Gastrointestinal parasites infecting ungulates, felids and avian species at National Zoological Gardens of South Africa

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that I have not previously in its entirety or in part submitted at any university for a degree. I furthermore cede copyright of the dissertation in favour of the North−West University.

Signature: ……..

Date: …….....

DEDICATION

This work is dedicated to my loving mother Gillian Mpotseng Mosala, for always believing in me and always wanting the best for me and my siblings and to my beautiful daughter Owaratwa Nomhle Mosala.

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ABSTRACT

Zoological gardens are a form of *ex-situ* conservation which involves keeping valuable animal species, especially wild animals alive outside their natural environment for educational, research and recreational purposes. South Africa is blessed with abundant wildlife species which need to be properly managed on a sustainable basis to prevent depletion. Parasites play a major role in the lives of animals, with effects ranging from negative impacts on host population size to the evolution of host behaviour to combat parasites. Gastro-intestinal tract (GIT) parasites are one of the leading factors that threaten health of wildlife, especially in captivity causing morbidity and even mortality and some are zoonotic with potential to infect staff and visitors.

Currently information on GIT parasites in wildlife is scarce in South Africa, such information is important for conservation of wildlife especially in captive environments. Animals should be monitored and managed regularly, it becomes costly to treat, the parasites become resistant to drugs, and there is reduced reproduction rate in infected animals and lastly death is sometimes the end result. Although the GIT parasites at the NZG are monitored regularly through the Preventative Medicine Program, there are no scientific research studies done on this subject, let alone DNA-based studies on this subject. This study is the first of its kind at the NZG. The study investigated the seasonal distribution of GIT parasites in selected captive animals at the National Zoological Gardens of South Africa (NZG) using both microscopic and molecular techniques.

A total of 772 faecal samples were collected from selected captive felids (n = 97), captive ungulates (n = 406) and captive avian species (n = 269) at the NZG between October 2015 and October 2016. Egg-floatation techniques (Faecalyser and McMaster) were used to estimate the parasite load in sampled animals whilst identity of helminth genera was confirmed by PCR.

Three hundred and thirteen (40.54%) out of 772 samples were positive for one or more GIT parasites. The prevalence in ungulates was 63.55%, 39.18% in felids and 6.32% in avian species. The most commonly observed eggs via microscopy were strongyles in ungulates, *Toxascaris* sp. in felids and *Capillaria* sp. in avians with prevalence levels

of 38.5%, 37.9% and 4.1% respectively. Faecal analysis revealed overall GIT parasite prevalence of 30.6%, 60.8% and 6.9% in felid, ungulate and avian species respectively over the study period. The average egg per gram (EPG) in the ungulate, felid and avian species sampled was respectively higher in warm summer months (63.7%; 47.2%; 10.8%) as compared to the colder winter months (60%; 27.3%; 1.0%). There were higher parasite loads in summer for felid avian species than in winter Warm and moist weather conditions facilitate development of parasitic eggs; hence the GIT parasite prevalence was higher in summer months. Majority of ungulates had mixed infections of strongyles type eggs. PCR detected for *Haemonchus contortus* in 51/107 (47.66%), 7/46 *Ostertagia ostertagii* (15.22%), 39/39 *Trichostrongylus* sp. (100%) and 13/30 *Nematodirus spathiger* (43.33%) from positive microscopy samples.

Our study suggests that among different helminthic infections, the prevalence of nematode infections was higher than that of cestodes and trematodes. Data from this study combined with regular monitoring and treatment of captive wildlife for GIT parasites is very important for understanding and maintaining the welfare of the animals, staff and visitors at the National Zoological Gardens of South Africa.

Keywords: Gastro-intestinal parasites, helminths, avian species, faecal samples, felids, ungulates, *Haemonchus*, *Toxascaris, Capillaria, Trichostrongylus.*

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ABBREVATIONS

°C: Degree Celsius

µl: microlitre

µm: micrometre

ARC-OVI: Agricultural Research Council - Onderstepoort Veterinary Institute

bp: Base pairs

DNA: Deoxyribonucleic acid

EPG: Eggs per gram

g: Grams

GIT parasites: Gastro-intestinal parasites

ITS: Internal transcribed spacer

L_3: Infective third stage larvae

mg: milligrams

ml: millilitre

Mm: millimetre

ng/µl: nano grams per microlitres

NZG: National Zoological Gardens of South Africa

OPG: Oocysts per gram

PCR: Polymerase Chain Reaction

RPM: Revolutions per minute

SG: specific gravity

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General overview of GIT parasites

