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SURVEY OF PARASITIC DISEASES IN AFRICAN-LIONS (*Panthera leo*) FROM NIASSA NATIONAL RESERVE, MOZAMBIQUE

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Dissertação de Mestrado Integrado em Medicina Veterinária

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"Os sonhos, quando verdadeiros, têm sempre pressa."

Mia Couto, *in "Revista Índico", Setembro, 2007*

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Resumo

O *Panthera leo* - o Leão-Africano - é uma espécie emblemática do continente Africano, classificada como Vulnerável pela International Union for Conservation of Nature (IUCN). Outrora visto como uma espécie abundante em África, o Leão-Africano encontra-se hoje extinto na grande maioria da sua área de distribuição na África Ocidental, e enfrenta declínios populacionais consideráveis na África Oriental e Austral. A Reserva Nacional do Niassa (RNN) é a maior área de conservação em Moçambique e das mais importantes em África. Esta Reserva concentra as maiores densidades de fauna selvagem no país. Esta área foi identificada como um das poucas "fortalezas do leão-africano", onde a espécie terá melhores hipóteses de sobreviver a longo prazo.

As doenças infeciosas são conhecidas por terem impactos negativos na sobrevivência de populações de leões e são consideradas uma das principais causas que pode condicionar os esforços de conservação na espécie. Contudo, o impacto das infeções de origem parasitária tem sido fracamente documentado em leões selvagens. Anteriormente, não foram realizados quaisquer estudos relativos a este tema nesta população de leões ou em qualquer outra em Moçambique. De modo a caracterizar a fauna parasitológica destes animais, foi realizado um estudo na RNN, com o total apoio do Projecto dos Carnívoros do Niassa (NCP) e a Administração da Reserva do Niassa. No total, foram colhidas 44 amostras fecais numa área de cerca de 600 km² (Concessão L5-Sul), que foram posteriormente processadas no Laboratório de Parasitologia e Doenças Parasitárias da Faculdade de Medicina Veterinária da Universidade de Lisboa. Os resultados demostraram que 65.9% (29/44) das amostras continham formas parasitárias, nomeadamente: 47.7% continham ovos de *Toxocara* sp., 31.8% com larvas L1 de *Aelurostrongylus* sp., 27.3% continham ovos de Taeniidae, 25% com ovos de *Spirometra* sp., 18.2% continham ovos de *Paramphistomum* sp., e 13.6% continham ovos de *Linguatula* sp. Nas 29 amostras positivas, 72% (21/29) foram observadas coinfecções de dois parasitas em 21% (6/29), três em 34% (10/29), quatro em 10% (3/29) e cinco em 7% (2/29). Estes resultados estão de acordo com estudos anteriormente realizados no continente Africano, com exceção de *Linguatula* sp., que não tinha sido reportado em leões selvagens. Estes resultados revelam a importância da continuação de estudos nesta área, com esta e outras espécies animais e as populações humanas. Para tal seria importante associar as técnicas de parasitologia clássica a técnicas de biologia molecular para proceder à identificação das espécies de parasitas encontradas. De futuro, seria também importante complementar este estudo com resultados relativos à presença de outras agentes como vírus e bactérias.

Palavras-chave: Leão Africano, parasitas, endoparasitas, *Linguatula*, Niassa, Moçambique.

Abstract

Panthera leo - The African lion - is an iconic species of the African continent, classified as vulnerable by the International Union for Conservation of Nature (IUCN). Once believed to be a widespread species throughout Africa, the African lion is now extinct in most of its range in West Africa and is facing considerable population declines in Eastern and Southern Africa.

Niassa National Reserve (NNR) is the largest conservation area in Mozambique and it is also one of the most important conservation areas remaining in Africa. It supports the highest densities of wildlife in the country. It has also been identified as one of the few "lion strongholds" where the species has a better chance of long term survival.

Infectious diseases are known to negatively impact the survival of lion populations and are considered a main threat that can significantly impair conservation efforts. However, the impact of parasitic infections has been overlooked and poorly documented in wild lions. To the best of the author's knowledge, no studies addressing this subject have been conducted on this lion population or any other in Mozambique. In order to characterize the parasitological fauna of these animals, a study was carried out in the Niassa National Reserve, with the full support of the Niassa Carnivore Project and in partnership with the Administration of the Niassa Reserve. In total, 44 lion faecal samples were collected in an area of 600 km² (Concession L5-South), and later processed and analyzed at the Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Lisbon University.

Results show that 65.9 % (29/44) of the samples were infected with parasites, namely 47.7 % for *Toxocara* sp., 31.8 % for *Aelurostrongylus* sp., 27.3 % for Taeniidae, 25 % for *Spirometra* sp.,18.2 % for *Paramphistomum* sp. and 13.6 % for *Linguatula* sp. Out of the 29 positive samples, 72 % (21/29) were co-infected, registering double infection in 21 % (6/29), triple in 34 % (10/29), quadruple in 10 % (3/29) and fivefold in 7 % (2/29). These results are consistent with previous studies performed in the African continent, with the exception of *Linguatula* sp., which had not yet been reported in wild lions. These results highlight the importance of further studies in this subject, not only with this and other animal species but on human populations as well. In order to carry out the identification of the species of parasites found it to would be important to associate classic parasitology techniques with molecular biology techniques. In the future, it would also be important to complement this study with results regarding the presence of other pathogens such as viruses and bacteria.

Key-words: African lion, parasites, endoparasites, *Linguatula*, Niassa, Mozambique.

Introductory Note

The present work was carried out in Niassa National Reserve in Mozambique as part of the author's training period during the 6^{th} year of the Integrated Master in Veterinary Medicine. This research has already resulted in:

- A full paper and poster communication in the Proceedings of the International Conference of Diseases of Zoo and Wild Animals 2015, 13^{th} to 16^{th} of May, 2015, Barcelona, Catalonia (Spain) (see Appendix A)

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List of Abbreviations and Symbols

µm – Micrometer

ANAC – National Administration of Conservation Areas

CDV – Canine Distemper Virus

CIISA – Centre for Interdisciplinary Research in Animal Health

CITES – Convention on International Trade in Endangered Species

cm - Centimeter

CPV – Canine Parvovirus

EPG – Eggs per gram of faeces

FCoV – Feline Coronavirus

FIP - Feline Infectious Peritonitis

FIV – Feline Immunodeficiency Virus

FMV – Faculty of Veterinary Medicine

GPS – Global Position System

IUCN – International Union for Conservation of Nature

MITUR – Ministry of Tourism of Mozambique

NCP / NLP – Niassa Carnivore Project / Niassa Lion Project

NNR – Niassa National Reserve

SGDRN / SRN – Sociedade para a Gestão e Desenvolvimento da Reserva do Niassa

TRT – The Ratel Trust

ULisboa – University of Lisbon

WCPA – World Commission on Protected Areas

WCS – Wildlife Conservation Society

Introduction

The definition of protected area is different all over the world, according to the legal systems of each country. Still, a protected area is currently defined by the International Union for Conservation of Nature (IUCN) and the World Commission on Protected Areas (WCPA) as "an area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means." Protected areas are considered the most important core units for in situ conservation (Chape, Harrison, Spalding, & Lysenko, 2005) and therefore, are utterly important for the safeguard and preservation of certain areas of the Planet, contributing to the conservation of habitats and species worldwide. However, these areas are often highly challenging and complex to manage, as there are very close connections between wildlife populations, human populations and the whole ecosystems.

Some species of mammals, carnivores in particular, are vulnerable to extinction in fragmented landscapes, due to their low densities, extensive spatial requirements and inevitable conflict with people (Purvis, Gittleman, Cowlishaw, & Mace 2000). Thus, the creation and maintenance of large protected areas is a highly relevant factor for the survival of several populations of carnivores (Balme, Slotow, & Hunter, 2010).

Over the recent decades Conservation Biology has been an area of increasing importance given the current situation of crisis the Planet is experiencing, with species extinctions all over the world, loss of habitats and biodiversity, climate change and pollution. Associated with this subject, Conservation Medicine has been defined as a multidisciplinary science dedicated to understanding how wildlife, human, and ecosystem health are related and to dealing with the threats that diseases pose to ecosystems (Scholoegel & Daszak, 2004).

The conservation and management of free-ranging wild felids in particular, relies on an effective integration of veterinary epidemiology and long-term research on cross-species and cross-pathogens (Cleaveland, Mlengeya, Kaare, Haydon, Lembo, Laurenson & Packer, 2007; Haydon, 2008). Therefore, in order to be successful, felid conservation will increasingly need to include assessments of disease risks and strategies for disease management (Macdonald & Loveridge, 2010). Also, it is now widely accepted that countries that conduct disease surveillance of their wild animal populations are better prepared to protect not only wildlife, but also domestic animals and human populations (Mörner, Obendorf, Artois, & Woodford, 2002). Disease risk modelling is recognized as the best method for predicting threats in free-ranging populations. However, the strength of these models is constrained by lack of knowledge regarding the prevalence and behaviour of infectious agents in free-ranging populations, susceptible species and reservoirs, and pathogen persistence in the environment (Cleaveland et al., 2007; Macdonald & Loveridge, 2010).

This study aimed to survey potential pathogens in the African Lions (*Panthera leo*) of Niassa National Reserve, Mozambique, through the collection of faecal samples, with the purpose of obtaining important data about the species. At the same time, this study also intended to contribute for a better understanding of the health status of lions in the Reserve, regarding their gastrointestinal parasitological fauna and other diseases, namely viral, that may be affecting the lion populations. A brief description about leopards (*Panthera pardus*) is also included since they are the only other large cat species in Niassa Reserve and as cohabitants they may share the same, or similar threats faced by lions.

Finally, this work is also expected to provide a base knowledge to local entities such as the Administration of Niassa National Reserve and the Niassa Carnivore Project, with the necessary data to enable the design and implementation of strategies they might consider necessary in the future.

The fieldwork was undertaken during the 6th year of the author's Integrated Master in Veterinary Medicine as a part of the author's training period and thesis, and was developed as an independent project which promoted a partnership between The Faculty of Veterinary Medicine of the University of Lisbon, the Administration of Niassa National Reserve, the Niassa Lion Project and the Lúrio University. This work was also sponsored by Grupo Entreposto of Mozambique.

1.1 - Niassa National Reserve

The Niassa National Reserve (NNR) is the largest Conservation Area in Mozambique, and supports the highest densities of wildlife in the entire country. The Reserve is located on the far north of Mozambique and stretches over 42,279km² divided into a Buffer Zone of 19,239 km² formed by hunting blocks for sport hunting, surrounding the core area of the Reserve with 23,040 km² (Branch, Roedel, & Marais, 2005) (Fig. 1). The core area of the Niassa Reserve lies between the Rovuma and the Lugenda rivers to the north and southeast respectively. The Niassa Reserve covers a large area in the Niassa Province and a smaller one in the adjacent Cabo Delgado Province. It is one of the largest protected areas in Africa and also one of the most undeveloped places in the continent (Begg & Begg, 2014). The Reserve still harbors the greatest diversity of flora and fauna in Mozambique (Leo Smith, Balson, Abacar. 1997). Niassa Reserve is also one of the largest protected Miombo forest ecosystems in the world (Branch et al., 2005).

Figure 1-Map of Mozambique highlighting the location of Niassa Reserve (Source: SGDRN 2010)

Together, the Niassa National Reserve in Mozambique and the Selous Game Reserve in Tanzania, are linked by the Niassa-Selous Ecological Corridor (Schuerholz & Baldus, 2007), representing one of the largest existing transfrontier conservation areas (Fig.2).

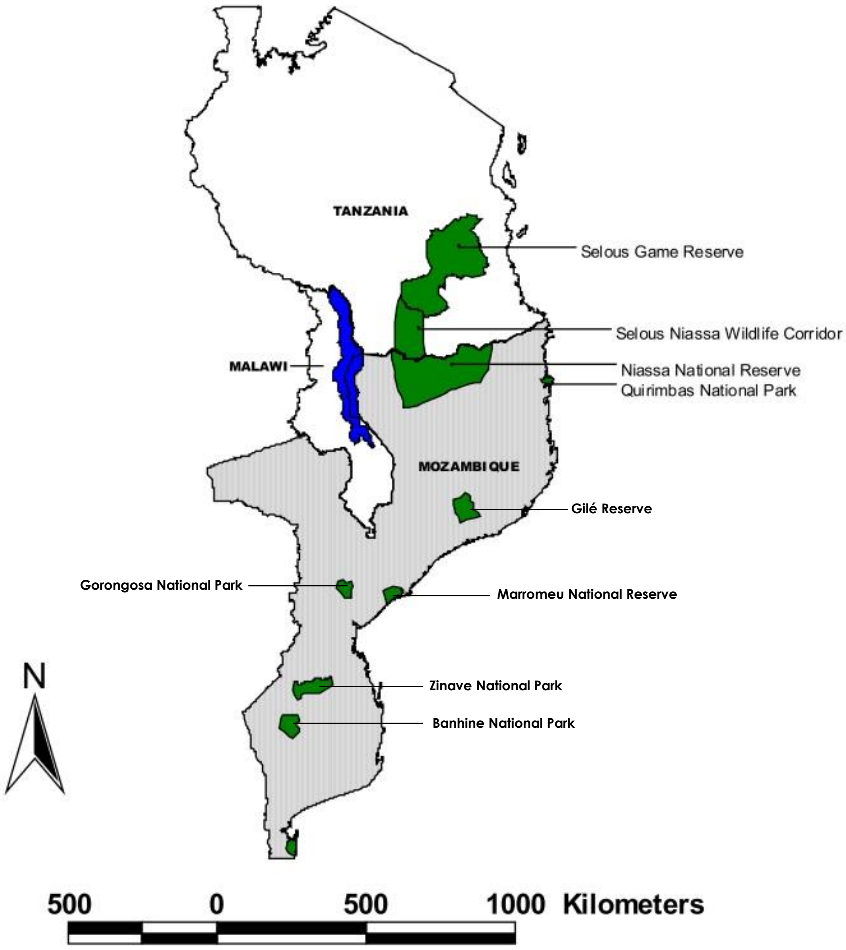


Figure 2 -Selous-Niassa Wildlife Corridor linking Niassa Reserve and Selous Reserve, and adjacent areas. (Adapted from: Begg & Begg, 2009)

Declared a protected area in 1954, but abandoned during the armed conflicts between 1975-1988 (Begg, Begg & Muemedi, 2007) the Reserve is currently managed by the Ministry of Tourism of Mozambique (MITUR) in a co-management agreement with the Wildlife Conservation Society (WCS) after 12 years of SGDRN (Sociedade para Gestão e Desenvolvimento da Reserva do Niassa) management, the first partnership between the public and private sectors for a conservation area in Mozambique. The Reserve is divided into variable-sized concessions, with a vast majority of those areas operating as concessions for sport hunting activities. The Reserve also includes over 42 villages with a growing population of about 40,000 people living inside the protected area (Begg & Begg, 2013). Still, the Niassa Province has the lowest population density in the country. The main activities of most inhabitants of these villages are fishing, hunting and subsistence agriculture. The coexistence of these growing human populations and wildlife in Niassa Reserve is perhaps the greatest challenge to the preservation of this site and its biodiversity. Mozambique is a country with a recent history of armed conflicts and has suffered 10 years of independence war (1964-1974), and 15 years (1977-1992) of civil war which have contributed significantly to the loss of wildlife populations throughout the country. The effects of the years of conflict are known for the Gilé Reserve in Zambézia Province, which suffered from a lack of management and financial support since 1982 resulting in a rapid decline in staff and consequently in patrol activities (Fusari & Carpaneto, 2006). Then, the associated worsening of human life conditions motivated the exploitation of the natural resources available inside the protected area. Even though, human intervention in Niassa Reserve during the wars in Mozambique was limited due to its remoteness the Reserve's wildlife populations have also decreased substantially during this period (Prin, Chamaillé, Grosbois, Fritz, Guerbois, Chardonnet, Cornelis, 2014). However, the Reserve is still considered one of the best preserved areas of the region (Ribeiro, Saatchi, Shugart, & Washington-Allen, 2008). It is believed that the black-rhinoceros (*Diceros bicornis*) was the only species to have disappeared entirely from Niassa and the decline of the species is considered to have been more pronounced during the years of conflict. A similar scenario was described in the Gilé Reserve where the black rhinoceros disappeared a few years earlier, in mid-1973 (Dutton, Dutton, & Balsinhas, 1973).

The elephant (*Loxodonta africana*) population inhabiting Niassa Reserve has been heavily targeted by illegal poachers to supply the illegal ivory trade (Fig.3). Most of the ivory obtained this way is then shipped to be sold mostly in Asia. Once known for its large elephant population, Niassa has been losing elephants at an alarming rate (around 2500 elephants lost in 2010-2011 alone) and it is estimated that just during the last 3 years the Reserve has lost around two thirds of its elephants to poachers. Poaching is currently the greatest threat to all the biodiversity of Niassa. The reserve also includes populations of several species of carnivores, with special emphasis on lions (*Panthera leo*), leopards (*Panthera pardus*), hyenas (*Crocuta crocuta*) and wild dogs (*Lycaon pictus*). Niassa's lion population is currently estimated at 1100-1200 individuals, representing one of the seven largest populations of the species (> 1000 individuals), remaining in Africa today (Begg & Begg, 2013). A recent study has considered this area as a "stronghold" for the species which means, this is one of the few existing areas in the Planet where the African Lion will have better chances of surviving in the long term due to existence of growing population of at least 500 individuals (Riggio, Jacobson, Dollar, Bauer, Becker, Dickman, Funston, Groom, Henschel, Iongh, Litchenfeld & Pimm, 2013).

Besides poaching there are other threats currently affecting the whole ecosystem of the Reserve. In 2014, alluvial gold and ruby mining was recognized as a new major threat to Niassa since it is not only disturbing rivers through digging and the use of mercury, but it is also contributing to an increase of movements of people from outside Niassa, inside the Reserve. This increase is also linked to an escalation in poaching and snaring rates and it is also destabilizing communities. In the last 2-3 years logging has also increased inside the protected area (Fig.4), and many hardwood trees are being taken out as more and more trucks are moving across the Reserve (Begg & Begg, 2014).

Niassa Reserve has a tropical climate, with a warm and rainy summer and a cool, dry winter. During summer, temperatures usually range between 15-30 °C and from 10-20°C in winter. The annual rainfall is usually less than 1000 mm. During winter it is very rare to have more than 2-3 mm per month of rain but in December-January the average rainfall may range between 200-230 mm per month (Branch et al., 2005).

Figure 3 - Poached elephant carcass (Source: Niassa Lion Project)

Figure 4 –Logging trucks in Niassa Reserve (Source: Niassa Lion Project)

1.2 - Niassa Carnivore Project (Projecto Carnívoros do Niassa)

Created in 2003 by Colleen Begg and Keith Begg, the Niassa Carnivore Project (NCP) has been operating in the Niassa Reserve aiming to promote co-existence between human populations of this Conservation Area and the species of large carnivores living side by side with them. The costs of living in such proximity with carnivores can sometimes be very high for local communities, but these same carnivores can provide significant cultural, economic and ecological benefits (Begg & Begg, 2014). The Project is managed through The Ratel Trust, a south-African non-profit conservation trust, created by Colleen and Keith Begg and Stephen Clark. The NCP has been monitoring the carnivore populations and implementing solutions to mitigate threats not only to lions, but also leopards, hyenas and wild-dogs.

The use of wire snares by local populations to catch wild animals (mostly large herbivores) for consumption and trade has been identified as the leading cause of death to large carnivores in the NNR. The use of these snares is widely distributed throughout the Reserve, resulting in the capture of a wide variety of species, some of them, like lions, as bycatch. Another consequence to lions and other carnivores, related to the use of these snares is the reduction in the number of available prey for the carnivores. The persecution and killing of certain animals (lions, leopards and hyenas) by the population in retaliation to attacks on people or livestock, is another threat that carnivores have been facing in Niassa Reserve. However this is not considered a major threat in Niassa when compared to other areas where retaliatory killing by pastoralists is the highest threat to carnivores (Kissui, 2008). Sport-hunting of lions in particular is also considered a threat to the survivability of the species in Niassa Reserve. The Administration of Niassa Reserve implemented specific regulations in collaboration with the NCP, for Lion trophy hunting (in 2006) and leopard (in 2010), which aimed to monitor the quality of hunted trophies in the various hunting concessions currently operating in NNR. Both regulatory systems were reviewed and updated in 2013 by WCS (Wildlife Conservation Society) and the Ministry of Tourism of Mozambique (MITUR). Through the implementation of such systems it is expected that no lions under the age of six are taken as trophies which will contribute to the reproduction and maintenance of existing populations, making a rational and sustainable management based on quotas defined by scientific criteria.

