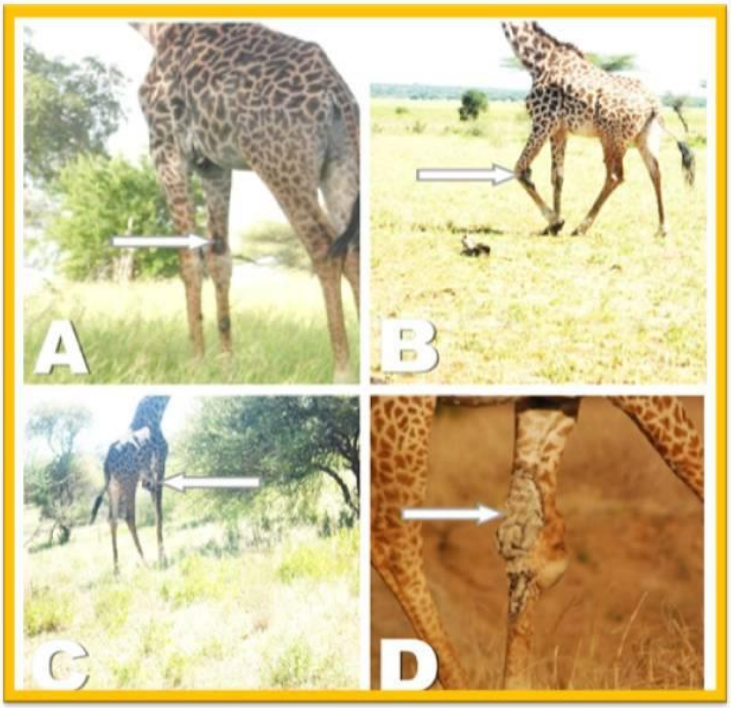

Figure 1: Characterization of GSD of lesions based on degree of severity

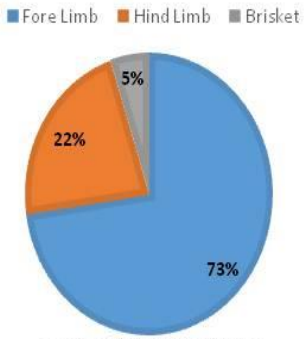

Figure 2:Location of Lesion

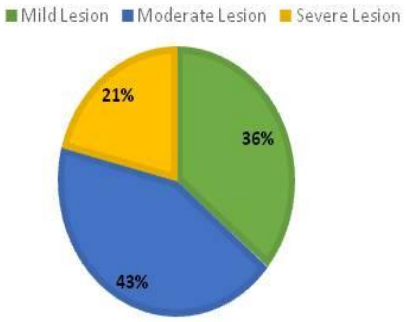

Figure 3:Severity of Lesion

Conclusion: Our findings suggest the involvement of fungal infection in GSD pathogenesis