Gastro-intestinal tract (GIT) parasites are microorganisms that survive on the nourishment they receive from their hosts as well as the protection they need to survive, all of which happens at the expense of the host (Hale 2006). These parasites live in the gastro-intestinal tract of animals, including humans. Some remain in the intestines while others may travel outside the intestines to invade other organs (Papazahariadou *et al*. 2007). They are known to be widespread in animals both wild and domesticated (Abouzeid *et al*. 2010). There are two main types of gastro-intestinal parasites, namely, protozoans and helminths. The protozoans are single-celled organisms that feed on organic matter (Hoorman 2011). There are over 30 000 different species of protozoa but they are not all parasitic. The helminths, also known as parasitic worms are multicellular organisms that are visible with the naked eye. Helminths are multicellular eukaryotic animals that generally possess digestive, circulatory, nervous, excretory, and reproductive systems. Helminths are often referred to as intestinal worms however not all helminths reside in the intestines. For example; schistosomes reside in the blood vessels. Helminths are parasitic because they benefit at the expense of the other, the host (Hale 2006).

Parasitic worms include the tapeworms, roundworms, lungworms, liver flukes, ring worms, hook worms and whip worms. Transmission of GIT parasites is fairly direct in most cases; the infective eggs or oocyst are passed with the faeces when the animal defecates, the next animal would be infected if they graze in the contaminated areas, and humans could be infected through ingestion of contaminated food and water and or through close interactions of humans with the infected animals. Some parasites can live in the soil for extended periods, and may penetrate though skin or may be ingested accidentally leading to GIT parasite infection. First signs of intestinal parasitic infection are dyspnoea, diarrhoea and weight loss, which are commonly associated with behavioural changes e.g refusal to feed, isolation and self-grooming, decision making and behaviour control mechanisms (Sanchez *et al*. 2009), however in severe GIT

parasite infections, symptoms such as blood loss, tissue damage, spontaneous abortions, congenital malformations, significant morbidity and mortality in both free ranging and captive wild animals are visible (Emikpe *et al*. 2007; Li *et al*. 2015). Gastro-intestinal parasites have a worldwide distribution, but they are more prevalent in countries with warm and tropical climates (Adeniyi *et al*. 2015). Factors such as light, temperature and humidity favour development of the parasite in the external environment and it is this climate that facilitates increased parasitic load in animals. There are a number of factors threatening the existence of wild animals; including wildlife diseases in particular those arising from GIT parasites; with that said helminthic infections are a major problem causing mortality in captive animals (Thawait *et al*. 2014). Parasite infections cause little or no distress at all to healthy animals in their natural habitat (Otegbade & Morenikeji 2014).

1.2 Gastro-intestinal parasites infecting captive animals

Zoos are areas in which a great number of valuable animals species; especially wild animals are kept within enclosures for studies, conservation and displayed to the public for recreational purposes (Adeyini *et al*. 2015). The spread of parasitic diseases in zoo animals reveals a number of negative effects such as infections with certain dangerous species which may lead to death in affected animals. Infected animals may have reduced reproduction rate which is a major factor in zoo where it is a common practice to conduct breeding programs. Lastly zoos are places where wild animals come in close contact with people which is lacking in their natural environment because most of the gastro-intestinal tract (GIT) parasites have zoonotic potential they pose a threat to human health (Chakraborty *et al*. 1994). Therefore, transmission of diseases to humans can occur when infected animals are handled especially in captive environments such as zoo where there is close interaction between animals, animal keepers and tourists. This factor increases the risk of zoonotic parasites spreading and posing a threat to health of animals, zoo keepers and the tourists (Panayotova-Pencheva 2013).

Factors such as pollution, deforestation, fragmentation and climate change among a few contribute to habitat loss for animals, as the habitat size shrinks, the number of the threatened species increases. Therefore, the animal species should be properly managed on a sustainable basis to prevent them from facing extinction (Opara *et al*.

2010). Conservationists are compelled to seek other secured facilities to maintain species, especially those who are threatened by extinction to prevent depletion. Zoological gardens are a platform that provides for this purpose the essential reservoir of genetic material for such species through captive breeding and then re-introduction to the wild (Mahmound 2015). Animals in zoological gardens are susceptible to almost all types of diseases. Parasitic diseases, especially helminthic infections can become a major problem and results in an outbreak especially in small zoos (Varadhajan & Kandasamy 2000). Parasitic infection may reduce competitive fitness especially in the wild, influencing population cycles and regulating host population abundance. The differing degree of resistance of the varying animal species to parasite infections presents another factor in the parasite - zoos system. Some parasites could be harmless for certain animals but life-threatening to others (Panayotova-Pencheva 2013). The modes by which parasites can be brought in the zoos vary and may either be: by the animal food (contaminated fruit and vegetables, infected meat or fish, etc), by intermediary and paratenic hosts (snails, ants, cockroaches, other insects, worms, rodents, etc), by newly acquired parasitized animals and by infected zoo staff and visitors.