NCP also acknowledges the impact that diseases may have on the carnivore populations inside the protected area which may impair conservation efforts. Disease risk to carnivores has been highlighted in every report of NCP (Begg & Begg, 2014). Although disease is a natural part of life in any ecosystem, the occurrence of some diseases may be related to human activities and human presence, and could affect not only wildlife populations, but human and domestic animal populations as well. According to recent data collected in 2014, the number of domestic dogs has been increasing (Fig.5) in Niassa Reserve. According no NCP data, domestic dogs are mainly used for protecting people and crops, but also for bushmeat hunting, resulting in high levels of contact with wildlife and people. An average of 4,2 dogs are owned by a single owner (Begg & Begg, 2014). Canine Distemper and Rabies are known to have considerable impacts on lion and wild dog populations. Rabies outbreaks are common in Niassa (last outbreak in 2005) and no dogs are vaccinated which has already resulted in human casualties (8 people died during the 2005 outbreak in one village and 500 dogs had to be destroyed) throughout the years also due to the impossibility of receiving proper treatment within 48 hours (Begg & Begg, 2007b;Begg & Begg, 2014). In 2006, NCP and the Alliance for Rabies Control created a rabies awareness poster which was distributed in villages, schools and clinics throughout Niassa Reserve. In order to monitor the disease risk to carnivores in the Reserve, the NCP has been collecting blood samples whenever a lion is captured and/or collared. Under the new regulations to trophy hunting proposed by NCP, hunting operators are also provided with the required materials to collect a blood sample from any lion or leopard hunted in hunting concessions. The blood is collected and preserved using Whatman® FTA® cards (Fig.6), which can be used later to search for specific pathogens. Through the collection of these samples, it was already possible to determine the positive FIV (Feline Immunodeficiency Virus) status of the lion population in Niassa Reserve.

Figure 5 - Local children running with several domestic dogs (Source: Niassa Lion Project)

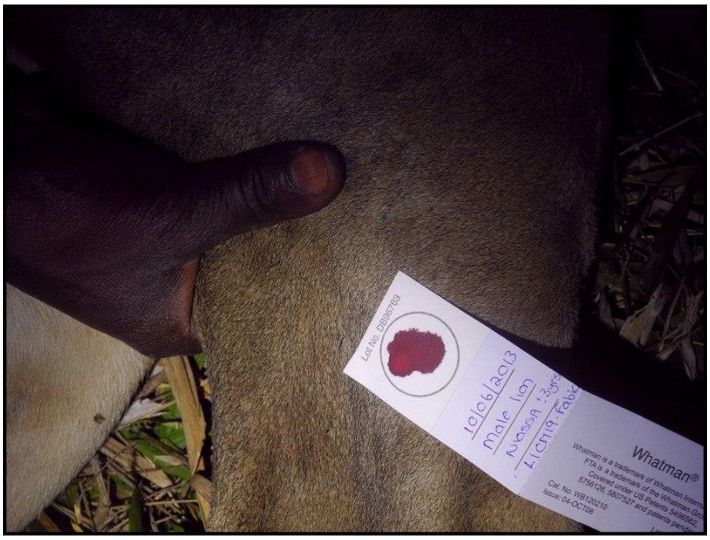


Figure 6 - Blood collection from captured lion using Whatman® FTA® cards. (Source: Niassa Lion Project)

Niassa Carnivore Project believes and applies a community-based conservation strategy aiming at improving human livelihoods inside Niassa Reserve while promoting culturally appropriate and locally derived conservation actions (Begg & Begg, 2014). NCP's approach to Conservation is accompanied by environmental education and community outreach actions as well, which are based in targeted ecological and socio-ecological research to provide baselines for future comparison and measurement (Begg & Begg, 2014). According to Begg & Begg (2014) the NCP is currently running several Community Programs in order to address specific issues related to carnivore conservation such as:

- Improving food security through conservation agriculture to sustainably increase productivity in fields;
- Reducing human-elephant conflict through the use of beehive-fences which provide protection against crop raiding by elephants but also produces honey;
- Providing alternative protein sources to reduce bushmeat consumption and increase the variety and quantity of meat available in villages. This voluntary program works like to a micro-credit scheme and is currently providing rabbits, ducks and domesticated Guinea fowl to households.;
- Reducing human-carnivore conflict identifying behaviours that increase the likelihood of being attacked while providing relevant education and support to improve corrals, shelters and other strategies to reduce conflict;
- Reducing snaring, mining and armed poaching through community supported anti-poaching while providing conservation-related employment.

Among the communities, the NCP has been also developing educational initiatives to raise awareness and promote behaviour changes (Fig.7) related to conservation in the Reserve, editing and providing books, funding scholarships for children and youth, and organizing the "Lion Fun Days", an initiative usually lasting 2 days in which the people from Mbamba village meet and play games, make dances, races, paintings (Fig.8) and other activities related to Conservation. Furthermore, the presence of the NCP and the activities the Project has been developing over the last 12 years, have created new jobs and opportunities for the people of the local villages. In fact, 98% of the staff hired by NCP comes from local communities, and their training is provided on the Project (Begg & Begg, 2014). Therefore, people who previously subsisted from poaching or activities related to the exploitation of natural resources of this area, are now able to subsist on jobs related to conservation in NNR. In addition, the NCP has established a partnership with the Mbamba village community, making them their legal partners in managing the L5-South Concession. The goal is that the results of this community based partnership will provide an example that can be scaled up in all villages of the Reserve in a near future.

NCP has a long term view of 25 years for Conservation in Niassa Reserve and is strongly committed to mentor the next generation of Mozambican Conservationists.

Figure 7- Safe-behaviours poster used in local schools (Source: Niassa Lion Project)

Figure 8 - Traditional games and paintings for children during the Lion Fun Days (Original)

1.3 - African Lion (*Panthera leo*)

The African lion, *Panthera leo* is perhaps the most iconic species in Africa. Originally described by Linnaeus in 1758 from a specimen in North Africa, the name lion originates from the Greek word for the species – leon (Skinner & Smither, 1990). The African Lion is classified as Vulnerable on the IUCN Red List of Species and listed in CITES Appendix II. Although it is very hard to know exactly how many lions still remain in the wild today, several assessments have been carried out to estimate the lion population in Africa (Chardonnet, 2002; Bauer & Van Der Merwe, 2004;Riggio et al., 2013). Still, the IUCN Cat Specialist Group indicates that the number of wild lions remaining in the wild today is close to 20,000 individuals. Overall populations of the species have suffered a sharp decline throughout their distribution (Fig.9), and are increasingly limited to protected areas (Riggio et al., 2013), with special emphasis on the remaining populations in West Africa which gradually became smaller and more isolated, and whose decline has been more pronounced (approximately 80% of its original distribution in this area). The results presented by Henschel et al. (2014) suggest that the remaining lion population in West Africa is formed by 406 (273-605) individuals of which less than 250 are adults. The same study indicates that the current distribution of the species in this area is 49,000 km^2 which represents 1.1% of its historical range. Also, based on genetic evidences, the same authors suggest a revision of the taxonomy of the lion population in West Africa, recognizing its uniqueness as a subspecies (Henschel et al., 2014). Apart from this revision, Henschel et al. (2014) recommend listing the lion as critically endangered in West Africa. The subspecies *Panthera leo persica*, the Asiatic-lion, which existed in some countries in the Middle East like Syria, Iraq, Iran and Pakistan, presently remains only in the Gir National Park, Gujarat state, in India, and surrounding areas (Wilson & Mittermeier,2009). This subspecies is fully protected in India where Asiatic lion numbers are rising. It is classified as Endangered by the IUCN and it is included in CITES Appendix I.

In some countries the direct hunting of the species (*P. leo*) is prohibited, whilst in others it is limited to the so called “problematic animals” (which often attack people or livestock), and there are also countries where the species has no legal protection. Due to their scavenging habits, lions are extremely vulnerable to snares set for bushmeat and poisoned baits (Hunter & Barrett, 2011). The Lion is a species with a strong positive socio-economic impact in many parts of Africa due to trophy hunting activities or due to safaris and ecotourism, and a negative impact as livestock predators (Wilson & Mittermeier, 2009). Trophy hunting of lions is a multimillion dollar industry in sub-Saharan Africa that provides tourists with the opportunity to hunt lions (mostly males) in ten African countries: Botswana, Burkina Faso, Central African Republic, Chad, Mozambique, Namibia, Republic of South Africa, Tanzania, Zambia, and Zimbabwe resulting in 600-700 male lions (mainly) being taken every year (Whitman, Starfield, Quadling, & Packer, 2007; Hunter & Barrett, 2011). Due do the concerns around the trophy hunting of lions, a proposal has emerged to list the African lion in CITES Appendix I during the 13th Conference of the parties in Kenya (Lindsey, Balme, Funston, Henschel, Hunter, Madzikanda, & Nyirenda, 2013). Such listing would not prevent the hunting of lions, but stricter measures would likely be implemented by importing countries once the species was listed in Appendix I and the proposal was rejected due to the beliefs that trophy hunting was not related to the declines of the species, and also because it was believed to generate funds for the conservation of lions and their habitats (Lindsey, Balme, Booth, & Midlane, 2012). Recent research in different areas has been highlighting the importance and impact of trophy hunting of African lions and this practice seems to be more detrimental to lion populations than previously thought, showing the most significant population declines where the species is hunted more intensively (Craig Packer et al., 2009).

In most species, trophy hunting of adult males would only reduce the overall population of the species if the removal rate was high enough to prevent females from finding a male to breed (Whitman, Starfield, Quadling, & Packer, 2004). Lions however, live in a stable social structure (pride) consisting of several females (usually related) and a coalition of pride males (or only one in smaller prides). The coalition sires all cubs born during their tenure period, which usually lasts long enough to raise a generation of cubs to independence. When new males takeover a pride, they usually kill all cubs under 9 months of age and evict other sub adult individuals. Therefore, trophy hunting of adult male lions increases the rate of male takeovers as prides become more vulnerable even with the loss of only one male (Whitman et al., 2004). Excessive trophy hunting can increase the rate of male replacements and associated infanticide of small cubs, preventing prides to raise cubs to adulthood (Whitman et al., 2004). Trophy hunting of lions has not only reduced lion populations significantly, but also led to shifts in sex ratios and spatial behaviours of the species in Zambia, Zimbabwe and Cameroon (Loveridge, Searle, Murindagomo, & Macdonald, 2007;Croes, Funston, Rasmussen, Buij, Saleh, Tumenta, & de Iongh, 2011; Davidson, Valeix, Loveridge, Madzikanda, & Macdonald, 2011;).

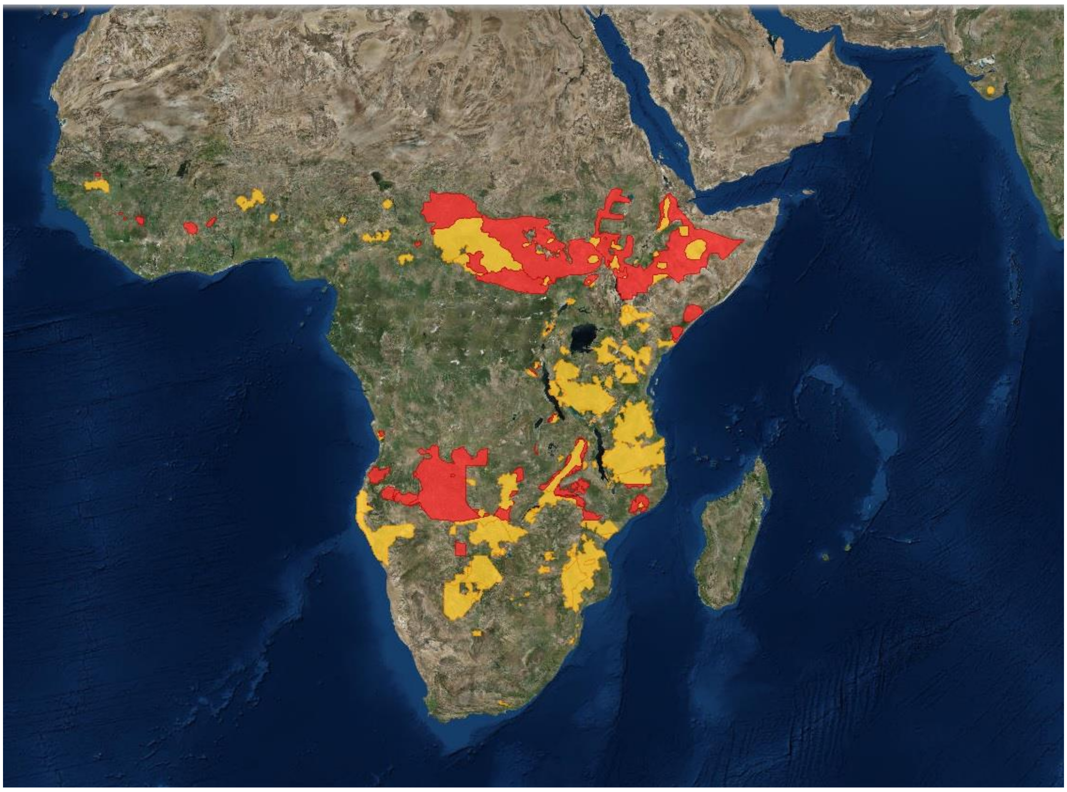


Figure 9 – Distribution map of the Lion showing the areas where the species still exists (yellow) and where it is possibly extinct (red). (Source: Cat Specialist Group Web Portal. Accessed on November 2015)

1.3.1 – Habitat and Distribution

Although Lions have a wide habitat tolerance, they have a patchy distribution south of the Sahara. The largest lion populations are found in Eastern and Southern Africa (Hunter & Barrett, 2011; Haas, Hayssen, & Krausman, 2005).They are found wherever suitable prey is available although they are absent from the dense lowland rain forests of West Africa and the Congo Basin and true deserts (Hunter & Barrett, 2011;Skinner & Smither, 1990; Wilson & Mittermeier, 2009). They are common in semi-desert areas like the Kalahari in Botswana. They will drink regularly, especially after feeding, if there is available water, but are not heavily dependent on it, as lions are adapted to get their moisture requirements from their prey and even some species of plants (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). The most relevant habitat requirements for lions are a mosaic of open woodlands, scrub, thick bush, and grass complexes that can provide ample supply of food such as medium and large-sized ungulates, also some shade for resting during the heat of the day, and the barest cover for lions to stalk their prey (Skinner & Smither, 1990;M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). The Lion was a widespread species which formerly ranged from North Africa (disappearing in in 1891 from Tunisia and Algeria and in 1920 from Morocco) through south-west Asia, where it disappeared from most countries within the last 150 years (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). In the Middle East, lions have once roamed throughout the forests of Syria, Iraq and southwest Iran but the species has disappeared as their habitat and prey declined. The last reliable account of a lion in this region was in 1942 in Iran (M. Sunquist & Sunquist, 2002). Lions were also found in Europe (Greece), but the last individuals were exterminated around the year of 100 AD (Skinner & Smither, 1990). The species was also present in Palestine until the 12th century (Skinner & Smither, 1990). More recently, lions inhabited the edge of the Air Mountains in the Central Saharan Desert, but went extinct nearly 60 years ago (Haas et al., 2005, Wilson & Mittermeier, 2009).

1.3.2 – Description

The Lion is the largest of the African carnivores (Skinner & Smither, 1990; Estes, 1991). According to Wilson & Mittermeier (2009) males are usually bigger and heavier than females with males reaching 172-250 cm (head-body) and weighing around 190 kg (150-225 kg) and females 158-192 cm and weighing around 126 kg (122-192 kg). The tails usually measure about 61-100 cm. Shoulder height is usually between 107-123 cm in both genders. The individuals of the Asian subspecies *Panthera leo persica* are usually smaller than their African relatives, and they have a distinctive longitudinal fold of skin along their bellies, rarely seen in African Lions (Skinner & Smither, 1990; Wilson & Mittermeier, 2009; Hunter & Barrett, 2011). The body color of adult lions ranges from a uniform sandy or tawny on the upperparts and flanks to white on the underparts. Coat colour results from a combination of short sandy-yellow hairs, mixed with long black guard hairs (Haas et al., 2005). Cubs and juveniles have heavily spotted fur with dark brown rosettes (that fuse to form stripes in some places) which tend to fade with age (Haas et al., 2005), but faint spotting may persist into adulthood to a lesser or greater degree (Skinner & Smither, 1990; Wilson & Mittermeier, 2009; Estes,1991;Sunquist & Sunquist, 2002) . Melanistic forms are very rarely seen, but very pale individuals are known from Kaokoland, Namibia and southwest Botswana and some almost white individuals, from Timbavati, South Africa (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). These nearly white lions are leucistic, not albino, and can be born to normally colored lions (Hunter & Barrett, 2011). Their appearance is a result of a recessive gene for coat colour and they have pigmented eyes, nose and pads (Hunter & Barrett, 2011). Adult male lions have a long mane of hair that can go up to 16 cm (Wilson & Mittermeier, 2009). The mane is located on the sides of the face and top of the head, extending onto the shoulders, around the neck and down the spine (Skinner & Smither, 1990). In sub-adults mane colors may range from sandy, yellowish, or tawny, and sometimes becoming black with advancing age (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Although uncommonly, “maneless” adult lions have been found in the Tsavo region, Tanzania and mostly in areas where temperatures are extremely high (Wilson & Mittermeier, 2009; Hunter & Barett, 2011). As mane development is highly influenced by testosterone it is considered to be an indicator of individual fitness, (Haas et al., 2005;Wilson & Mittermeier, 2009), and therefore advertises the condition of the male to females and also to other males (F. Sunquist & Sunquist, 2014). The mane is a distinguishing feature of the males serving as a sexual signal to females, providing protection to the head and neck when fighting, and allowing recognition between individuals from a distance (Skinner & Smither, 1990; Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). Although young males begin to grow a mane when they mature (around 3-4 years of age), the growth rate of the mane varies with each individual (M. Sunquist & Sunquist, 2002). Some lions have whitish whiskers which are arranged in parallel rows on the sides of the upper lip, with each whisker arising from a black spot, although the top row of spots has no whiskers (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). It is possible to recognize individual lions by the arrangement of these spots as there are no two lions with spots arranged in the same way (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Lions have five digits on the front feet and four on the hind feet, each digit armed with an extremely sharp retractile claw (Skinner & Smither, 1990).

1.3.3 – Feeding ecology and feeding habits

The Lion is an opportunistic and generalist species, preying on virtually everything from mice to young rhinos, hippos, elephants (only health and mature individuals are invulnerable to lion attacks), birds up to the size of ostriches, as well as reptiles, fish, and even insects (Skinner & Smither, 1990; Hunter & Barett, 2011). However, lions prefer large herbivores (Hunter & Barett, 2011) and ungulate species including a wide variety of bovids like Blue Wildebeest, African Buffalo, Waterbuck, Hartebeest, Kob, Sable Antelope, Gemsbok, Eland, Impala, Springbok, Giraffe, Warthog and Zebra in Africa, and also deer and a high percentage of cattle in Asia (Wilson & Mittermeier, 2009; Hunter & Barett, 2011). Although males take little part in the hunt, leaving it to females, they are usually the first to take part in feeding once the kill is made. Pride males may be more tolerant to young cubs sharing his meal but lionesses usually have to wait until the pride male has eaten his part (Skinner & Smither, 1990). Though females make most kills, males are also capable hunters that increase hunting success of larger prey which is usually hunted as a cooperative effort, with males being bigger and stronger, tackling larger animals more readily than females (Estes, 1991; Hunter & Barett, 2011). Smaller prey, mainly warthog, usually accounts for a considerable part of the Lion's diet during the dry season when larger prey is migrating or absent (Hunter & Barett, 2011). Lions may cause serious losses to livestock and may also occasionally prey on humans and become “man-eaters”, when their normal prey is absent or scarce (Wilson & Mittermeier, 2009). In Serengeti National Park in Tanzania, lions have been recorded to prey on cheetah (*Acinonyx jubatus*) cubs and may be responsible for reducing cheetah density in that area (Haas et al., 2005). Lions are also scavengers, and males, more frequently than females, acquire up to 53% of their food in the plains of the Serengeti this way (Wilson & Mittermeier, 2009). There are individual differences in prey selection, based on sexual and social differences within the pride as well as seasonal weather patterns (Haas et al., 2005), and killing techniques may also be different between prides in the same area, suggesting that the Lion’s hunting behaviour is most likely influenced by learning (Wilson & Mittermeier, 2009). For example, in Kruger National Park male lions fed mostly on African buffalo whereas females fed on the most abundant medium-sized ungulates, such as Blue Wildebeest and Zebra (Wilson & Mittermeier, 2009). The hunting behaviours are influenced by prey and cover availability, as well as prey body size (Skinner & Smither, 1990). In the Serengeti, 30% of stalks succeeded when two or more lions joined the hunt, whereas the success of solitary hunters was only 17-19% (Estes, 1991; Wilson & Mittermeier, 2009). When chasing prey (Fig.10), lions are able to achieve speeds of 45-50 km/h in a rush for up to a few hundred meters (no more than 100-200 m). Once within catching distance of its prey, a lion has to overpower and kill its prey without hurting itself (Estes, 1991). The attack is delivered to the rump or shoulders, with the weight of the Lions often bringing the prey to the ground. Sometimes the prey’s neck is broken in falling. Once the prey is down the lion lunges for its throat or muzzle in order to strangulate it, maintaining a firm grip until its prey stops moving (Estes, 1991; Wilson & Mittermeier, 2009). Lions may feed at the site where the prey has fallen (usually happens with larger animals), or may drag it to a nearby cover before starting to eat. In fact, when compared to other wild felids like tigers or leopards, lions eat almost immediately after they kill and eat very quickly, which is probably due to the presence of scavengers and other pride members (M. Sunquist & Sunquist, 2002). If the carcass is not fully eaten by the pride, lions may try to protect it lying down nearby, or even burying the remains which tends to be ineffective due to the numerous amounts of scavengers in their habitats (M. Sunquist & Sunquist, 2002). As scavengers, lions may also steal other animal’s kills whenever possible (e.g. spotted-hyenas) rather than hunting for themselves.

Figure 10– Camera trap footage of a lioness running towards a zebra (Source: Niassa Lion Project)

The presence of the pride males, as well as, the number of individuals involved, are very important factors regarding the interactions with Spotted Hyena clans (Estes, 1991; Skinner & Smither, 1990). Large packs of Spotted Hyenas can intimidate lionesses into leaving kills, however, protection of kills against hyenas, is greatly enhanced when males are present (Skinner & Smither, 1990; Wilson & Mittermeier). As a group, lions can deter most scavengers but solitary males often have their prey stolen (Skinner & Smither, 1990;M. Sunquist & Sunquist, 2002). In Botswana's Chobe National Park, prides females lost up to 20% of their kills to Spotted Hyenas and 17% to unrelated Lions.

1.3.4 - Activity patterns

Lions are usually more active around sunrise and sunset and are considered to be mainly nocturnal. However, lions are often observed moving or hunting at daylight hours as well (Skinner & Smither, 1990; Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). The species activity patterns depend on environmental factors such as habitat type, season, ambient temperatures, availability of stalking cover, and prey abundance (Estes, 1991;Sunquist & Sunquist, 2002). The Lion is considered to be the least active of all the felids (Sunquist & Sunquist, 2002) typically spending 20-21 hours a day resting (Estes, 1991). In the Serengeti, lions seem to have two distinctive hunting peaks occurring between 02:00h and 04:00h, but in Etosha, Namibia, most hunts occurred from 21:30-22:30h. Lions seem to be less successful when hunting on moonlight hours than when no moonlight is present, which suggests they take advantage of the absence of light

(Skinner & Smither, 1990). It is also believed that Lion's hunting levels may be higher during storms, when prey has a harder time to detect the lion's presence due to noise, wind and waving vegetation. Resting periods are interrupted by short periods of intense activity related to hunting or exhibiting aggression between individuals, but lions usually rest together in groups. Lions avoid the heat of the day usually by resting in the shade (Fig.11) and although they are considered a terrestrial species, lions are also good climbers and have been seen resting along branches of trees (Skinner & Smither, 1990;Sunquist & Sunquist, 2002;Wilson & Mittermeier, 2009). Some authors suggest this behaviour might also be a way of avoiding biting insects like flies and as a mean to escape from buffalo and elephants (Skinner & Smither, 1990; Sunquist & Sunquist, 2002).

Figure 11– Two lionesses and a cub from the "M-Pride" resting in the shade (Original)

The Lion's roar has been often considered as one of the most impressive natural sounds (Estes, 1991). Under optimal conditions a lion's roar can be heard from 5 km away (Wilson & Mittermeier, 2009), and it appears to serve to demarcate territory, location of pride members, intimidating rivals during aggressive interactions, and strengthening social bonds since prides frequently indulge in communal roaring (Estes,1991;Wilson & Mittermeier, 2009). Lions also use scats, urine spraying (mainly a male behaviour, although females occasionally do it as well) and scrapes to define boundaries of their territories. Pride males usually spend much more time patrolling, roaring and scent-marking when compared to females, so they can warn others demonstrating their right to the territory and avoiding encounters (Skinner & Smither, 1990; Estes, 1991; Wilson & Mittermeier, 2009).

1.3.5 - Social and spatial behaviour

The Lion is the only communally living cat with gregarious habits (Estes, 1991; Hunter & Barrett, 2011). Sociality in this species was probably a way to increase hunting success on larger prey, defence of cubs and kills, maintenance of territories for longer periods, and insurance against individual injury or incapacity (Wilson & Mittermeier, 2009). Lions have a matriarchal society whose core unit is the pride with lionesses being the nucleus of the society. Prides usually include a group of related females, none dominant, their cubs and the pride male(s) of which, one is usually dominant. The size of the pride is usually measured by the number of adult females (Wilson & Mittermeier, 2009). Prides are considered "fission-fusion" social units where membership is unstable, especially for males, whereas females may only occasionally leave the pride due to emigration as sub-adults or after a takeover, becoming peripheral, unless they mate with the incoming males (Skinner & Smither, 1990; Estes, 1991; Haas et al., 2005; Wilson & Mittermeier, 2009). However, pride members can often be found inside the pride's range, distributed into small subgroups, operating in different parts of the territory, and not necessarily moving all together (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Lions are mostly sedentary and territorial although some populations move seasonally following migratory prey. While most lions live in resident prides, some individuals are nomadic, especially males and sub-adults (Haas et al., 2005; Wilson & Mittermeier, 2009). Females actively defend their range against other prides and strange males. The pride male(s) are initially immigrant males, unrelated to the breeding females (although they may exceptionally breed with related pride females), who gain tenure over one or more prides and actively defend them against strange males. In average, male lions hold tenure over a pride for only 2-3 years but coalitions of males (usually consisting of 2-4 males but can go up to 9) can maintain their tenure more than twice as long as 1-2 males (Skinner & Smither, 1990;Estes, 1991; Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009;). Due to the intolerance of adult males, male offspring have to leave their original pride usually around 3 years of age (25-48 months) entering a nomadic phase lasting 2-3 years until they acquire their own prides (Estes, 1991; Hunter & Barrett, 2011) . Males forming larger coalitions (more than 4 individuals) are usually related but smaller coalitions of 2-3 individuals are formed by unrelated individuals. Coalition males are highly cooperative. They usually remain together for life, hunt and scavenge cooperatively, compete for tenure over a new pride against resident males and defend their tenure against foreign males. Fights between rival males attempting to take over a pride are very serious and may result in death (Skinner & Smither, 1990) .In order to propagate their genes and assure paternity during their reproductive lifetime, infanticide is common and it is considered a vital part of male reproductive strategy (Estes, 1991), when new males take over a new pride. Therefore, a month after takeover most females with dependent cubs end up losing their young whereas pregnant females lose cubs shortly after giving birth (Wilson & Mittermeier, 2009). Females can be wounded and even killed while defending their young from infanticidal males and older juveniles may escape with their lives but are not able to survive on themselves unless their mothers leave with them as sometimes happens (Estes, 1991). Females show a burst of heightened sexual activity for an average of 3 months following a takeover, initiating more copulation and attracting more partners than normal. Even pregnant females go through an apparent oestrus while others seem to fail to ovulate during this time although they come into heat every few weeks (Estes, 1991; Haas et al., 2005; Wilson & Mittermeier, 2009). During this period lionesses remain infertile, and tend to breed in synchrony, when the tenure has stabilized. This mechanism contributes to bond the new males to the pride reducing the likelihood of desertion, while providing time to ensure that the fittest coalition is able to gain tenure (Estes, 1991; Wilson & Mittermeier, 2009). Female pride members share several cooperative behaviours unique among felids such as giving birth at about the same time, caring for the cubs communally allowing allosuckling of cubs and cooperative hunting (Estes, 1991; Wilson & Mittermeier, 2009). Litters born synchronously seem to have a higher survival rate and show a sex ratio biased towards male cubs (Wilson & Mittermeier, 2009). Prides occupy home ranges varying from 25 - 226km^2 (Wilson & Mittermeier, 2009), but can be over 400km where prey density is low (Estes, 1991). In some types of habitats overlap of home ranges can happen but in others ranges are largely exclusive, and actively defended. The defence of the territories appears to be related to the movement of the lion's prey species (Skinner & Smither, 1990; Wilson & Mittermeier, 2009).

1.3.6 – Reproduction

Lions have no fixed breeding season but females are usually synchronized within prides. Birth peaks have been recorded in certain areas, probably related to prey availability and seasonal weather conditions (Haas et al., 2005; Wilson & Mittermeier, 2009). According to Estes (1991) females start breeding when they are around 4 years old (43-53 months) and continue until they are about 15 years old, usually giving birth to a litter every two years (Skinner & Smither, 1990; Haas et al., 2005; Wilson & Mittermeier, 2009). Females come into ooestrus in response to within-pride mechanisms such as estrus of other lionesses in the pride as well as loss of cubs due to male infanticide at pride takeovers (Haas et al., 2005). Lions are polyoestrous and oestrus lasts usually around 4-16 days. Inter-oestrus interval may last from a few days to over a year, on average 16 days. Both male and female may start the courtship and remain close to each other during the mating period. The female shows her availability for copulation by lordosis and the male follows and rests with her at all times (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). If there are other male members of the pride close by, they are usually tolerated and multiple-mate copulations may occur, but foreign lions are not allowed nearby (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). However, not every male in the coalition may have a chance to mate regardless of being relatives or not (M. Sunquist & Sunquist, 2002). Unlike other carnivores, there is very little aggressive behaviour during the mating period and pairs may copulate as often as every 15 minutes, and each copulation lasts usually up to one minute (Skinner & Smither, 1990; Estes, 1991; Wilson & Mittermeier, 2009). Many copulations appear to be necessary to induce female ovulation but after ovulation fertility is very high (Estes, 1991). Most copulation do not result in pregnancy (Skinner & Smither, 1990). Pregnancy lasts a mean of 110 days (100-114) and litter size averages 2, 5 – 3 although up to 6 cubs have been recorded in a litter. Still, 89% of litters have 1-4 cubs (Skinner & Smither, 1990; M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). Lionesses usually give birth to a litter every 20 months in average (range 11-25) if the previous litter survives to maturity, or 4-6 months if the previous litter is lost (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Females usually leave their pride for 4-8 weeks to give birth and later re-join the pride with their 4-8 week-old cubs (Wilson & Mittermeier, 2009). Usually any lactating female in the pride will allow suckling from cubs other than her own and cubs will suckle regularly during a period of 6 – 7 months, suckling less after 7 months and usually ceasing to suckle around twelve months of age. Young lions stay with their mothers for 21-30 months and grow rapidly during the first three years (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Cub mortality is considerably high, with a 40-50% survival rate, depending on factors such as food supply and pride takeover as smaller prides usually do not have males in constant attendance and therefore are more likely to lose their cubs (Skinner & Smither, 1990; M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). Packer and Mosser (2009) also showed that lioness's reproductive success and survival is negatively influenced by the presence of neighbors. Lion cubs, stay with the maternal group much longer than any other cat (Estes, 1991). Young individuals leave their original pride only around the age of 24-42 months. Males become sexually mature at 26 months, but will usually mate for the first time only when they are about five years old, usually when they gain tenure over a pride. Females have their first pregnancy around 43 months of age and continue to breed until they are about 15 years old, producing a litter every two years (Skinner & Smither, 1990; M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009).

1.4 - Leopard (*Panthera pardus*)

The Leopard is the largest spotted cat in Africa. They are not very specific regarding their habitat requirements and their diet can be made of any type of prey. Leopards are considered to be the most widespread of all felids after the domestic cat (Estes, 1991). They are very tolerant to human activities and are able to persist near human populations where other large carnivores cannot (M. Sunquist & Sunquist, 2002; Hunter & Barett, 2011). The main threats to the species survival are the loss of wild prey and habitat (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). Leopards are heavily hunted mostly in southern Asia for their skins and parts to be sold for Chinese traditional medicine and also in West and Central Africa for their skins, canines and claws (Hunter & Barett, 2011). As many as 24 subspecies are recognized nowadays. For this reason, recent morphological and genetic analysis have suggested a new classification including all African leopards as *pardus*, all populations in India as *fusca* and all Central Asian leopards as *saxicolor* (Wilson & Mittermeier, 2009). Due to its wide geographic distribution and variety of subspecies, the Leopard is considered endangered in some parts of its range while being seen as a problem animal in others.

- 4 subspecies are now listed in CITES Appendix I (Arabian, Amur, North African and Anatolian Leopard) and classified as Critically Endangered on the IUCN Red List.

- 4 subspecies listed as Endangered (Caucasus, Sri Lankan, North Chinese and Javan Leopard)

- Others subspecies are classified as Near Threatened on The IUCN Red List.

1.4.1 – Habitat and Distribution

Leopards seem to be able to inhabit almost every type of habitat being absent only from open interiors of true deserts and are often able to survive in close proximity with humans (M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009 ;Hunter & Barett, 2011). It is described as the least specialized of the big cats being able to persist wherever diversified habitats afford a variety of small to medium-sized mammals (Estes, 1991). Leopards range from Russian boreal forests where winter temperatures can reach -30ºC to desert areas with summer temperatures of nearly 70ºC. Studies have shown that leopards can survive without drinking for ten days and may use caves, burrows or the shade of thick vegetation to avoid the heat. On the other hand, in areas where snow is abundant throughout the year, leopards seem to prefer habitats where snow depth does not exceed 15 cm (Wilson & Mittermeier, 2009).

The species was once distributed over much of Sub-Saharan Africa and throughout northern Africa. In Asia, the species range extended East through India, Sri Lanka, China, Tibet, the Russian Far East and Southeast Asia. Leopards are found in Java but are absent from Sumatra and Borneo. Although they are still quite widespread (Figs. 12 and 13), their historic distribution was probably much larger as Pleistocene fossil records have been found in Europe, Middle East, India, Africa and Java (M. Sunquist & Sunquist, 2002).

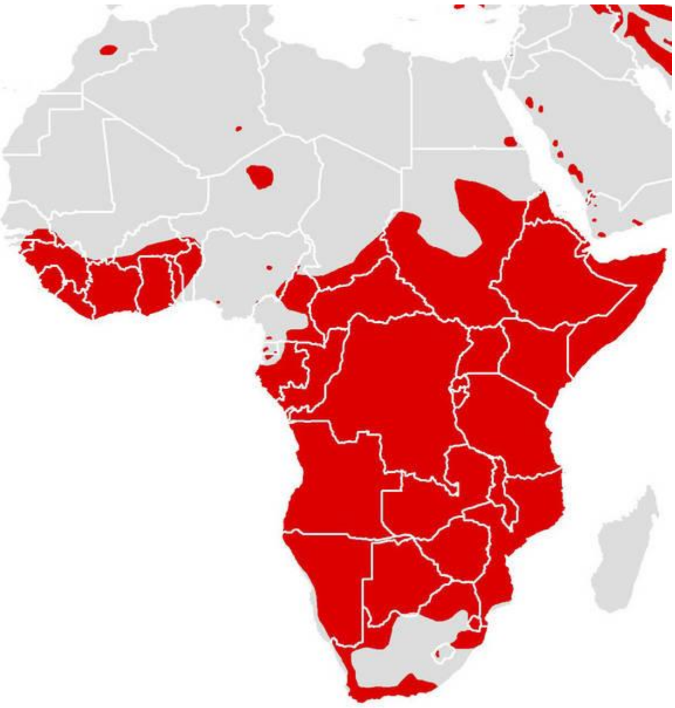


Figure 12 – Present distribution of the Leopard in Africa (Source: Cat Specialist Group Web Portal. Accessed on March 2015)

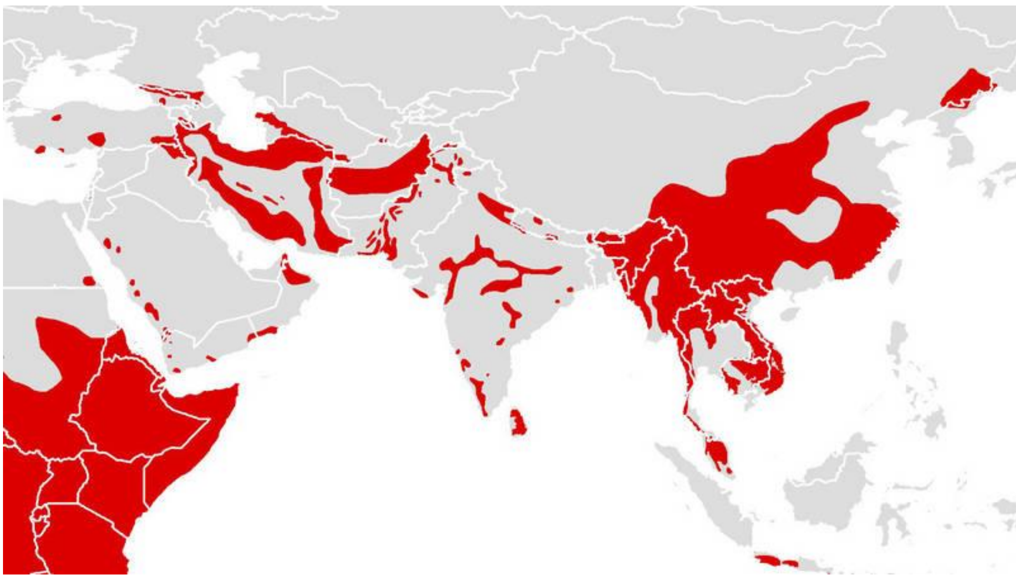


Figure 13 - Present distribution of the Leopard in Asia (Source: Cat Specialist Group Web Porta. Accessed on March 2015)

1.4.2 – Description

Leopard's background coat colour varies according to each region and may range from a bright golden yellow to a pale cream or a wide variety of shades of orange as well as dark-brown, with white underparts. Some areas of their coat are also covered with black spots (head, neck, shoulders, limbs and hindquarters) which form broken circles or rosettes on the sides and back of the animal (Wilson & Mittermeier, 2009). Each of these rosettes are made of small black spots around a normally unspotted centre which is usually darker than the body colour (Hunter & Barett, 2011). The pattern of markings on each individual is unique (Fig14). Body size also varies considerably among the different regions where leopards occur, but adult males are larger than adult females. Female head-body length may range from 95-123 cm whereas males can reach 91-191 cm. The tail may range from 51-101 cm in length. Leopards usually weigh between 21-71kg but some males in particular can weigh up to 90 kg whereas females usually don't weigh more than 43kg (Wilson & Mittermeier, 2009; Hunter & Barett, 2011). The skull is also different between both genders with males having a larger, longer and more angular skull with a well-developed sagittal crest which is almost absent in the females' skull, and larger teeth (M. Sunquist & Sunquist, 2002).

Figure 14 -Female leopard from Niassa Reserve (Photo: Colleen Begg)

1.4.3 – Feeding ecology and feeding habits

Leopards are adaptable generalists and can prey on a wide variety of animals from insects to very large antelopes like Elands but usually prefer medium-sized ungulates. In sub-Saharan Africa there at least 92 prey species that are known to be part of the Leopard's diet (Estes,1991;M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009). The typical prey of the Leopard varies throughout the species range but may include Steenbok, Duikers, Impala, Gazelles, Nyala, Muntjacs, Chital, Roe Deer, Bushpig and Warthog and younger individuals of other larger species such as Kudu, Wildebeest, Oryx and Hartebeest. African Leopards from rain forest areas prey significantly more on a wide variety of primates, but still prefer medium-sized ungulates. The Leopard's diet can also include some carnivore species such as Aardwolf, Cheetah, Genet and Bat-eared Fox. In areas where Leopards live in close proximity with human populations, they can prey on dogs, cats, sheep, goats, calves and pigs and sometimes can even hunt humans (Wilson & Mittermeier, 2009; Hunter & Barett, 2011). Leopards are considered to be stalk-ambush predators, as they prefer to approach and stalk their prey as close as 4-5m before launching an attack. Hunting usually takes place at night or at crepuscular hours and most hunting attempts during the day are unsuccessful. In order to avoid getting robbed of their kills by scavengers, leopards can go to great lengths to find areas with dense cover and they can also climb trees dragging the carcasses to the top. This habit is more common in Africa where other large carnivores like hyenas and lions would readily steal the leopard of its kills (M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009; Hunter & Barett, 2011).

1.4.4 – Activity patterns

Leopards are solitary animals except during the mating periods and when females are accompanying their cubs, and are mainly nocturnal (Fig.15) although they can be occasionally seen moving during the day. During the day when temperatures are higher, leopards will often lie down in areas with dense cover, in the shade of rocks, caves and also on top of trees to where they usually drag their kills (Skinner & Smither, 1990). In Sri Lanka, where the Leopard is the only large carnivore, they are often very active during the day which suggests their activity patterns might be influenced by the presence of other large carnivores such as lions and tigers (Wilson & Mittermeier, 2009). Also, in some areas where other large carnivores are absent the Leopard seems to be less nocturnal and more terrestrial (M. Sunquist & Sunquist, 2002). Leopards are also very good swimmers (Skinner & Smither, 1990).