Early literature about parasites in the zoo animals were in the early 1970s, and these publications directed all the attention to helminthoses as an important factor for animal health condition (Panayotova-Pencheva 2013). The entrance, development and spreading of the parasitic diseases is accounted for by interrelationships among the parasites, their host and their surroundings.

1.2.1 Nematodes

Nematodes infect a wide range of hosts including humans, domestic and wild animals, and plants (Walker & Morgan 2014). The phylum Nematoda is the second largest in the animal kingdom, encompassing up to 500 000 species. Many nematodes are free living in nature, however it is estimated 60 species are parasitic to mammals (). Parasitic nematodes of small ruminants and other livestock have a major economic impact worldwide (Roeber *et a*l. 2013). In many parts of the world grazing land is shared between wild and domestic species, leading to potential transmission of parasitic nematodes between these groups (Rose *et al*. 2014).An example are the Trichostrongyloid nematodes, they are extremely diverse with equally diverse host

range. Primarily they affect production in livestock and infect free-ranging ruminants worldwide. For example, majority of helminths that infect Saiga antelope in Kazakhstan are shared with livestock. This includes several species that have major economic importance (Rose *et al*. 2014). Therefore studying GIT parasites is important for both captive and free ranging animals as it provides broader view of the epidemiology of the diseases caused by the parasites.

Anatomy

The adult nematodes are slender, brown-red in colour and can range from 5 – 10 mm. long depending on the different species. The body is covered with flexible cuticle and has no visible external signs of segmentation. These worms have a complete digestive system with 2 openings (mouth and anus) however they have incomplete nervous system and no excretory and circulatory system. The female ovaries are large with an opening called vulva, and the males have 2 spicules used for attachment during copulation. The eggs are ovoid, with a very thin shell and the egg may measure around 85 – 115 µm, the egg is elongated and pointed at one or both ends; and the eggs have embryo when shed (Thienpont *et al.* 1979).

Life cycle

Parasitic helminths are highly modified as compared to the free-living helminths. The lifecycles of the nematodes are similar and direct and require no intermediate host. They have four larval stages, and an adult stage (Figure 1). The eggs are passed with the faeces when the animal defecates and contaminate pastures. When the eggs hatch, they release the L_1 (first larval stage) which later moults into L_2 (second larval stage). Both the L_1 and L_2 are free-living stages that only feed on bacteria contained in the faecal pellets. After moulting to L_3 (infective stage, non-feeding), the L_2 cuticle remains for protection of the larvae. The L_3 remain alive until stored nutrients are exhausted, this stage can stay alive for weeks to few months depending on the weather conditions. When the weather conditions are warm and moist, the L_3 leaves the faecal pellet, migrate up the grass blades. The L_3 are ingested with the grass blades. Third larval stage moult to L_4, this occurs in the GIT. Depending on the weather conditions, L_4 becomes hypobiotic, arrested in its development. Fourth larval stage feeds on the protein and or blood, if the weather conditions are temperate and moist; it moults to adult worms which will produce eggs (Hale 2006).