Figure 15 - Camera-trap footage of a Leopard moving during the night (Source: Niassa Lion Project)

1.4.5 – Social and spatial behaviour

The Leopard is a territorial species and adults defend their territories against other individuals of the same sex while allowing some degree of overlap at the edges of their territories. Still, territorial fights are rare as individuals usually try to avoid encounters but may result in death for both females and males. The territory tenure system of leopards is similar to other solitary cats with males usually occupying larger areas which may overlap the territories of one or more females. For example, female territories in Kruger National Park averaged 14.8 km^2 in size while males in the same area occupied territories two and sometimes three times bigger than females. In terms of size, Leopard's territories seem very similar throughout their distribution except in more arid areas such as the Kalahari where home range sizes can be a lot higher due to low densities of prey. In the Kalahari a male has occupied a 2710 km^2 territory while a female in Tsavo only had a territory of about 5,6km^2 (Skinner & Smither, 1990; M. Sunquist & Sunquist, 2002; Wilson & Mittermeier, 2009;Hunter & Barett, 2011).

Leopards seem to never form family groups. Adults only remain together for short periods of time during mating periods and cubs become independent as sub-adults around 22 months of age (Estes, 1991). In some parts of Africa females appear to be philopatric as daughters usually establish territories close to their mothers' (Wilson & Mittermeier, 2009).

The principal mean of communication used by Leopards seems to be through olfactory information carried in urine, faeces and anal sac secretions. Although scent-marking seems to be the most used method of communication among leopards, visual markings such as scrapes and scratches in trees or on the ground and auditory signals such as growling or grunting are also used (Skinner & Smither, 1990; M. Sunquist & Sunquist, 2002).

1.4. 6– Reproduction

Leopards are considered to have no fixed breeding season but in most of their northern range (China, Korea, Russia) the extreme winters may induce seasonality (Hunter & Barett, 2011). Individuals of both genders reach sexual maturity around 24-28 months of age. Females usually have their first pregnancy around 30-36 months and may have litters until they are as old as 16 years. On the other hand, males usually breed for the first time at around 42-48 months of age (Wilson & Mittermeier, 2009; Hunter & Barett, 2011).

The oestrus usually lasts around 7-14 days and reoccurs around every 46 days until there is a successful pregnancy (Estes, 1991). In Leopards, gestation period ranges between 90-106 days and females usually give birth in caves, sheltered places among rocks, hollow trees or in holes in the ground. Females may produce a litter every 16-25 months (Skinner & Smither, 1990; Hunter & Barett, 2011). Litters usually have between 1-4 cubs weighing approximately 400-600g at birth (Estes, 1991). During the first few days, the mother stays at the den most of the time nursing her cubs but after this period she can leave the den for longer periods (24-36 hours). Leopard cubs are very vulnerable to predation especially by lions and most cub mortality occurs in the earliest months of life. The cubs open their eyes for the first time at 6-10 days but only leave their hiding place around 6 weeks when they also start to eat some meat while following their mother on short excursions (Estes, 1991; Hunter & Barett, 2011). Weaning period usually starts at around 8-10 weeks and most cubs will stop suckling before the age of 4 months. The cubs become independent at around 12-18 months and while females tend to inherit part of their mothers' territory, males usually disperse more widely. Still, the timing of the cubs' dispersal varies from 15-36 months (Wilson & Mittermeier, 2009; Hunter & Barett, 2011).

1.5 - Diseases and their influence on the conservation of wild felids

Diseases are an important part of ecosystems and have the potential to influence not only individuals but whole populations and even their evolutionary process. Felids are no exception and they are often targeted by specific pathogens which are either present in the environment – endemic pathogens – or circulate periodically among felids and other hosts in the ecosystem – epidemic pathogens. Still, due co-evolution mechanisms, felids have been well adapted to the presence of these endemic infectious pathogens in stable ecosystems, and while these agents may be able to trigger disease in some individuals, infection does not always result in disease. Therefore, in such conditions, where endemic pathogens and their hosts have evolved together, felids have either developed individual immunity, or as a population, and consequently disease usually does not occur (Macdonald & Loveridge, 2010).

The outcome of an infection in an individual animal, as a result of the contact between an infectious agent and its felid host, depends on the amount and virulence of the acquired pathogen on one hand, and the host's immunocompetency and immune status on the other. Epidemics arise when this host/pathogen balance is disrupted or when high virulent pathogens are introduced in the ecosystem. It is widely recognized that most felid populations are now at risk of exposure to new pathogens since the ecosystems they inhabit are changing dramatically. Behaviour is also an important part of the ecology of infectious diseases, since some more social species like the lion are more likely to acquire infectious agents through direct contact within the prides (Macdonald & Loveridge, 2010). Under normal circumstances, populations can withstand losing some individuals to diseases without major consequences for their conservation. Still, some diseases and epidemics have already had considerable effects in populations of free-ranging felids. Such effects are even more relevant when concerning highly endangered felid populations like the Iberian lynx (Meli, Cattori, Martínez, López, Vargas, Palomares, López-Bao, Hofmann-Lehmann, & Lutz, 2010).

In 1994 the Serengeti lion population lost one third of its individuals, and such high mortalities coincided with a major epidemic of Canine Distemper Virus (CDV). Later, in 2001 another high mortality epidemic affected the nearby Ngorongoro Crater lion population. Retrospective studies indicate that CDV infection alone was not responsible for such high mortalities as serological evidence has shown that between 1976 and 2006 the same two lion populations have been affected by five silent-epidemics of CDV without any visible clinical symptoms or fatal consequences. However, clinical and pathology findings suggested that the lions were also infected with haemoparasites during the fatal epidemics and as a result of 22 years of blood sample collection it was possible to lions find significantly higher levels of *Babesia* sp. infection during the fatal epidemics. Additionally, severe droughts have been recorded right before the fatal epidemics. During the droughts, many herbivores species, mostly buffalos, have also experienced severe mortalities. It is believed that after the resumption of rains, heavy tick infestations in starving buffalos, made lions more likely to be infected with higher levels of *Babesia* sp. The immunosuppressive effects of CDV associated with unusually high loads of *Babesia* sp. resulted in the mass mortalities experienced during those outbreaks (Munson, Terio, Kock, Mlengeya, Roelke, Dubovi, & Packer, 2008).

Endangered felid species and/or populations are often also affected by low genetic variability which is not considered to be a disease in itself but is sometimes the underlying cause enhancing the impact of certain pathogens in felid populations. Low genetic variability due to a very restricted habitat and close proximity with human populations in the Ngorongoro Crater in Tanzania, has made the lion population unable to recover from three epidemics since 1994. The simultaneous infection with Canine Distemper Virus (CDV) and high loads of *Babesia* sp., reduced the lion population from 124 individuals to less than 40. On the other hand the less inbred Serengeti lion population was able to recover from the same epidemics much easier. It has been suggested that the slow recovery faced by the Ngorongoro Crater lions is most likely related to poor sperm quality and impaired testicular function probably resulting from inbreeding (Macdonald & Loveridge, 2010).

The impacts, exposure and relevance of Canine Parvovirus (CPV) have not been studied as intensively as other pathogens in some African carnivores, but it has already caused considerably high cub mortalities in wolves (Johnson, Boyd, & Pletscher, 1994) and could affect recruitment of wild dogs as well (Creel & Creel, 1998). Still, some studies on the prevalence of this virus in free-ranging African carnivores show a considerable prevalence of CPV exposure in such species (Driciru, Siefert, Prager, Dubovi, Sande, Princee, Munson, 2006; Berentsen, Dunbar, Becker, M'soka, Droge, Sakuya, Hanlon, 2013). In most situations, an increase in human settlements and domestic dogs around protected areas, plays an important role in disease transmission to carnivores (Berentsen et al., 2013). Feline Coronavirus (FCoV) is another pathogen known to affect a wide spectrum of wild felids, causing a disease known as Feline Infectious Peritonitis (FIP). Cheetahs have been found to be very susceptible to FCoV infection and disease (Evermann, Heeney, Roelke, McKeirnan, & S.J. O'Brien, 1988). Studies on the prevalence of antibodies to FCoV on free-ranging lions from different parts of Africa have shown a low number (3%) of FCoV positive lions in Etosha, none in Kruger National Park and 57% in a broader study in Tanzania's National Parks (Hofmann-Lehmann et al., 1996).

Another example of a disease influencing the conservation of wild felids is Bovine Tuberculosis (BTB). This disease is caused by *Mycobacterium bovis,* a cosmopolitan pathogen and it is considered to be a livestock associated disease that has also been widely recorded to affect several species of felid hosts. The disease has become common amongst wild animals who live closer to humans, like those in zoos (De Vos et al., 2001), but it has also been an increasingly relevant threat to wild felids due to the infection of their prey (Michel et al., 2006Macdonald & Loveridge, 2010). The infection of wildlife with *Mycobacterium bovis* is most likely the result of an increased contact with livestock in some areas. Some consider that the introduction of this pathogen in African ecosystems was a result of the importation of infected cattle from other areas, mainly from Europe. Tuberculosis has been reported to affect several species of free-ranging wild felids such as lions (Maas, Keet, Rutten, Heesterbeek, & Nielen, 2012), cheetahs (Keet, Kriek, Penrith, Michel, & Huchzermeyer, 1996) and Iberian-lynxes (Meli et al., 2010). The felids are not considered to be a reservoir for *Mycobacterium bovis* and the infection of such species is thought to be sporadic and accidental (Macdonald & Loveridge, 2010). As for lions, the tuberculosis epidemics in the Kruger National Park, South Africa, became one of the most studied scenarios regarding the transmission of this pathogen in wild animals. The disease usually has a slow progression providing more opportunities for a broader dissemination within the population. In the year of 2000, almost 80% of the lions in the south of Kruger National Park (KNP) were infected with BTB (Maas et al., 2012). Lions and other felids acquire the infection mostly through ingestion of infected prey but transmission between individuals is possible through the aspiration in aerosols.

Anthrax is a disease caused by *Bacillus anthracis* which is another relevant pathogen for wild felids that can be acquired through ingestion of infected prey or inhalation. This bacteria can also persist for long periods in the environment and has been known for affecting many different species of wildlife (Good, Houser, Arntzen, & Turnbull, 2008). In Namibia's Etosha National Park, anthrax is suspected to be the reason why viable cheetah populations are hard to maintain (Macdonald & Loveridge, 2010). The species well known lack of genetic diversity is also responsible for significant lack of variation in the major histocompatibility complex (MHC) and therefore may be influencing their ability to mount a strong immune response to infectious agents (Turnbull et al., 2004).

Mange is an ectoparasitic skin disease caused by mites like *Notoedres cati* and *Sarcoptes scabiei* and it is also a disease with recorded impacts on wild felids. The occurrence of the disease depends on many factors such as poor health condition, lack of immunity or other environmental, social or nutritional stressors. This disease has been recorded to affect many wild felid species such as Eurasian lynx, Bobcat, Leopard, Cheetah, Ocelot, Puma, Snow leopard, Lion and Serval. Although not associated with high mortalities, mange appears to be an unlikely threat to stable wild felid populations whereas other fragmented, endangered or genetically inbred populations may be affected by drastic short-term effects of this disease, and therefore it should be monitored in such cases (Macdonald & Loveridge, 2010).

1.6 - Lion Gastrointestinal Parasites

The prevalence and consequences of parasite infection in large wild carnivores is poorly understood. Parasites are usually considered to be endemic pathogens which coexist in a fine balance with their hosts and ecosystems and although parasites are not always a cause of disease in wild animals they may influence competitive fitness at an individual level and may even predispose their hosts to infection from other pathogens such as virus and bacteria. Over the years, there have been numerous reports regarding lion endoparasites but most of them have been a result of studies in captive lions from zoos, game reserves or recreational parks. Such reports usually lacked important information about the animal's diet, movements and the way they were being managed. Therefore, this information is not reliable for the purpose of assessing the endoparasite diversity of wild lions. However, there are also some studies which surveyed the gastrointestinal fauna of free-ranging wild lions and the currently available information shows that lions can be hosts of many different parasites (Mukarati, Vassilev, Tagwireyi, & Tavengwa, 2013). Since wild lions usually prey on a wide spectrum of species, their diverse endoparasite fauna is most likely a result of their very diverse diet. Some of the endoparasites found in free-ranging lions are true lion parasites whereas others are considered to be accidental parasites. Müller-Graf (1995) has described 15 different parasite taxa in wild lions in northern Tanzania, from the Serengeti National Park and the Ngorongoro Crater Conservation Area. Later, Bjork et al. (2000) identified nineteen different parasite species in the same lion populations. Smith & Kok (2006) conducted a study on a small population of lions in the Kalahari where they identified three species of nematodes and two species of coccidian. More recently, Berentsen et al. (2012) conducted a gastrointestinal parasite infection survey in Luangwa Valley, Zambia, in which they also found some parasite species infecting both lions, hyenas and wild-dogs. Some of the parasite species found are common to all areas that have been surveyed. Although some of the parasites species found were already known and were readily identified others could not be identified beyond the genus level. The parasites described so far in wild African lions are: *Eimeria* sp., *Giardia* sp., *Cystoisospora felis, Cystoisospora rivolta, Sarcocystis* sp., *Toxoplasma gondii*, *Aelurostrongylus* sp., *Ancylostoma* sp., *Ancylostoma paraduodenale, Capillaria* sp., *Habronema* sp., *Toxocara cati, Trichuris* sp., *Ancylostoma braziliense*, *Gnathostoma spinigerum*, *Uncinaria stenocephala, Spirometra* sp., Taeniidae, *Physaloptera* sp. Paramphistomatidae. In order to put the results of the present study into context, a more detailed revision about the six parasite species found is required and will be developed in the following subchapters.

1.6.1 - *Linguatula* sp.

Linguatula sp is a parasite genus belonging to the *Pentastomida* Phylum which consists of about 100 species of linguatulids, also called tongueworms (Ravindran, Lakshmanan, Ravishankar, & Subramanian, 2008). They are highly specialized crustaceans and endoparasites of a wide variety of predatory mammal, reptile and bird hosts (Bowman, 2014). Amongst mammals, carnivores are the most common definitive hosts for Pentastomids. The adult stages typically inhabit the respiratory passages, nasal sinuses and nasopharynx of their definitive hosts where the females lay their eggs. The eggs containing fully developed larvae are consequently discharged from the respiratory tract in mucus and nasal secretions or they can be swallowed and passed in the faeces. Many domesticated and wild species of herbivores serve as intermediate hosts of these parasites and will ingest the eggs via contaminated water or food. Humans may also acquire the eggs accidentally through the consumption of contaminated plants or vegetables, or the larvae and nymphs while ingesting raw meat and periorganic tissues from animals acting as intermediate hosts like the domestic and wild artiodactyl ungulates. After ingestion, the eggs will hatch in the intermediate hosts' digestive tract from where the larvae will invade other tissues and organs, mainly the mesenteric lymph nodes where they encyst, and complete their development to nymphs. Nymphs can grow up to 60 mm (Rezaei, Tavassoli, & Javdani-Gandomani, 2012). Except for their mature reproductive organs, nymphs and adults are very similar. Both have four hooks surrounding a central mouth and possess a flat segmented body with transversally striated spines (Fig.16) (Sivakumar, Sankar, Nambi, Praveena, & Singh, 2005). However, adults have a highly pronounced sexual dimorphism with females growing up to 130mm whereas males only grow up to 20mm (Rezaei et al., 2012). *Linguatula serrata* can lead to nasopharyngeal linguatulosis in human and dogs resulting in a condition called *halzoun* or marrara syndrome (Sivakumar et al., 2005).

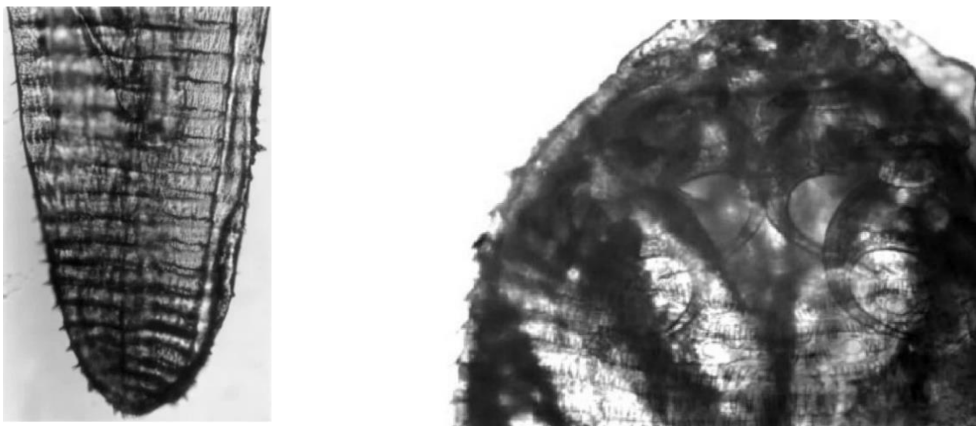
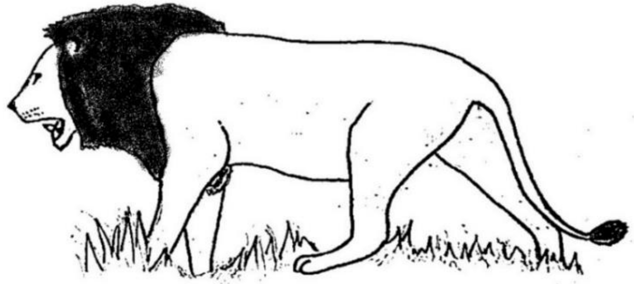


Figure 16 – Nymph body segments and spines (left), mouth with four hooks (right) of *Linguatula serrata* - Adapted from: Sivakumar P. et al. (2005)

Linguatula sp. life cycle in Lion

Mammalian carnivores and even humans acquire the nymphs through the ingestion of raw or undercooked viscera. The nymphs will then invade the nasal cavities and complete their development to adults within 6-7 months.

The adult females lay eggs containing fully developed larvae on the nasal sinuses and nasopharynx of carnivores which are then released to the environment through nasal secretions or swallowed and passed in the feces.



Linguatula sp. egg (40x) -Original

A wide variety of domestic and wild herbivores serve as intermediate hosts after ingesting the eggs through contaminated food or water. Humans can also ingest the eggs accidentally.

In the herbivore's digestive tract, the eggs hatch and complete their development to nymphs migrating and encysting in various organs, mainly mesenteric lymph nodes.

Mesenteric lymph node containing a nymph stage (black arrow). Adapted from: Sivakumar P. et al. (2005)

Figure 17 - *Linguatula* sp. life cycle in Lion (Original)

1.6.2 - *Aelurostrongylus* sp.

Aelurostrongylus abstrusus also known as feline lungworm is a nematode species from the *Metastrongylidae* family known to affect both domestic and wild felid species (Di Cesare, Crisi, Di Giulio, Veronesi, Frangipane Di Regalbono, Talone, & Traversa, 2013). This parasite has an indirect life cycle. Adults can be found in the terminal respiratory bronchioles, alveolar ducts, and alveoli of the felid definitive host where they also mate and lay their eggs. The eggs hatch in the airways and the first-stage larvae (L1) are carried up the tracheobronchial tree to the pharynx, and swallowed in order to be released in the host's feces (Fig.18). After being released into the environment, these larvae will only complete their development if they enter a suitable host. In this case, a wide variety of slug and snail species such as *Helicella* sp., *Limax flavus*, *Theba pisana, Cernuella virgata, Achatina fulica* and *Helix aspersa* serve as intermediate hosts where the L1 larvae undergoes two molts, to reach its infecting L3-stage. In domestic cats, the ingestion of slugs or snails is uncommon and therefore infection occurs mostly through predation of paratenic hosts like mice, birds, lizards, snakes and frogs that usually include snails and slugs in their diet. After ingestion by a paratenic host, the third-stage larvae (L3) encyst and stop its development until being ingested by a felid host. The larvae will then reach the cat's lungs through the bloodstream and lymphatic vessels showing up in the host's faeces 5-6 weeks later (Bowman, 2014). The disease is caused by the presence of the adults, eggs and larvae resulting in damage to the alveolar ducts and the alveoli. In domestic cats the disease is characterized by a wide variety of symptoms that are not always detectable and can range from minimal respiratory signs such as occasional coughing to interstitial bronchopneumonia with open mouth abdominal breathing, sneezing, dyspnoea, intense coughing, and even hydrothorax in the most severe cases (Traversa, Lia, Iorio, Boari, Paradies, Capelli, Avolio, & Otranto, 2008). The severity of the infection depends not only on the parasite load of the host but also its age, immune status and presence of other diseases.

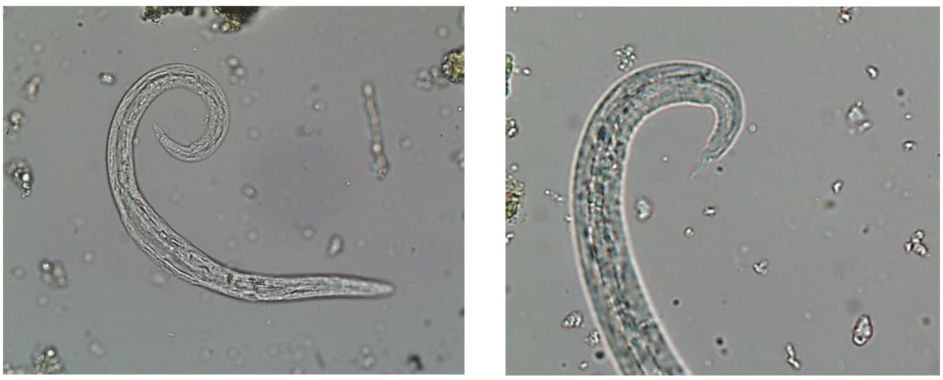


Figure 18 - L1 Larvae of *Aelurostrongylus* sp. (left) (40x) and detail of the characteristic "kinky tail" (right) (53x) - Original

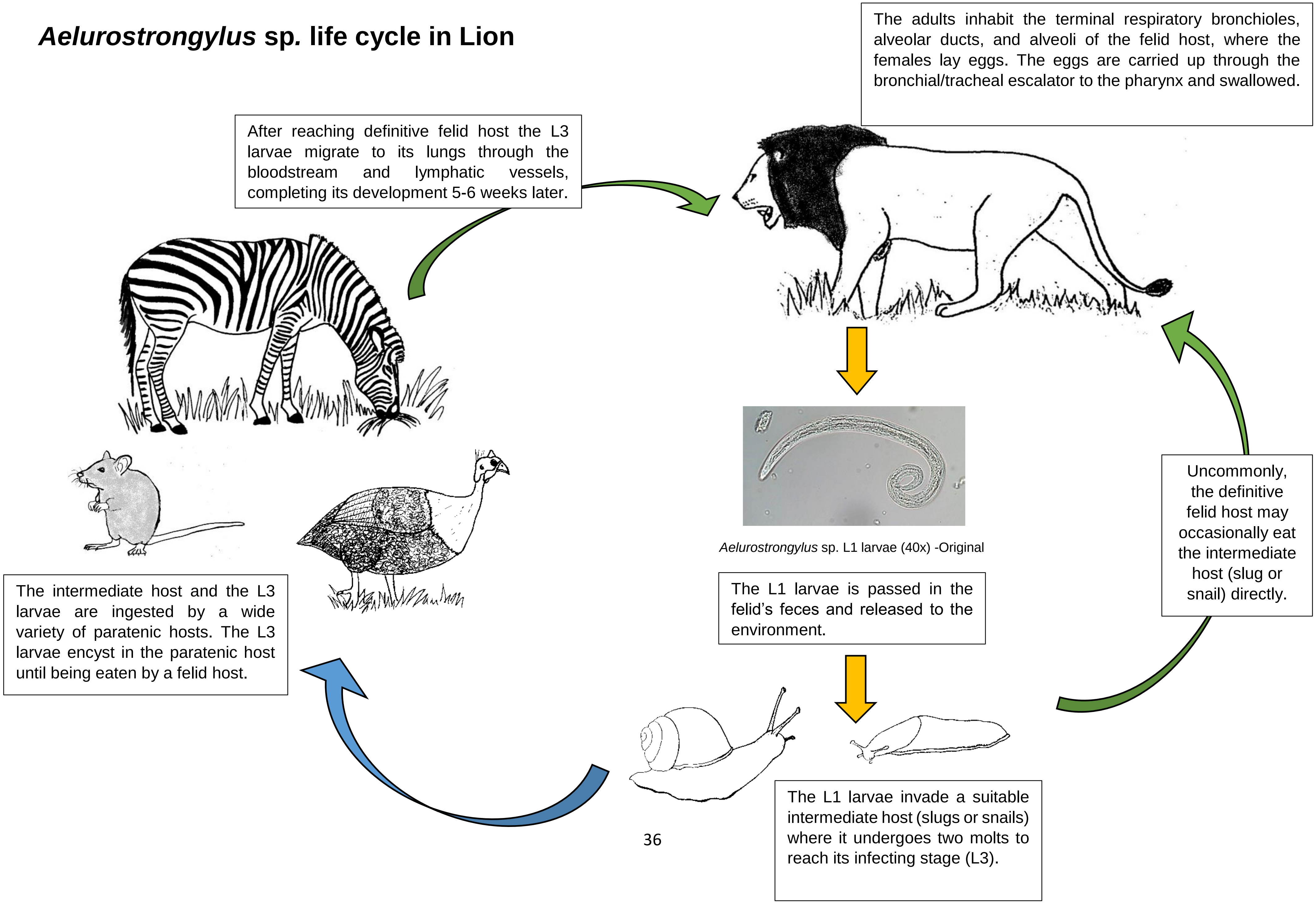


Figure 19 - *Aelurostrongylus* sp. life cycle in Lion (Original)

1.6.3 - *Spirometra* sp.

Spirometra and *Diphyllobothrium* are the two most important genera amongst pseudophyllidean tapeworms in veterinary medicine. Both belonging to the Diphyllobothriidae Family they share a typical pseudophyllidean morphology with only two shallow, longitudinally grooved bothria for locomotion and attachment and no hooks. Apparently the necessary traction to keep the parasite in place is afforded by the long chain of broad body segments which provide a considerable area of contact between the intestinal mucosa and the tapeworm (Bowman, 2014). Unlike other tapeworms of dogs and cats, *Spirometra* and *Diphyllobothrium,* do not detach whole body segments but instead release their eggs through the uterine pores of their mature segments. These two genera are also similar in their life cycles but *Diphyllobothrium* species use only aquatic intermediate hosts (copepods and fish) while *Spirometra* species use amphibious and terrestrial intermediate hosts (copepods, amphibians, reptiles, mammals and birds)(Bowman, 2014). In both cases, the life cycle of the parasite involves the ingestion of eggs by copepods. Copepods are crustaceans with a shrimp-shaped body that often ingest parasite forms like nematode larvae or tapeworm coracidia, serving as either transport or necessary intermediate hosts. Of the three major groups of existing copepods, cyclopoids (Genus Cyclops) are the most commonly involved in parasite life cycles. *Spirometra* adults inhabit the small intestine of some carnivorous mammals (dog, cat, bobcat, raccoon and even humans), where they lay eggs that are released to the environment with their definitive host's faeces. The eggs deposited in water hatch and release the coracidium (oncosphere with ciliated embryophore) that will swim until being ingested by a copepod. In the body cavity of the copepod, the coracidium sheds its ciliated coat and develops into a second larval stage called a procercoid. Then, a second intermediate host ingests the infected copepod and the procercoid invades its musculature or connective tissues and develops further into a third larval stage called a plerocercoid. There is a wide variety of vertebrate species (except fish) serving as second intermediate hosts. The definitive host becomes infected after ingesting a second intermediate host. However, plerocercoids are able to survive predation of their hosts and remain in this development stage in new paratenic hosts until they reach a suitable definitive host. In humans the plerocercoids cause a condition called sparganosis (Bowman, 2014).*Spirometra* sp. eggs have also been detected frequently in wild African lions (Berentsen, Becker, & Stockdale-walden, 2012; Kavana, Kassuku, & Kasanga, 2015) and in some studies it has been referred as the most prevalent parasite (Müller-Graf, 1995).

Spirometra sp. life cycle in Lion

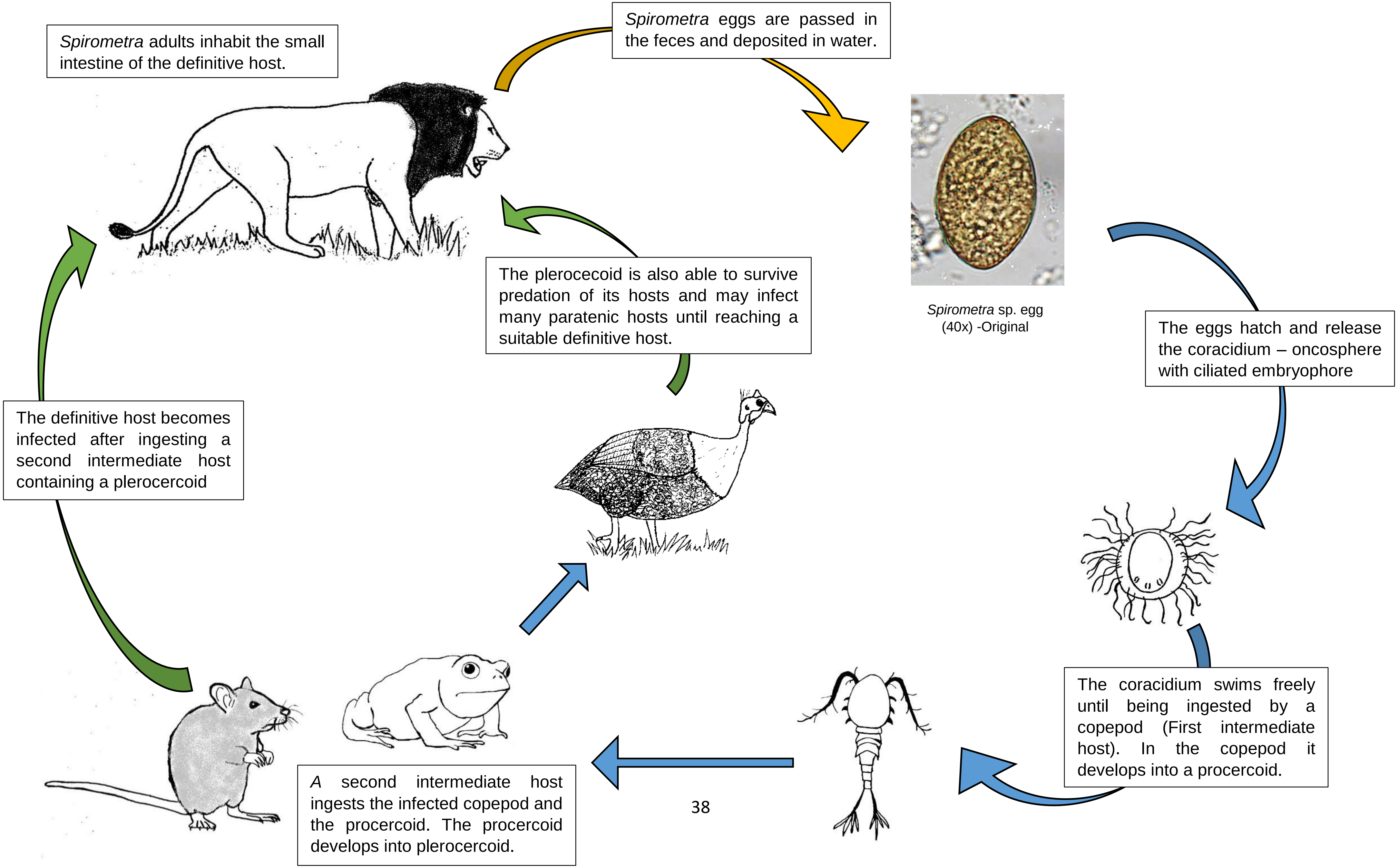


Figure 20 - *Spirometra* sp. life cycle in Lion (Original).

1.6.4 - *Toxocara* sp.

The *Toxocara* genus is a large group of ascaridoid nematodes and a very important one in veterinary medicine and public health. *Toxocara cati* and *Toxocara canis* adults are two of the most common parasites of the small intestine of cats and dogs and both are widely distributed due to human settlement of nearly all land masses (Despommier, 2003). *T. cati* and *T. canis* are also recognized as dangerous zoonosis since the infection with migrating *Toxocara* larvae, can often lead to ocular larval migrans and visceral larval migrans in humans. Human infection usually occurs as a result of ingestion of embryonated eggs from the environment although there is also a possibility of ingesting larvae within small paratenic hosts or in undercooked meat (Fisher, 2003). There are also other species of mammals affected by parasites belonging to this genus, such as calves (*Toxocara vitulorum*) and even elephants, hippos, bats, civets, coatis, rats, mongooses (Bowman, 2014) and caracal (Macchioni, 1999). Previous studies have shown that African lions can also be hosts of *Toxocara* sp and although both *T. canis* and *T. cati* have been reported (Müller-Graf, 1995), *T.cati* seems to be more associated to lions so far (Berentsen et al., 2012; Bjork, Averbeck, & Stromberg, 2000). Regarding their morphology, *Toxocara* adult worms have three large lips, a glandular oesophageal bulb and cervical alae. The eggs have pitted surfaces, are very resistant to environmental conditions and remain infective for years (Bowman, 2014).The lifecycles of *T. canis* and *T.cati* are very similar although there are some differences regarding their migration patterns. Unlike *T. canis*, the pre-natal infection through the placenta does not occur on *T. cati* while transmammary transmission may still occur if cats are heavily infected during the last part of the pregnancy (Bowman, 2014). Also, paratenic hosts serve as an important reservoir of infection for adult cats. For the purpose of the present study, the lifecycle of *T. cati* will be explained with more detail: The adults of *T. cati* inhabiting the small intestine of the felid host produce eggs containing one cell that are released in the cat's faeces. Then, after a period of about 4 weeks an infective larval stage develops inside the egg. From this point, the egg containing the infective larvae may be ingested directly by a cat or by a paratenic host. When ingested by a paratenic host, the egg hatches and the larvae undergo a somatic migration and become arrested in its tissues until the host is eaten by a cat. Most of the larvae acquired this way (or from cats infected acutely during pregnancy) will develop to maturity after a brief period in the stomach of the definitive host. On the other hand, if the egg is ingested directly by a cat host, the larvae will undergo a tracheal or a somatic migration and develop to maturity or stay arrested in the host's tissues (Bowman, 2014).

Toxocara sp. life cycle in Lion

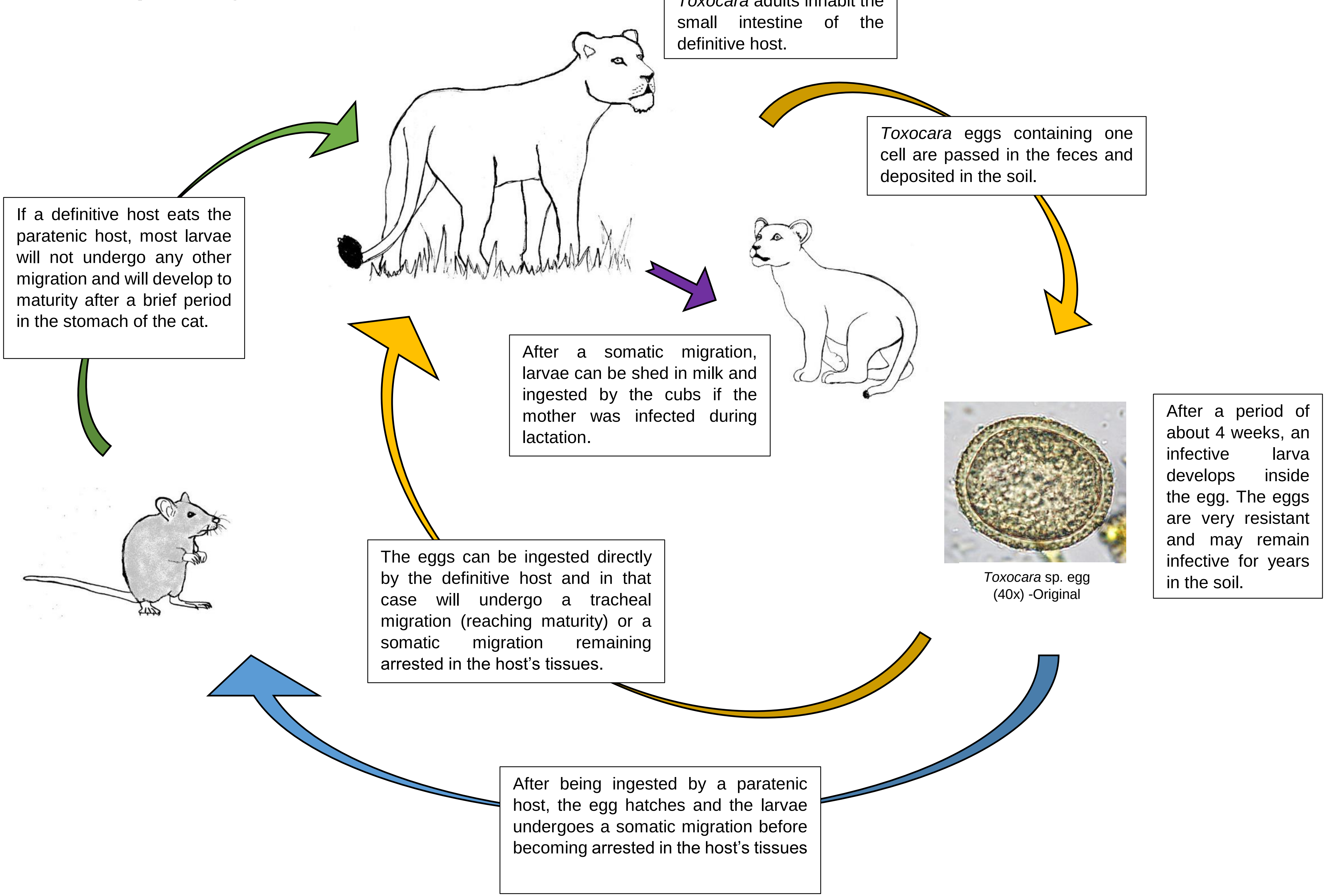


Figure 21 - *Toxocara* sp. life cycle in Lion (Original)

1.6.5 - Taeniidae

Cyclophyllidea is the largest taxonomic group within the Cestoda Phylum and this order includes at least 15 families with several thousands of species. Amongst these families, the Taeniidae family consists of only two genera: *Taenia* and *Echinococcus* (Knapp, Nakao, Yanagida, Okamoto, Saarma, Lavikainen, & Ito, 2011). In general, taeniid parasites, also called tapeworms due to their body shape, require two mammalian hosts to complete their life cycles. A terrestrial carnivorous or omnivorous predator acts as definitive host harbouring the adults in its intestine, and usually its herbivorous prey acts as intermediate host for the cystic larvae. Humans can also be definitive hosts of tapeworms (Hoberg, 2002; Knapp et al., 2011) . However, the two genera included in the Taeniidae family are very different from each other:

The genus *Echinococcus* is a rather small taxonomic group as recent phylogenetic studies revealed that the genus includes a minimum of 9 species. All the species belonging to this genus are morphologically similar to each other. As adults, all species have few body segments (usually four or five), also called proglotids, and usually with only the last segment being gravid. The adults are very small (measuring about 2-8 millimeters in length) when compared to adults of the *Taenia* genus. They inhabit the small intestine of carnivorous hosts belonging to the Canidae, Felidae and Hyaenidae families. Both *Echinococcus* and *Taenia* species have a scolex with four suckers and a nonretractable rostrellum armed with two rows of hooks (Bowman, 2014; Knapp et al., 2011). Also, their larvae are able to multiply asexually in the vesicular cysts.

Among the few known species of *Echinococcus* one was first described in lions. *Echinococcus felidis* was found for the first time in African lions from South Africa by Ortlepp (1937) and it was the first species of *Echinococcus* found in a felid definitive host (Hüttner, Nakao, Wassermann, Siefert, Boomker, Dinkel, & Ito, 2008). For many years this species has been considered as belonging to the *Echinococcus granulosus* complex, but recent work confirmed it as a different species belonging to a sister taxa and not to the *E. granulosus* complex. There is little information available regarding other possible definitive hosts (felids, canids or hyaenids). Although there are no studies on the pathogenicity of this species on lions or humans, the close evolutionary relationship with *E. granulosus* suggests a possible zoonotic potential (Hüttner et al., 2008).

The genus *Taenia* is a much larger taxonomic group and consists of a minimum of 42 recognized species. Among the *Taenia* species, only *Taenia saginata*, *Taenia solium* and *Taenia asiatica* use humans as definitive hosts (Knapp et al., 2011). The adult tapeworms of the genus *Taenia* can reach considerable lengths according to their degree of maturity.

The life cycle of tapeworms is very similar in both genera and it is ecologically linked to specific predator-prey associations (Hoberg, 2002) . The segments containing the eggs (gravid segments) are released through the anus of the carnivorous definitive host and attach to the pelage or the surface of the faecal mass where they are emptied of their content. Unlike *Taenia*, *Echinococcus* segments are rarely seen due to their small size and also because usually only the eggs are shed in the host's faeces. Then, if the egg (oncosphere) is ingested by a suitable intermediate host, the eggs hatch, releasing the hexacanth embryo that will migrate from the intestine to other organs (mainly liver, peritoneal membranes or muscles) and develops to the second and infective larval stage called the bladderworm. A bladderworm is a structure filled with fluid, containing one or several scolices and surrounded by a connective tissue membrane. In case of being ingested by a definitive host, the membrane is digested and the scolices attach to the mucosa of the small intestine and they start to grow developing new segments. The second larval stages of *Taenia* are named cysticercus, coenurus and strobilocercus according to each species whereas the second larval stage of *Echinococcus* is called hydatid (Bowman, 2014).

Morphologically, taenid eggs are very similar between each other and are microscopically indistinguishable. So far, at least six taenid species have been recorded from the *Panthera* genus (Hüttner et al., 2008).

Taeniidae life cycle in Lion

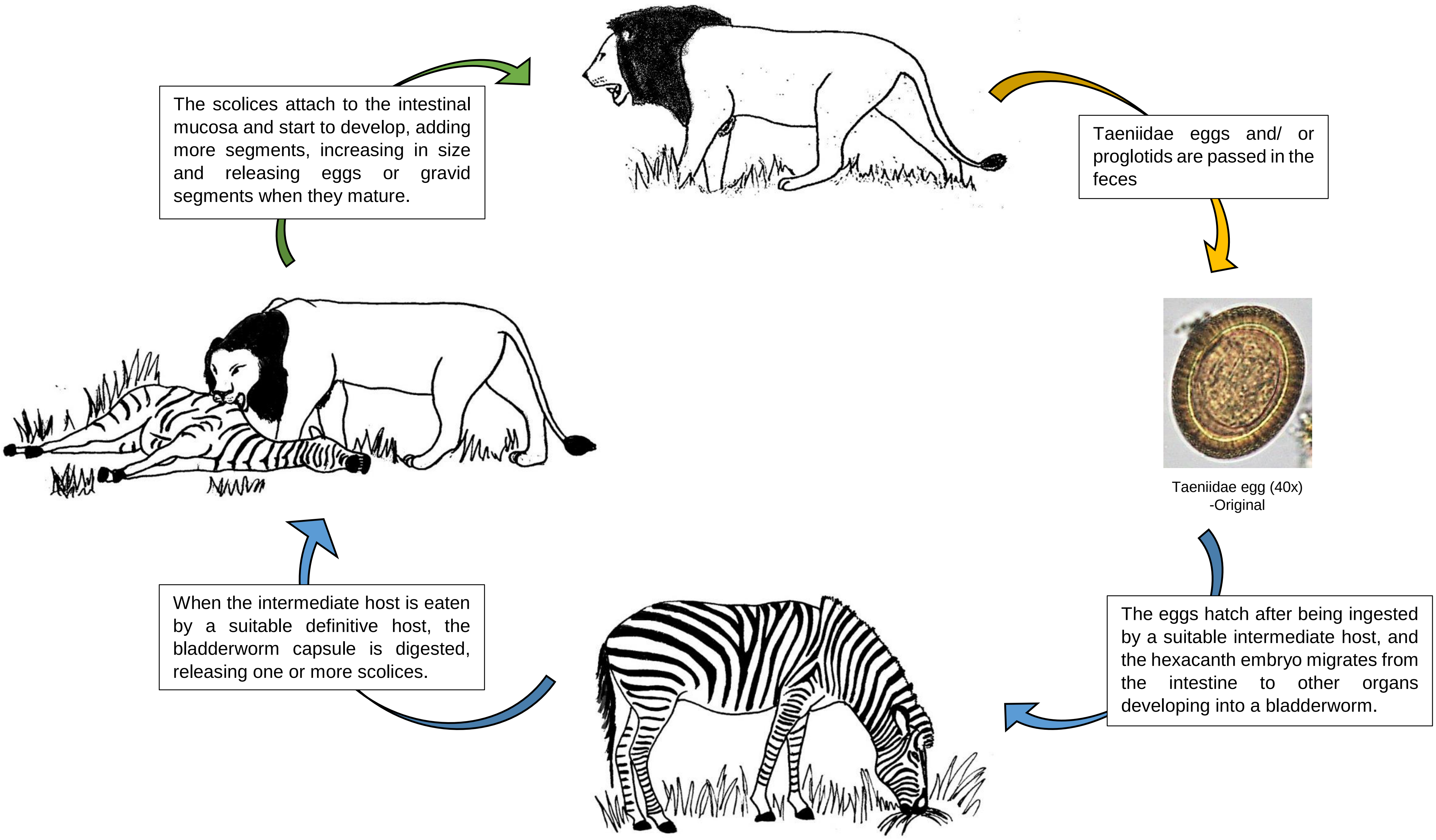


Figura 22 - Taeniidae life cycle in Lion (Original)

1.6.6 - *Paramphistomum* sp.

The *Paramphistomum* genus also called paramphistomes is a widespread and abundant group of trematodes and known parasites of domestic and wild ruminants worldwide. They are part of the broader Paramphistomatidae family which also includes other genera and species such as *Calycophoron*, *Cotylophoron*, *Gastrodiscoides hominis* (a parasite of humans and other primates) and *Megalodiscus* species which are parasites of the cloaca and colon of frogs (Bowman, 2014). In cattle, paramphistomosis can be caused by different species of *Paramphistomum* (*P. cervi, , P. ichikawai, or P. microbothrium)* and it can be responsible for many economic losses due to lower nutritious conversion, loss of weight and/or a decrease in milk production (Rangel-Ruiz, Albores-Brahms, & Gamboa-Aguilar, 2003). Although the disease occurs all around the world, the highest frequencies are registered in tropical and sub-tropical regions. The pathogenicity of *Paramphistomum* sp. is mostly related to the migrations carried out by the most immature stages of the parasite whereas the adult parasites inhabiting the reticulum and rumen of the host, are relatively harmless once they reach their typical locations (Hanna, Williamson, Mattison, & Nizami, 1988; Junker, Horak, & Penzhorn, 2014; Mage, Bourgne, Toullieu, Rondelaud, & Dreyfuss, 2003; Rangel-Ruiz et al., 2003).

Morphologically, the members of the Paramphistomatidae family are different from other trematodes with the main difference being the location of their ventral sucker at the posterior end of the body, which in other trematodes is either absent or located on the ventral surface (Bowman, 2014).

The eggs of *Paramphistomum* sp. are passed in the faeces of ruminants and will develop into miracidia if deposited in water. After hatching from the eggs, the miracidia invade snails (*Physa, Bulinus, Galba* and *Pseudosuccinea*) and develops to cercariae after one sporocyst and two rediae occur. The tailed cercariae leave the snail or it is excreted by the snail through slime balls, and encysts in water vegetation developing into a metacercariae. If the metacercariae is ingested by a suitable definitive host, it will excyst in the ruminant’s duodenum and will migrate to the rumen through the abomasum (Mehlhorn, 2001).

Although *Paramphistomum* sp. is not a true parasite of wild felids, some stages of the parasite can be found in these hosts as accidental or pseudo-parasites due to predation of the definitive hosts. Therefore, no life cycle of this parasite in lions is presented in this document.

2 - Material and Methods

The present research was carried out with the full collaboration and support of the Niassa Carnivore Project and all its collaborators especially Euzebio Waiti, a local field tracker and research assistant of NCP. Euzebio's experience and field knowledge allowed us to follow radio-collared lions daily and to collect the needed faecal samples.

2.1 - L5- South Concession – Study site

This study took place in the L5-South Concession of Niassa National Reserve which is also the smallest concession in the Reserve with approximately 580 km^2 (Fig. 23). L5-South is located on the south-eastern section of Niassa Reserve, along the north-eastern bank of the Lugenda River and it has been the intensive study area of Niassa Carnivore Project since 2003. In 2012, The Ratel Trust (TRT) won the tender for the management of L5-South for 25 years. This 25-year lease has allowed the Niassa Carnivore Project to develop its community based conservation approach by making the Mbamba village and its people their legal partners in managing this concession (first concession managed by communities). The NCP has built the Mariri Environmental and Skills Training Centre in L5-South that will be a place for building a relationship between the communities and Conservation while providing, education, and skills training to local people. The Mariri Environmental Centre is also the headquarters of NCP and the hotspot of the Project's activities in L5-South.

Figure 23 - Map of Niassa Reserve's concessions highlighting the location of L5-South (Source: Niassa Lion Project)

Geographically, the L5-South concession presents many features common to all Niassa with vast miombo woodland, acacia woodland, plains, open wooded grassland and the typical granite island mountains – "inselbergs" (Begg & Begg, 2009) (Fig. 24). The highest mountain in Niassa is the Mecula Mountain with 1,442 m which forms a totally different ecosystem with unique vegetation and also unique endemic animal species not found anywhere else in the Reserve like a newly described girdled-lizard (Branch et al., 2005). The southern boundary of L5-South is a 37 km stretch of the Lugenda River that has been identified as the most intensively fished area in the 350 km course of the river inside Niassa National Reserve (Begg & Begg, 2012) (Fig. 25).

Figure 24 - Niassa landscape with "inselbergs" and miombo woodland features in L5-South. (Original)

Figure 25 - Aerial view of the Lugenda River bed during the end of the dry season in L7-Concession (Original)

In 2010-2011, L5-South was identified as one of the worst areas for elephant poaching and bushmeat snaring in Niassa and although the concession is small when compared to other concessions, L5-South presents all the challenges faced by the whole Reserve in a smaller scale with the exception of sport-hunting (Begg & Begg, 2012).

2.2 - Lions in Niassa Reserve and L5-South

Lions have been monitored in L5-South since 2005 using specific features for individual recognition (scars, freckle patterns) and radio-collars. Since 2005, the Niassa Carnivore Project has collared 36 lions (15 females and 21 males) aiming to assess density, mortality, turnover, cub recruitment, movement patterns around villages and density (Begg & Begg, 2014). The density of the lion population in L5-South in particular is assessed every year in November calculating the number of adult lions/100km^2. On the other hand, the call up surveys across the whole Reserve, to assess the population trends of lion and spotted-hyaena have been done in 2005, 2008, 2012 and will be done again in 2015. In 2014, the lion density in L5-south was 0.04 lions / km^2 (Begg & Begg, 2014). Of the 36 collared lions, 10 (28%) have died in bushmeat snares and 50% of those died inside the concession between 2005 and 2012 (Fig. 26). No known lion has died as a result of snaring in L5-South since 2012. Snaring is the most common cause of death impacting lion and leopard survivability in Niassa Reserve although snares are usually not being placed to directly hunt lions in the first place (Begg & Begg, 2014). However, different snares targeting leopards specifically are being used mainly to obtain their valuable skins. Bushmeat snaring is not only affecting lions and leopards directly when they are caught as bycatch, but it is also reducing their available prey. Lions in L5-South prey on a wide variety of species (zebra, impala, sable antelope, eland, kudu, waterbuck, bushbuck, elephant and hippo calves, baboons) but warthog, bushpig and buffalo seem to be the most predominant species in their diet (Begg & Begg, 2009).

Figure 26 - Male lion caught in a bushmeat snare (Source: Niassa Lion Project)

At the moment, five apparently stable prides inhabit L5-South whereas only two were present in 2010. This was probably related to an increase in prey density in the area and resident prides have adapted their home ranges as the new ones moved in. However, prides are generally small, consisting of about 2-4 individuals except one that has six females. In total, eighteen females currently inhabit L5-South and nine (from all prides) are radio-collared. Four adult male lions currently inhabit the L5-South concession. They have been seen with multiple prides and usually don't remain with the females for more than 1-2 days. Therefore it is hard to know to which pride they belong to. In 2014, there were only six cubs and three sub-adult males associated with the eighteen females in the concession. Other non-territorial (nomadic) males are often seen and sometimes collared in L5-South and in 2010 a coalition of two nomadic males was collared to track their movements. The results showed that they were occupying a home range five times larger than territorial pride males. Their movements seemed to be related to the movements of a buffalo herd that was being followed by Prin et. al (2014) for research purposes (Begg & Begg, 2012). Having collared individuals in each pride makes it possible to monitor not only the collared animal but also other unknown animals in the same pride and obtain important information regarding their mortality, recruitment and turnover. NCP is currently using Radio and GPS collars (Fig.27) to track lions (Begg & Begg, 2014).

Figure 27 - Keith Begg and Eusebio Waiti collaring a male lion (Source: Niassa Lion Project)

Cub mortality is still high and no females have been able to rear more than two cubs to more than eight months of age, and usually only one cub is raised at a time in L5-South. This is most likely related to an also high mortality of adult males, leading to a constant male turnover in prides and infanticide of cubs. Outside the concession, mortality of male lions is still very high whether due to snaring or sport-hunting (Begg & Begg, 2014).

Human-carnivore conflict is an important threat faced by lions and other species across their range. In other areas of lion range, retaliatory killing due to attacks on people or livestock is sometimes one of the biggest threats to the survivability of the species. However, cattle is absent form Niassa due to the widespread presence of tsetse flies (*Glossina* spp.) and trypanosomiasis, but smaller livestock such as goats and chickens can be found in the villages (Begg & Begg, 2009). This threat has been monitored by the Niassa Carnivore Project through questionnaire surveys in most of the villages inside the

Reserve. Results have recorded 89 lion attacks from 1970-2010, resulting in an average of two attacks each year. The results also showed that 86% of the victims were males and the majority of attacks (76%) occurred in the village fields – locally called “machambas”. Also, it was during the wet season that most people have been killed by lions (67%). Still, there is a strong link between attacks and risk behaviours such as sleeping outdoors or in inadequate shelters (Fig.28) with no roof and doors, sitting around fires at night and walking alone at night (Begg & Begg, 2012). The majority of attacks occurring during the wet season are also due the presence of lion prey (mainly high concentrations of warthogs and bushpigs) feeding in the fields. Lions are then attracted to these areas and are more likely to come into contact with people chasing warthogs and bushpigs out of the fields, which can lead to attacks (Begg & Begg, 2009). Another aspect of conflict with carnivores was the use of inadequate corrals in the villages. According to NCP, although 87% of goat owners used some kind of goat corral to keep their goats, only 26% of those corrals were considered to be “predator-proof” (Begg& Begg, 2011) (Fig.29).

Figure 28 - Examples of a safe shelter (left) and an unsafe shelter (right) used during the wet season in the "machambas" (Adapted from Begg & Begg, 2009)

Figure 29 - Examples of inadequate goat corral (left) and a predator-proof corral (right) (Adapted from Begg & Begg, 2011)

Other solutions to mitigate conflict between people and carnivores are being used such as the creation of living fences around the crops using cuttings of *Commiphora africana*, a thorny African plant, to keep pests out of the fields while also preventing lions to follow them (Begg & Begg, 2011).

2.2.1 - Trophy hunting

Lions, leopards and other species have been hunted in Niassa Reserve for years whether by local people for skins or by sport hunters for trophies. However, this situation is currently hard to justify, since sport-hunting for trophies is considered a legal activity in Niassa while for local residents, hunting a lion for its skin is considered illegal (Begg & Begg, 2012). Still, both are an additional threat to the survivability of these species and they both add to the already high total mortalities in the area. In most places where sport-hunting of lions and leopards is allowed, the quota settings are rarely based on actual carnivore densities in those areas and the criteria used to assess trophy quality and sustainability are often inadequate (Begg & Begg, 2012). Therefore, sport-hunting of lions based in wrong off-takes, quotas and with inadequate age restrictions is most likely contributing to the decline of the species. In Tanzania, inside and outside of protected areas, trophy hunting has been identified as the leading cause for the decline of lion populations (Packer et al., 2010). In Niassa Reserve, trophy monitoring of lion and leopard trophies began on 2004 when 75% of the monitored trophies were clearly identified as below 6 years of age. The monitoring of lion and leopards was implemented by SGDRN in 2006 following a NCP proposal, along with a Points System based on trophy quality and enforcing a 6 year minimum age limit through independent trophy monitoring (Begg & Begg, 2007a). The 6-year minimum is not only supported by lion researchers (Whitman et al., 2004) but also by major hunting associations and Niassa became one of the few areas where this is actually monitored and enforced. Aging of lions in three age categories (< 4 years of age, 4-6 years and older than 6 years) is possible since there is correlation between nose pigmentation (according to Whitman et al., 2004) noses get darker with age, reaching 50% at 5-6 years), mane development, tooth wear and closure of the pulp cavity (completely closed by 4.5 years) in Niassa's lions. The number of underage trophies taken has been reduced significantly after the Points System was implemented. Although trophy hunting of lions in Niassa can be considered as sustainable on its own when carefully monitored, it is not the only factor contributing to the decline in the species numbers. While it may seem better to completely stop trophy hunting, the reality is that the funds currently obtained through sport-hunting activities are essential for conservation management in the Reserve. In fact, sport hunting is responsible for around 30% of NNR's annual operating budget (Begg & Begg, 2012).

2.4 - Sample collection and analysis

In order to characterize the gastrointestinal parasitological fauna of lions (*Panthera leo*) in Niassa National Reserve, forty-four lion faecal samples were identified and collected from the field, in the L5-South Concession of Niassa National Reserve, between October and November 2014 (30 days). The collection of samples was made during daytime on a daily basis during this period.

The samples were collected from the field on a daily basis by the author with the invaluable help of Euzebio Waiti after locating and/or following the radio-collared groups in L5-South (Fig. 30). During the fieldwork, the author also assisted in other daily routines such as collecting GPS data about the different prides and maintenance and management of camera-trap information.

Figure 30 – The author radio-tracking lions in L5-South (Original)

The typically small size of prides, their behaviour and the geography of the terrain (with very few usable roads) in Niassa Reserve, made it very hard to follow the prides into very thick bush areas where they often rest during the day, and made it impossible to collect fresh samples and to track down each individual in order to know the source of each sample (Figs. 31 and 32).

Figure 31 - Lioness (right) and two juveniles (left) from the F-Pride resting in the shade (Original)

Figure 32 -Radio-collared lioness from the M-Pride moving to a thicker bush area (Original)

The collected faecal samples were stored in dry conditions, transported in plastic bags to the base camp of Niassa Lion Project at Nacatopi and kept in a cool place until transported by plane to Portugal under the necessary legal permits from both Mozambican and Portuguese authorities. Samples were analysed between 15 to 30 days after collection at the Laboratory of Parasitology and Parasitic Diseases, CIISA-FMV-ULisboa, using the following coprological methods according to Thienpont, Rochette e Vanparijs (1986) and Bowman (2014): qualitative natural sedimentation and Willis flotation (for isolating helminth eggs and protozoan oocysts); McMaster quantitative method (to assess the number of eggs per gram of faeces, EPG); and Baermann technique (for the search of first stage larvae (L1) of pulmonary nematodes).

2.5 - Coprological methods

Several coprological techniques are currently used in parasitological diagnosis in Veterinary Medicine for detecting and/or assessing the number or eggs and/or larvae in faeces. A brief description of each of the techniques used by the author, during the 2 months of laboratory work is provided below.

2.5.1 - McMaster technique – Quantitative method

In order to quantify the number of parasite eggs shed per gram of faeces the McMaster technique proposed by Thienpont et al. (1986) and Madeira de Carvalho (2001) was used. The technique starts by adding two grams of the faecal sample to 28 ml of a saturated sucrose solution at 25%. The solution is then homogenized using a glass rod and filtered through a strainer into a beaker. From here the solution is carefully poured, in order to fill the two compartments of the McMaster chamber. The McMaster chamber has a capacity of 0, 30 ml distributed in the two compartments which are covered by a slide on the upper part of the chamber. The slide has a grid in two areas (above each of the compartments) to assist at counting the eggs when the chamber is observed on a microscope usually using a 10x objective (Madeira de Carvalho, 2001). The eggs will float on the saturated solution and become attached to the slide. Only the eggs observed inside the limits of the grids are counted, and this number is then multiplied by a correction factor of 50 for the purpose of determining the real EPG (eggs per gram of faeces) on that sample.

2.5.2 - Willis floatation and Natural Sedimentation – Qualitative methods

The Willis floatation is a qualitative coprological method which takes advantage of parasite egg's specific gravity (>1) by adding the faecal sample to a saturated sucrose solution. Due to its qualitative nature, the technique does not require a very exact weighing of the faecal sample, but around 2 grams of faeces are usually used. The sample is added and diluted in 30 millilitres of saturated solution and passed through a strainer into a test tube. By doing so, the lighter parasite eggs, usually nematode eggs, will float whereas the heavier eggs will sink in the bottom of the water column due to their higher specific gravity, when compared to the saturated solution. A cover slide is then placed on the top of the test tube and after a period of about 15-20 minutes (the time required for the eggs to attach to the cover slide) it is placed on a slide and observed on a microscope using a 10x objective. When performing this technique, a sucrose solution was used rather than a salt solution since the first allows a better and longer observation of the eggs without any deformations (Madeira de Carvalho, 2001).

The natural sedimentation method is usually performed in the same test tube after the Willis floatation, making use of the sediment on the bottom of the tube. The supernatant solution is decanted and the sediment is carefully homogenized in order to collect 2-3 drops using a Pasteur pipette. Then, the collected material is placed on a slide to be observed on a microscope. In order to ease the observation of the sediment a drop of methylene blue can be added to the slide. This compound cannot usually penetrate the parasite eggs if they are intact and will stain all the debris in blue contrasting with the bright gold or brown eggs (Fazendeiro, 1989). According to Bowman (2014) this method can be used for trematode and acanthocephalan eggs, amoebas, ciliates, and formalin-fixed *Giardia* cysts.

2.5.3 - Baermann method

The Baermann technique is used specifically for concentrating and collecting nematode larvae (usually L1 stage), taking advantage of the larvae's inability to swim against gravity in a considerable body of water (Bowman, 2014). There are many variations and refinements of this technique but all of them are based on the same principles. Between 5 and 15 gram of faeces should be used to perform this technique. In the present work, the faeces were wrapped in gauze and unlike the funnel described by Bowmam (2014), this gauze was placed in a conical beaker with lukewarm water. The warmth of the water stimulates larval mobility and some larvae migrate to the surface of the faecal mass falling off and sinking in the bottom of the water column. Samples were allowed to rest overnight although in heavier infections larvae can be found after an hour (Bowman, 2014). After a period of about 24 hours, the samples were collected after removing the supernatant and the pellet was placed in centrifuge tube using a Pasteur pipette. The tubes were centrifuged for a minute and the supernatant was removed to collect a portion of the sediment to a microscope slide which was observed under an optical microscope at a total magnification of 100x or 400x.

2.5.4 - Identification

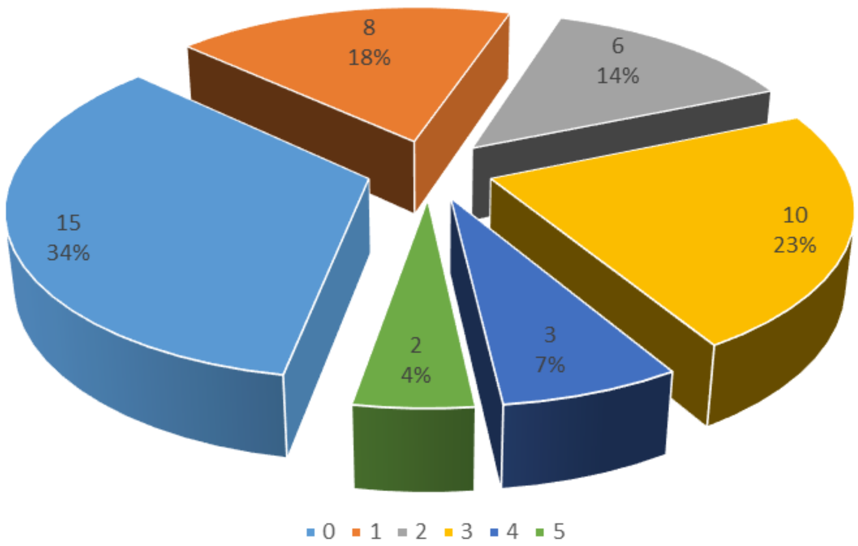
All the eggs were photographed and measured in order to allow a correct identification (until genus level) and the results were compared to those obtained on previous studies on wild lion parasites (Müller-Graf, 1995; Bjork et al., 2000; Berentsen et al., 2012) .

3 - Results

The results obtained in the present work show that lions in L5-South are hosts of a wide variety of parasite species (Graphs 1 and 2).

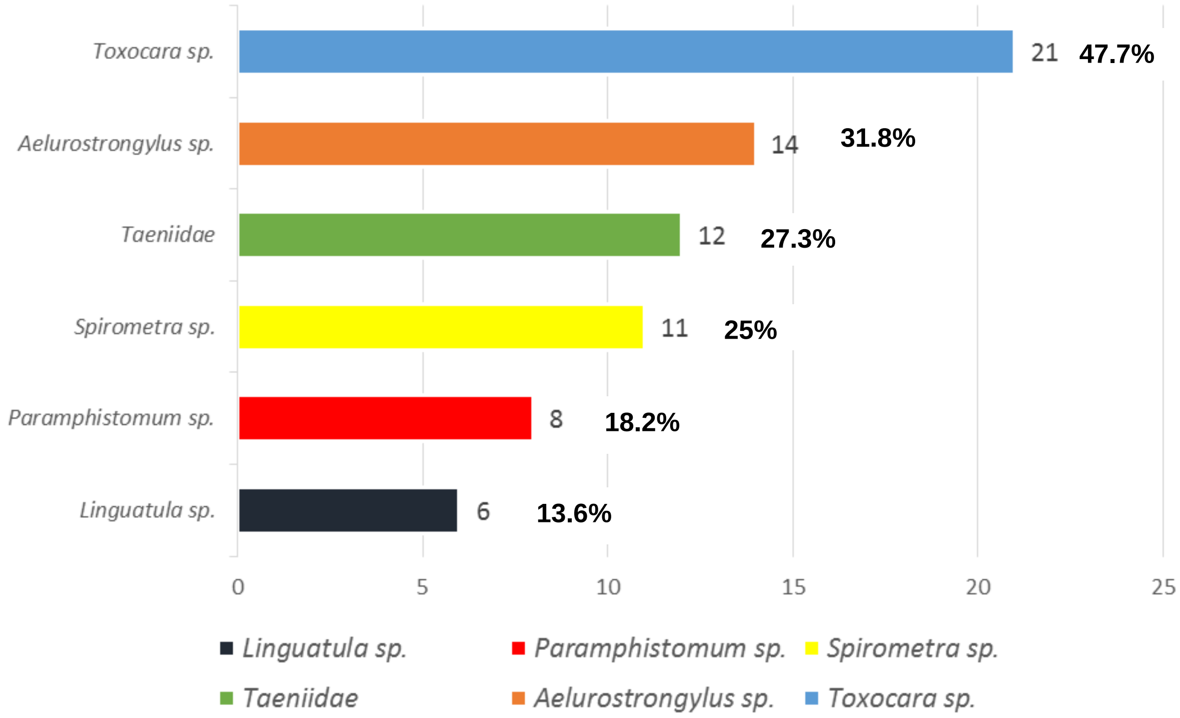
Through all the coprological techniques used for assessing the presence of parasites in the forty-four faecal samples, it was possible to identify eggs and larvae of six different parasite species: *Toxocara* sp., *Aelurostrongylus* sp., Taeniidae, *Spirometra* sp., *Paramphistomum* sp. and *Linguatula* sp.(Graph 2).

A total of 66% (29/44) of the samples were positive considering all the coprological techniques, while in 34% (15/44) of the samples, no parasite species were found. Also, only one species of parasite was found in 18% (8/44) of the samples, two species in 14% (6/44), three species in 23% (10/44), four species in 7% (3/44) and five parasite species in 4% (2/44). These results are presented in the Graph 1 below.



Graph 1 - Proportion of positive and negative samples using all the coprological methods. Number and proportion of parasite species found in all forty-four samples tested.

As it is shown in Graph 2, *Toxocara* eggs were recovered in 47.7% of the collected samples (21/44), *Aelurostrongylus* sp. in 31.8% (14/44), Taeniidae eggs in 27.3% (12/44), *Spirometra* sp. in 25% (11/44), *Paramphistomum* sp. in 18.2% (8/44) and *Linguatula* sp. in 13.6% (6/44). *Toxocara* sp. eggs were the most prevalent parasite forms found in this study.

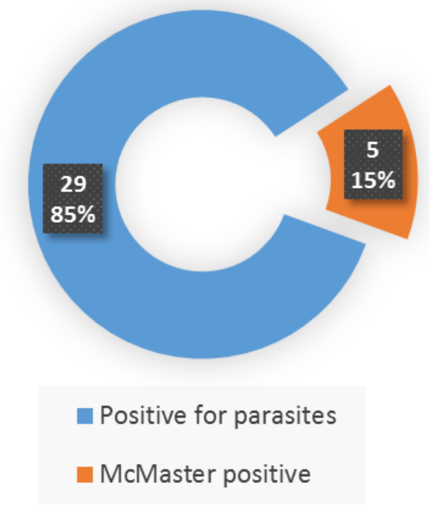


Graph 2 - Graph showing the proportion of each parasite species found in all the 44 collected samples

Although every sample was tested using all the qualitative and quantitative methods described above, it was only possible to determine the EPG (eggs per gram of feces) in five (17.2%) of the 29 positive samples tested using the McMaster technique, since the remaining thirty-nine samples revealed no eggs to be counted when the technique was performed (Graph 3 and Table 1). The McMaster technique has a detection threshold of 50 EPG and so a negative result does not correspond to a negative individual. To be considered as such, a negative result must be obtained in all the other coprological techniques (Fazendeiro, 1989; Madeira de Carvalho, 2001). The positive results for McMaster egg counting showed that 80% (4/5) of the eggs belonged to *Toxocara* sp. And 20% (1/5) to *Linguatula* sp.

Table 1 - Egg counts of the only five positive samples in the McMaster method

Sample nº	**EPG**
3	50 (*Toxocara* sp.)
10	100 (*Toxocara* sp.)
20	50 (*Toxocara* sp.)
29	50 (*Toxocara* sp.)
37	350 (*Linguatula* sp.)



Graph 3 - Proportion of McMaster positive samples in all the twenty-nine positive samples

The results also show that co-infections of multiple parasite species were commonly found. In 28 % (8/29) of the positive samples, only a single parasite species was found and the remaining 72 % (21/29), were co-infected with two, 21 % (6/29), three, 34 % (10/29), four, 10 % (3/29) and even five, 7 % (2/29) distinct parasites. Overall, the most common co-infection found in all the positive samples was the one caused by *Aelurostrongylus* sp. and *Toxocara* sp., 41 % (12/29). The number of samples where each association of two parasite species was found is presented on Table 2.

	Toxocara sp.	*Linguatula sp.*	*Aelurostrongylus sp.*	*Spirometra sp.*	*Taeniidae*	*Paramphistomum sp.*
Toxocara sp.						
Linguatula sp.	5					
Aelurostrongylus sp.	12	4				
Spirometra sp.	7	2	4			
Taeniidae	7	2	6	2		
Paramphistomum sp.	7	4	4	2	5	

Table 2 - Number of samples where each of the presented parasite associations were found.

Apart from the McMaster method, all the coprological techniques used on the present study have been successful at recovering parasite forms. As it was expected, the Baermann method was the most successful coprological technique to recover the highest densities of L1 larvae of *Aelurostrongylus* sp., although these larvae have also been found in the Willis floatation and natural sedimentation methods. All other species of parasites were also recovered using the Baermann method. Molecular techniques have been used in order to identify the parasite species involved but the results were not conclusive.

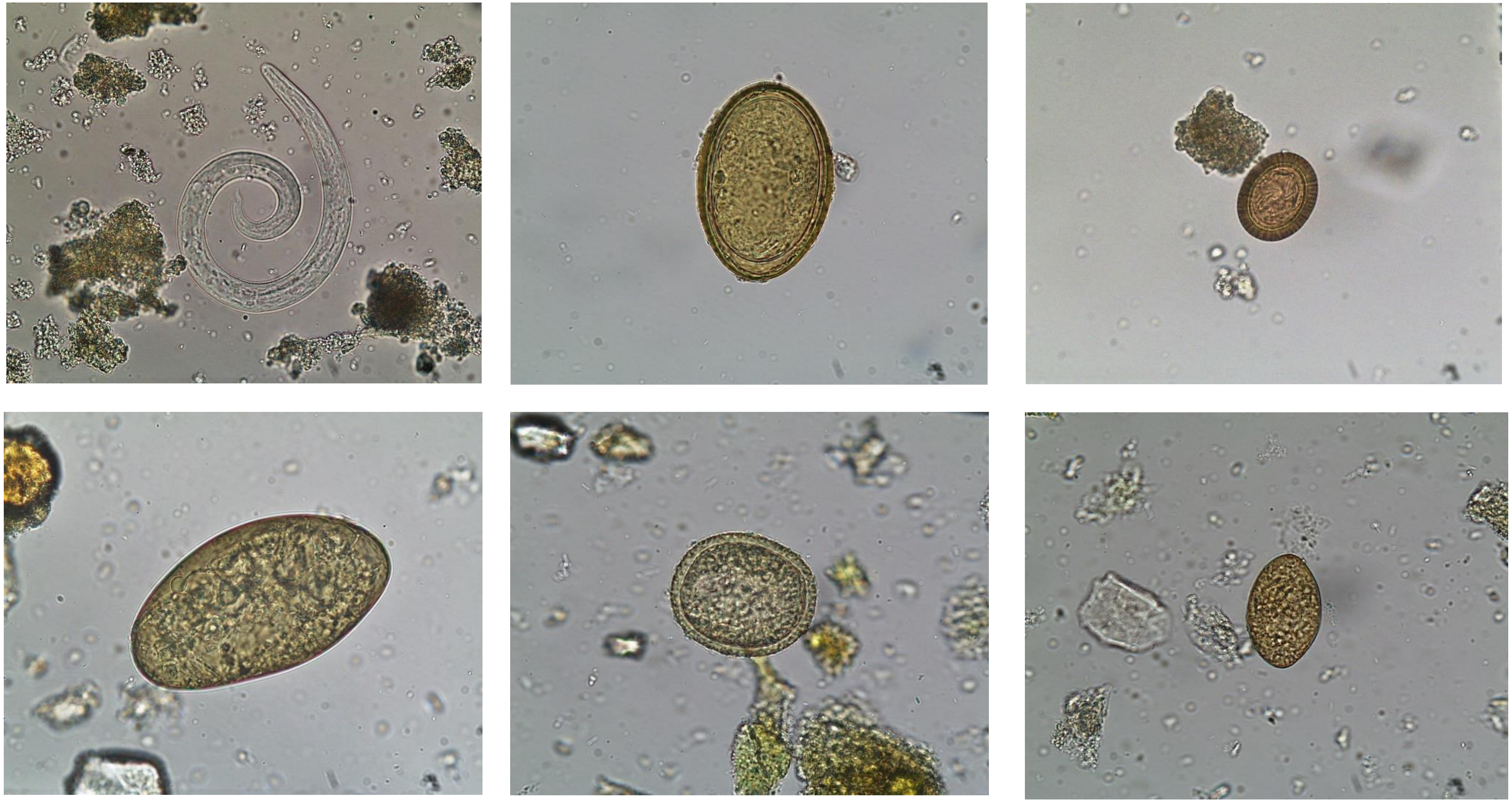


Figure 33 – Parasite forms recovered from the samples. Left to right, top row: *Aelurostrongylus* sp. (L1 Larvae - 260 µm x 2.5 µm, 40x), *Linguatula* sp. (Egg - 80 µm x 53 µm, 40x), Taeniidae (Egg - 39 µm x 32.5 µm, 40x). Bottom row: *Paramphistomum* sp. (Egg - 120 µm x 67.5 µm, 40x), *Toxocara* sp. (Egg - 63 µm x 55 µm, 40x), *Spirometra* sp. (Egg - 57 µm x 35 µm, 40x). (Original)

4 - Discussion

Overall, the species found in the present study were in accordance with what has been described, in the few previous studies (Table 3) carried out in wild lion populations. This study revealed that *Toxocara* sp. eggs were the most common parasite form found, with a prevalence of 47.7% in all the collected samples (n=44). The presence of L1 larvae of *Aelurostrongylus* sp. was recorded in 31.8% of the faecal samples, followed by a prevalence of 27.3% of Taeniidae eggs, 25% of *Spirometra* sp. eggs, 18.2% of *Paramphistomum* sp. eggs and 13.6% of *Linguatula* sp. eggs. The possibility of multi-sampling from the same individual or even the same prides can never be discarded, and consequently, exact conclusions regarding parasite loads or their effects on a specific individual cannot be drawn. Also, only lion morphometrically compatible samples were collected in order to reduce the possibility of collecting samples from other carnivores. Leopard scats are smaller than lion scats and it was unlikely that any sample from hyenas have been collected since hyenas use latrines, their faeces have a markedly white coloration due to high calcium content (Hulsman et al., 2010).

The parasite species found in this study may also have been influenced by the weather condition of the dry season (with extremely high temperatures and very low humidity), causing dehydration of the samples taken from the field. This fact probably mirrors a potential sub characterisation of parasitic load, suggesting that the prevalence of some parasites found in this work could be higher and other species could have been found if the samples were freshly collected. The reason behind the absence of these parasites is probably linked to the effects of high temperatures which made impossible the survival of hookworm eggs by damaging their thin capsules. It is also important to remark that usually parasitic infections tend to be over dispersed, to where many individual hosts have low parasite intensities and only a few individuals have high parasite loads (Scott 1988; Müller- Graf 1995; Junker et al. 2008).

	Toxocara sp.	*Linguatula* sp.	*Spirometra* sp.	Taeniidae	*Aelurostrongylus* sp.	*Paramphistomum* sp.
Patton & Rabinowitz (1994) (n=54)	33.3%	-	35.2%	5.5%	55.5%	-
Müller-Graf (1995) (n=112)	4.5%	-	63.4%	58%	29.5%	-
Bjork et al. (2000) (n=33)	9%	-	33%	15%	21%	-
Berentsen et al. (2012) (n=15)	13%	-	87%	80%	20%	-
Kavana et al. (2015) (n=7)	71.4%	-	100%	42.8%	-	-
Present study (n=44)	**47.7%**	**13.6%**	**25%**	**27.3%**	**31.8%**	**18.2%**

Table 3 – Comparison between results obtained in the present study and previous ones. First row shows results obtained from wild leopard scats in Thailand.

Nevertheless, the number of parasite species identified in this study was higher than previously found in Namibia by Smith and Kok (2006) and lower than those found in Zambia (Berentsen et al., 2012) and in Tanzania (Müller-Graf, 1995; Bjork et al., 2000).

Toxocara sp. (47.7 %) was the most prevalent parasite in our study, in comparison with the studies of Müller-Graf (1995), Müller-Graf et al. (1999), Bjork et al. (2000) and Berentsen et al. (2012), who documented *Spirometra* sp. as the most common parasite. In Tanzania, Bjork et al. (2000) found a prevalence of 9% of infection by *Toxocara cati* in a sample size of 33 known lions, while Müller-Graf (1995) recorded a 4.5% prevalence of this parasite in a sample size of 112 known individual lions in the same country. Berentsen et al. (2012) recorded a prevalence of 13% of *Toxocara* sp. eggs in 15 lion faecal samples collected in Zambia. In our study *Toxocara* sp. was the most prevalent parasite, probably due to its direct life cycle, but also due to the chance of lions ingesting paratenic hosts with larval stages. Such a widespread presence of *Toxocara* sp. eggs in the samples can also be a result of the high resistance of the eggs in the environment (Bowman, 2014) unlike other parasite species found. Also the possibility of environmental contamination from other domestic and non-domestic carnivores to lions and paratenic hosts can never be excluded as a source of infection. Contamination from the soil to the faecal samples must also be taken into consideration as a source of infection. In fact, infected paratenic hosts play a very important role in the life cycle of *Toxocara cati*, and have been recognized as very important source of infection to adult cats, especially those with well-developed predatory habits (Bowman, 2014).

It is likely that the odds of a lion directly ingesting *Toxocara* sp. eggs are high, since lions are the only communal living cats, and show all sorts of social behaviours within the pride such as grooming themselves and/or other pride members (Estes, 1991). In domestic cats grooming behaviours also increase the likelihood of ingesting embryonated eggs (Martínez-Barbabosa, Vázquez Tsuji, Cabello, Cárdenas, & Chasin, 2003). Although we cannot link the collected faecal samples to a particular individual it would not be surprising that females and cubs would probably be at a higher risk of ingesting *Toxocara* sp. eggs than older adult males (Smith & Kok, 2006), since they spend much more time together carrying out these behaviours within the pride (Skinner & Smither, 1990; Wilson & Mittermeier, 2009). This is also supported by the fact that *Toxocara* sp. can be shed in milk and transmitted from the mother to her cubs (Bowman, 2014), which could create a scenario of permanent infection within the pride. Although no reports on the pathogenesis of *Toxocara* sp. for lions are available, the known effects and consequences of heavy infections in other cat species, suggests that cubs are more prone to develop more severe symptoms such as vomiting (sometimes containing immature larvae), abdominal discomfort as well as intestinal obstructions and rupture (Bowman, 2014). In other words, while in adult cats the infection is usually asymptomatic, in cubs it can even lead to death (Ghaemi, Sadr-Shirazi, & Ghaemi, 2011). However, larval migrations within the individual's tissues and their consequences cannot be discarded as a potential health risk. In the case of free-ranging wild felids, heavy parasite infections can also lead to a more delayed development of cubs due to parasitic exploitation and may also be responsible for reducing competitive fitness (Brassard et al. 1982; Scott 1988) and consequently their ability to survive in a challenging ecosystem.

Spirometra sp. has been recognized as the most common lion parasite by several authors (Macdonald & Loveridge, 2010), since nearly all studies on lion gastrointestinal parasites, have recovered *Spirometra* sp. eggs. Different results regarding this parasite have been recorded: Müller-Graf et al. (1995) found out that 63.4% (n=112) of the lions in the Serengeti and Ngorongoro Crater were infected with this parasite. Bjork et al. (2000) recorded a prevalence of 33% (n=33) in a sample size of 33 known lions in Tanzania; Berentsen et al. (2012) recorded prevalence of 87% (n=15) of *Spirometra* sp. eggs in Zambia. In a more detailed study of this cestode, Müller-Graf (1999) concluded that lions inhabiting the Ngorongoro Crater in Tanzania were more heavily infected with this cestode than lions inhabiting the nearby Serengeti. Samples were collected from 112 individually known lions and over 60% were infected with *Spirometra* sp. eggs. However, Müller-Graf (1999) concluded that there were no significant correlations between individual parasite load and rainfall season, age, sex, reproductive status or pride size (Müller-Graf, 1999). The same author suggests that these results could be related to ecological differences, namely swampy vs. dry habitat and abundant vs. sparse prey. In the present study we have recorded a prevalence of 25% (n=44) of *Spirometra* sp. egg, which could have been related to the time of the year when the samples were collected. Since *Spirometra* sp. lifecycle involves amphibious and terrestrial hosts and part of its cycle takes place in water, one would expect a higher prevalence of this parasite during the wet season, when more waterholes and rivers are available, which would result in more intermediate and definitive hosts being infected. In order to prove or disprove this hypothesis, samples would have to be collected in the same study area during both seasons to establish a comparison. However, the results of Müller-Graf (1999), who stated that differences in parasite loads could be due to several ecological factors such as habitat type and prey abundance, could explain the relatively low prevalence found in this study when compared to previous ones. Niassa Reserve's habitat can be considered more of a dry habitat rather than a swampy one, and lions inhabiting this area have much wider territories than in other areas of the species' range also due to low prey density. The infection with *Spirometra*.sp. is usually harder to detect when compared to other cestodes since the adult parasite does not release entire body segments in its host's faeces. The eggs are continuously discharged through the uterine pores of the parasite's mature segments making *Spirometra* sp., less obstructive than other tapeworms. In their definitive cat hosts the plerocercoids will develop into adult parasites and will start exploiting the host but even the procercoids of *Spirometra* sp. can be ingested by a cat host, that may support their development into plerocercoids which usually appear in the flat muscles of the body wall and subcutaneous fascia (Bowman, 2014).

The registered prevalence of 32 % for *Aelurostrongylus* sp. in this work was higher than the described prevalence of 21% (n=33) by Bjork et al. (2000) in Tanzania, and the 20% (n=15) described by Berentsen et al.(2012) in Zambia. As it was mentioned above, the highest densities of *Aelurostrongylus* sp. were observed when the Baermann method was carried out, although this parasite has also been found in all other coprological techniques. The principles behind the Baermann method take advantage of an increase in larvae mobility and of their inability to swim against gravity. However, in the present study, all larvae observed were dead when the coprological methods were performed since the collected faecal samples were not fresh. This fact is probably the reason why these larvae were also found in the other coprological methods. Although some larvae were already much damaged at the time of observation, others were in a perfect condition which allowed a more accurate observation and identification.

The effects and consequences of *Aelurostrongylus* sp. infection in felids can range from asymptomatic, self-limiting, and subclinical forms to severe (and possibly life-threatening) respiratory signs (Traversa et al., 2008; Di Cesare et al., 2013). Although *Aelurostrongylus abstrusus* has been recovered from scats of a wide diversity of wild felid hosts (tigers, lions, cheetahs, cougars, jaguars) from all over the world (Di Cesare et al., 2013), little is known regarding how disease occurs in such species. However, it is likely that heavy infections of *Aelurostrongylus* sp. might be able to induce disease in both domestic and wild felids alike, impairing their respiratory functions significantly (Traversa et al., 2008; Di Cesare et al., 2013) and therefore decreasing individual fitness. Ultimately, we consider that such effects could even lead to a decrease in the animal's ability to chase and hunt prey, which in predatory species like lions could directly affect their survivability.

The prevalence of Taeniidae eggs, 27.3 % (n=44), found in this study is intermediate between what was found by Berentsen et al. (2012) in Zambia (80 %), Müller-Graf et al. (1999) (58 %) and Bjork et al. (2000) (15 %). Although the presence of structures such as the hexacanth embryo made it possible to identify the Taeniidae eggs, it was not possible to determine to which species of Taeniidae the eggs belonged to. *Echinococcus* and *Taenia* eggs are morphologically similar and practically indistinguishable. Also, the wide spectrum of prey included in a lion's diet and the existence of *Echinococcus felidis*, recognized as a true lion parasite, make it even harder to speculate about source of the Taeniidae species involved. The transmission of taeniids is ecologically-based, with lifecycles linked to very specific predator-prey associations (Hoberg, 2002). Nevertheless, and as highly adapted as taeniids are to their definitive and intermediate hosts due to millions of years co-evolutionary processes (Hüttner et al., 2008), they are relevant human and animal pathogens (Guerra et al., 2013) and the infection with such parasites cannot be considered harmless, since taeniid infection may result in considerable exploitive and obstructive effects for the host (Bowman, 2014). The infection for the intermediate hosts is often associated with more severe health risks such as the one caused by the presence of hydatids (Bowman, 2014).

The presence of *Paramphistomum* sp., was considered pseudo-parasitism, as a result of hunting wild ruminants, in particular buffalos. However, other ruminant species such as antelopes, like impala, waterbuck, kudu, eland or bushbuck could be hosting this and other endoparasites, but no studies have been conducted on their parasite fauna in Niassa Reserve. Therefore, it is possible to say that the diet of these felids is probably the source of most of the parasitic forms found in the present study, although it is not possible to establish a direct link between the identified parasites and the prey species they belong to. Müller-Graf (1995) recorded heavy infections with nematodes *Trichuris* sp. and *Trichostrongylidae* eggs which the authors did not consider as true lion parasites. Other situations of pseudo-parasitism are likely to be recorded due to the wide diversity of prey included in a lion's diet. Throughout the year, several parasite forms associated with true parasitism in lion's prey species may cross the lions' digestive tract. Bjork et al. (2000) recorded the presence of an oocyst belonging to *Eimeria* spp. which was considered to be a case of pseudo-parasitism since this genus usually does not parasitize felids (Bjork et al., 2000).

The prevalence of 13.6 % (n=44) for *Linguatula* sp. recorded in this study represents the first documentation of this parasite species' prevalence, recovered from wild lions' scats, since only Young (1975) has reported this parasite as a potential cause of disease in African lions, and Mukarati et al. (2013) who recovered *Linguatula* sp. eggs from captive lions in a recreational park in Zimbabwe. Although this was the parasite with the lowest prevalence in all the forty-four collected samples, it was a very interesting finding. Again, the diet of lions in Niassa, is known to include a wide variety of herbivores in a greater or lesser degree and these herbivores are most likely hosting the nymphs of *Linguatula* sp., acting as intermediate hosts for this parasite. Pentastomids such as *Linguatula* sp. are certainly not harmless parasites, even for lions, since adults inhabit the nasal and paranasal sinuses of their definitive hosts, causing bleeding catarrhal inflammation, and some impediment to respiration (Bowman, 2014). The nymphs of *Linguatula* sp. can also be responsible for considerable damage due to their migrations to various internal organs and tissues in some intermediate hosts (Ravindran et al., 2008).

It is also interesting to note that out of the 29 positive samples 34% (n=10) were co-infected with three parasite species which is in accordance to what has been described by Bjork et.al (2000) who recovered an average of three parasites species per lion in 33 fecal samples. The same authors also mentioned that there seems to be no connection between the number of parasite species found and the sex, habitat or age of the lions they sampled.

During the fieldwork in Niassa Reserve, prides inhabiting L5-South have been observed, and although we cannot confirm that all the samples belonged to lions inhabiting the concession, we can say that most of them would surely belong to the resident territorial prides. However, and as subjective as it can be, all animals observed during the daily fieldwork, looked healthy and seemed to be in good overall condition and most prides had cubs or juveniles

Lions and leopards are the only species of large felids (Genus *Panthera*) inhabiting Niassa's ecosystem and both species share similar predatory habits and often compete for the same prey species, although leopards may prey on smaller species when interspecific competition for larger prey is high. In fact, interspecific competition is considered to be a main driver for the Leopard's habit of dragging kills to the top of trees, which occurs mostly in Africa, and it is not observed where leopards are the only large predators (Estes, 1991; Sunquist & Sunquist, 2002). Although leopards have a much broader distribution worldwide, phylogenetic estimates indicate that modern leopard lineages originated 470 000–825 000 years ago in Africa followed by their migration into and across Asia (Uphyrkina et al., 2001). However, some few studies on the Leopard's gastrointestinal parasites in different parts of the species' range have showed that leopards are hosts to many common felid parasites such as *Toxocara* sp., *Aelurostrongylus* sp., *Paragonimus* sp., Ancylostomatidae, Spiruroidea, Taeniidae, Pseudophyllidea, *Capillaria* sp. and *Sarcocystis* sp. (Patton & Rabinowitz, 1994; Ghaemi, Sadr-Shirazi, & Ghaemi, 2011), and even some less common ones such as *Trichinella britovi* (Mowlavi et al., 2009). In fact, amongst the 20 parasite species recovered by Patton & Rabinowitz (1994) in Thailand, the most common parasite species found in the leopard scats, were *Aelurostrongylus* sp. which was recovered in 55,5% of the samples (n=54) followed by *Paragonimus* sp. (51.8%), Ancylostomatids (50%), *Capillaria* sp. (48.1%), Pseudophyllidea (35.1%), *Toxocara* sp. (33.3%), Spiruroidea (28%), and *Sarcocystis* sp. (20.4%). Based on this information, and although no leopard scats were collected for the present study, we believe that leopards in Niassa Reserve may also share several parasite species with lions since they are both top predators in the ecosystem, and frequently feed on the same prey. Therefore, we consider it is also likely that the infection with these parasite species may have similar consequences for lions and leopards alike.

Anyway, the high prevalence of *Toxocara* sp. (a parasite with direct life cycle) and *Aelurostrongylus* sp. (with indirect life cycle) is remarkable. This fact can be related with a high animal density in the area where the samples were collected, or probable inter-transmissibility of these nematodes among the big carnivores living in this ecosystem. Nevertheless, due to their life cycles and host-parasite interactions, it can be considered that such a high prevalence of *Toxocara* sp. and *Aelurostongylus* sp. could be a potential cause of disease in the big cat species of Niassa. Further studies on the pathological effects of these parasites in wild lions and leopards are needed in order to assess how disease occurs in such species as well as more information on individual parasite loads. However, the ecology of endemic diseases (such as those caused by macroparasites) in free-ranging populations is difficult to study without controlled experiments and interventions (Macdonald & Loveridge, 2010). Additionally, studying diseases in wildlife is not easy, since sick animals and/or carcasses are hard to detect and pathogens are often hard to isolate (Macdonald & Loveridge, 2010).

Although wild animals are expected to be natural hosts of many parasite species and other infectious agents, most of the times these agents are also expected to not cause disease until other triggering factors arise (Munson et al., 2008). As it was reported by Munson et al. (2008) regarding the Serengeti lion CDV epidemics, the unusually high mortalities were most likely a result of several host, prey and environmental factors. For that reason it is possible to expect normal and seasonal fluctuations regarding parasite infection morbidity, without significant mortality due to parasite infections, as lions seem to be constantly infected and their population and gastrointestinal parasite fauna remains near equilibrium (Macdonald & Loveridge, 2010). However, the presence of other contributing factors that could interfere with the host's ability to tolerate these parasite species such as the presence of infectious diseases, the host's immune status, nutritional deficits or dramatic environmental changes such as severe droughts have the potential to disturb this balance leading to higher mortality scenarios.

The identification of the parasitic elements in this work was based on morphometric analysis and comparison with current literature, which allowed the identification until genus level. However, further work on the molecular analysis of both parasites, viruses and also bacteria should be carried out in order to allow a more detailed identification of all the elements. This would also contribute for a better understanding regarding the potential sources of both viruses and parasites.

Although our results were surprising considering the poor state of some of the samples, any further studies aiming at detecting lion parasites from faecal samples should use freshly collected samples whenever possible.

We hope the results of the present work can provide a baseline for future studies in Niassa Reserve in order to find answers and design strategies to mitigate threats to the populations of lions and other carnivores, while contributing to a better understanding of the impact of diseases in these populations.

We also highlight the importance of studies in the scope of Conservation Medicine such as the present one, in order to gain a better understanding about the role of parasites as well as other pathogens, and how they impact wild animal populations, and consequently the Health of such complex ecosystems.

5 - Conclusion

This study represents the first survey on the population of African lions in Niassa National Reserve and Mozambique and is also one of the few ever undertaken within the parasitology of African lions in the wild. To the best of the author's knowledge this was also the first conducted study in Niassa Reserve, based on the collection of fecal samples from wild lions.

Our results showed that a total of 65.9% of the collected samples were positive for gastrointestinal parasites considering all the coprological techniques used. Out of all the positive samples we were able to identify six different parasite species: *Toxocara* sp., *Aelurostrongylus* sp., Taeniidae, *Spirometra* sp., *Paramphistomum* sp. and *Linguatula* sp. The most common parasites forms found in this study were *Toxocara* sp. eggs (47.7%) and L1 larvae of *Aelurostrongylus* sp (31.8%). Unlike what has been suggested in previous studies on this subject, *Spirometra* sp. was not the most common parasite in our samples. Still, the presence of this parasite in almost every study ever carried out in wild lions is remarkable. Finally, and although *Linguatula* sp. eggs were only found in 13.6% of all the forty-four samples, we also consider it a very interesting finding since to the best of our knowledge, this was the first documentation of this parasite's prevalence in wild lions. We also highlight the importance of distinguishing between cases of pseudo-parasitism and true parasitism such as the presence of *Paramphistomum* sp. in the present study, when dealing with carnivores with such a wide spectrum of prey species.

Some of the parasite species found in the lion scats in this study namely *Toxocara* sp. *Aelurostrongylus* sp. *Spirometra* sp. and Taeniidae, have also been found in leopards. Although we have not included leopard scats in our study, the ecology of some of these parasites indicates that they might also be infecting leopards in Niassa.

This work also showed how challenging it can be to work with such species in Africa. We acknowledge the constraints of working with free-ranging populations in such remote areas where logistics are often a problem, and the complexity of the interactions between all the elements involved is sometimes overwhelming. Nevertheless, the lack of knowledge in this field can often be frustrating, but very challenging at the same time.

The existence of large protected areas such as Niassa Reserve supporting high densities of wildlife is something that we should all cherish to maintain and help to preserve as they are an invaluable natural and cultural legacy of great importance not only for Mozambique, but for the World.

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APPENDIX A – Full paper presented in the Proceedings of the International Conference of Diseases of Zoo and Wild Animals 2015, 13th to 16th of May, 2015, Barcelona**,** Catalonia (Spain)

GASTROINTESTINAL AND RESPIRATORY PARASITES SURVEY IN WILD AFRICAN LIONS (PANTHERA LEO) FROM NIASSA NATIONAL RESERVE, MOZAMBIQUE – PRELIMINARY RESULTS

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Summary

The African lion (Panthera leo) is an iconic species of the African continent, classified as vulnerable by the IUCN. Infectious diseases are one main threat for the survival of this species. However, the impact of parasitic infections has been overlooked and poorly documented. In order to characterise the parasitological fauna of these animals, a study was carried out in the Niassa National Reserve (NNR), Northern Mozambique, where a large population of lions lives. In partnership with the Niassa Lion Project and the administration of the Niassa Reserve, 44 lion faecal samples were collected in an area of 600 km² (Concession L5-South), and later processed and analysed at the Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Lisbon University. Results show that 65.9 % (29/44) of the samples were infected with parasites, namely 47.7 % for Toxocara sp., 31.8 % for Aelurostrongylus sp., 25 % for Spirometra sp., 27.3 % for Taeniidae, 18.2 % for Paramphistomum sp. and 13.6 % for Linguatula sp. Out of the 29 positive samples, 72 % (21/29) were co-infected, registering double infection in 21 % (6/29), triple in 34 % (10/29), quadruple in 10 % (3/29) and fivefold in 7 % (2/29). These results are consistent with previous studies performed in the African continent, with the exception of Linguatula sp., which had not yet been reported in wild lions.

Introduction

The Niassa National Reserve (NNR) is one of the largest, most remote and least known conservation areas in the World, located in the far North of Mozambique. This Reserve stretches over 42,000 km2, representing the conservation area with the highest density of wildlife in the country, with particular relevance for its African lion population (Panthera leo), with an estimated size of 1000 to 1200 individuals (NIASSA CARNIVORE PROJECT, 2013). For this reason, the Niassa National Reserve is considered a stronghold for the species (RIGGIO et al., 2012) and represents one of the few areas in the World where the species has a chance of long-term survival. According to The IUCN Red List of Threatened Species, P. leo is mostly in decline throughout its range. The African lion is a species classified as vulnerable by the IUCN and is protected by CITES Appendix II. The main threats to its survival are conversion and destruction of habitat, prey depletion and indiscriminate killing (IUCN, 2014). Diseases may contribute to the reduction of animal populations and biodiversity loss. There is currently little information on parasitic diseases in wild lions, although their parasitological fauna is considered to be very different from that reported in lions kept in zoos (SCOTT, 1988; MÜLLER-GRAF, 1995). In order to characterise the diversity and the level of parasitism of African lions in NNR, a study was developed in a partnership with the Niassa National Reserve Management authority (a comanagement agreement between Wildlife Conservation Society and the Ministry of Tourism) and the Niassa Lion Project, which has been based in Niassa National Reserve since 2003.

Material and methods

Between October and November 2014, 44 lion faecal samples were identified and collected from the field, in an area with approximately 600 km2 (L5-South Concession) of the NNR. Multiple sampling of the same animal(s) was possible, as the source of faecal samples was not known. Samples were located by a local tracker and lion field worker, stored in dry conditions, transported in plastic bags to the base camp of Niassa Lion Project and kept in a cool place until transported by plane to Portugal. Samples were analysed between 15 to 30 days after collection at the Laboratory of Parasitology and Parasitic Diseases, CIISA-FMV-ULisboa, using the following coprological methods according to THIENPONT et al. (1986) and BOWMAN (2014): qualitative natural sedimentation and Willis flotation (for isolating helminth eggs and protozoan oocysts); McMaster quantitative method (to assess the number of eggs per gram of faeces, EPG); and Baermann technique (for the search of first stage larvae (L1) of pulmonary nematodes).

Results

A total of 65.9 % (29/44) of the samples were positive considering all the coprological techniques. A wide range of parasites was observed (figure 1), belonging to genus Toxocara 47.7 % (21/44), Aelurostrongylus sp. 31.8 % (14/44), Spirometra sp. 25 % (11/44), Taeniidae family 27.3 % (12/44), Paramphistomum sp. 18.2 % (8/44) and Linguatula sp. 13.6 % (6/44).

Out of the 29 positive samples, 28 % (8/29) were infected by a single species of parasite and the remaining 72 % (21/29), were co-infected with two, 21 % (6/29), three, 34 % (10/29), four, 10 % (3/29) and even five, 7 % (2/29) distinct parasites. The most common co-infection was the one caused by Aelurostrongylus sp. and Toxocara sp., 57 % (12/21).

Discussion

The number of parasite species identified in this study was higher than previously found in Namibia by SMITH and KOK (2006) and lower than those found in Zambia by BERENTSEN et al. (2012) and in Tanzania by MÜLLER-GRAF (1995) and BJORK et al. (2000). Toxocara sp. (47.7 %) was the most prevalent parasite in our study, in comparison with the studies of MÜLLER-GRAF (1995), MÜLLER-GRAF et al. (1999) BJORK et al. (2000) and BERENTSEN et al. (2012), who documented Spirometra sp. as the most common parasite. The registered prevalence of 32 % for Aelurostrongylus sp. in this work was higher than previously described 20 % (BJORK et al. 2000) and 21 % (BERENTSEN et al., 2012). The prevalence of Taeniidae eggs, 27.2 %, is intermediate between BERENTSEN et al. (2012) (80 %), MÜLLER-GRAF et al. (1999) (58 %) and BJORK et al. (2000) (15 %). The prevalence of 13.6 % for Linguatula sp. represents the first documentation of this parasite species in P. leo in the wild, since only two authors reported this parasite as a potential cause of disease in African lions

(YOUNG, 1975; MUKARATI et al., 2013). The presence of Paramphistomum sp., was considered pseudo-parasitism, as a result of hunting wild ruminants, in particular buffalos. The diet of these big cats is probably the source of most of the parasitic forms found, although it is not possible to establish a direct link between the identified parasites and the prey species they belong to. Toxocara sp. was the most prevalent parasite, probably due to its direct life cycle, but also due to the chance of ingesting paratenic hosts with larval stages. The parasite species found in this study may have been influenced by the weather condition of the dry season (with extremely high temperatures and very low humidity), causing dehydration of the samples taken from the field. This fact probably mirrors a potential subcharacterisation of parasitic load, suggesting that prevalence of some parasites found in this study may be higher Ancylostomatid eggs with its thin capsule were not found, probably because high temperatures made impossible the survival of hookworm eggs. However, the high prevalence of Toxocara sp. (a parasite with direct life cycle) and Aelurostrongylus sp. (with indirect life cycle) is remarkable. That could be related also with high animal density in that area, or probable intertransmissibility of these nematodes among big carnivores living in this ecosystem. Wild animals can be natural hosts of many parasite species and other infectious agents, which usually do not cause disease until other triggering factors arise (MUNSON et al., 2008). Due to their life cycles and hostparasite interactions, we consider high loads of Toxocara sp. and Aelurostongylus sp. to be a potential cause of disease in lions. Further studies on the pathological effects of these parasites in wild lions are needed. The identification of the parasitic elements was based on morphometric analysis and comparison with current literature, which allowed the identification until genus level. Molecular analysis is under progress in order to enable an accurate and complete identification. This qualitative study represents the first survey on the population of African lions from NNR and Mozambique and is also one of the few ever undertaken within the parasitology of African lions in the wild.

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