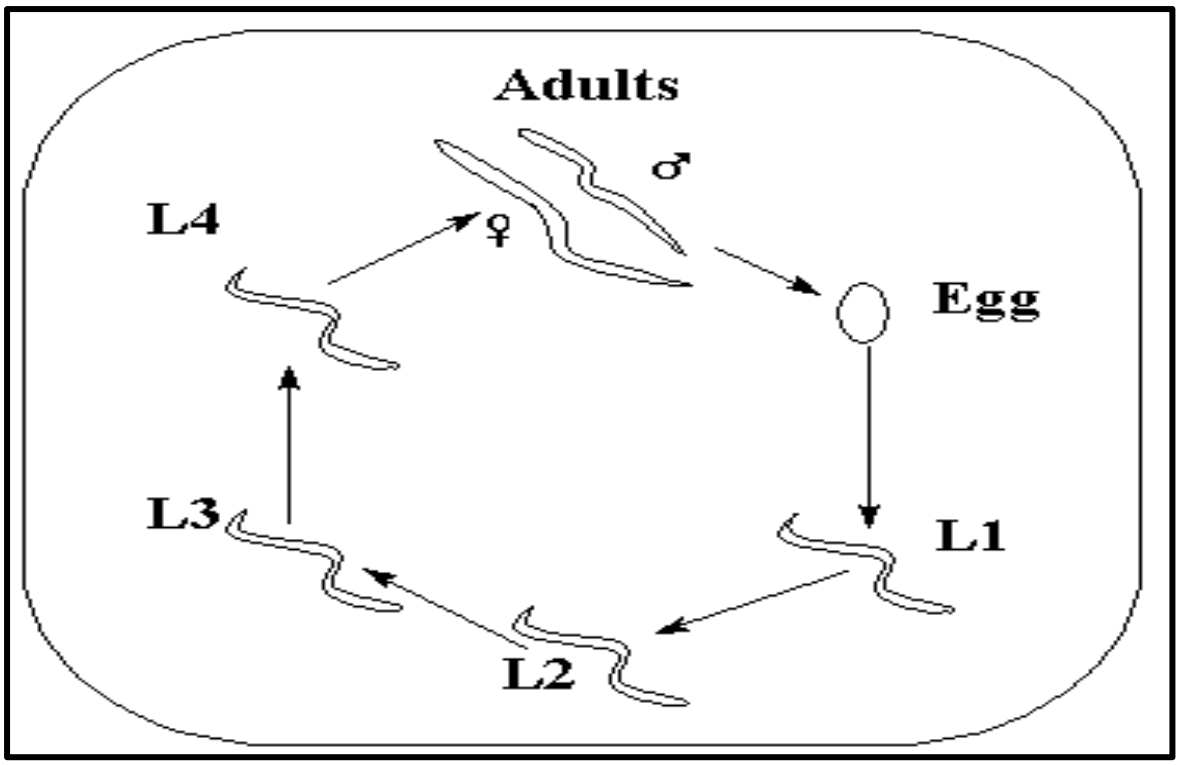


Figure 1: Basic lifecycle of the parasitic nematodes (http://soilcrawlers.weebly.com/life-cycle.html)

Signs and symptoms

In humans, the majority of infections are asymptomatic or a patient may present mild symptoms. Reported symptoms for the infections include abdominal pains, nausea, diarrhoea, flatulence, dizziness, generalized fatigue and malaise (Ralph *et al*. 2006). *Trichostrongylus* nematodes infection damages the lining of small intestine of the infected host, this occurs when there is heavy infestation of the parasitic worms; this may lead other effects such as diarrhoea or constipation, general weakness and wasting disease. Loss of appetite, acute severe infection in young animals can be fatal. Lesions have not been described in wild bovids or cervids but may be expected to resemble those typical of trichostrongylosis in cattle. Under heavy infestation, there may be hyperemia of the abomasum and development of whitish, necrotic plaques (Hoberg *et al*. 2001).

During infection with *Ostertagia* sp., two major clinical developments occur; Type I Ostertagiasis also known as the Summer Ostertagiasis which affects calves and young ruminants during grazing season and affects these young animals first time. This is from maturations of larvae shortly after ingestion (Conti & Howerth 1987), Type II Ostertagiasis also known as the Winter Ostertagiasis which affects the adult

ruminants as they become sick (Myers & Taylor 1989). The arrested larvae resume development during winter and early spring. This is from sudden and spontaneous maturation of larvae after a period of arrested development (Conti & Howerth 1987). Clinical signs are mostly observed in young animals but have also been seen in adult who not previously exposed to the infection show signs. The *Ostertagia* sp. infection is characterized by watery diarrhoea which usually persistent, there are however periods of constipation as well. Lesions can be readily seen in the abomasum and small petechiae may be visible were the worms have been feeding. Characteristic lesions of *Ostertagia* sp. infection are small, umbilicated nodules 1 – 2 mm in diameter (Myers & Taylor 1989).

Most often, the symptoms of *Trichuris ovis* infection are confused with coccidiosis because of the bloody diarrhoea it is associated with. The larvae that penetrate the lining of the intestines cause irritation and the parasitic adult roundworms damage the wall the cecum. Most of the *Trichuris* sp. infection shows no clinical signs (Wideman 2004). A light infection usually does not cause much damage but sometimes acute infections cause colitis, chronic diarrhoea and other disorders related to intestinal tract (Thapar *et al* 1954). Under heavy infestation, *Trichuris* sp. infection can cause the intestines' inflammation, ulceration, bleeding and subsequent anaemia, and bloody diarrhoea. Fatalities occur, mostly in young infected animals but they are not very common. Most cases of trichuriasis are asymptomatic, although some animals may be in poor condition or have reduced performance. Outbreaks of severe of bloody diarrhea with mucous associated with anorexia, depression and deaths have been reported in pigs. Trichuriasis can be particularly serious in pigs up to 3 months old, and they are susceptible to other intestinal infections including salmonellosis and swine dysentery (CFSPH 2005).

In livestock, *Haemonchus* remains the most damaging gastro-intestinal helminth in tropical and subtropical regions mainly for sheep and goats (Qamar *et al*. 2009). Both the larvae and the adult feed on blood cause tremendous damage to the intestinal tissue particularly the stomach. Blood loss is often observed which results in anaemia. While feeding, they release anticoagulants which delays blood clotting (Geldhof & Knox 2008). Other effects of infections are edema (a typical example is bottle jaw, whereby the liquid accumulates in the tissue around the neck), weak and listless behaviour and ultimately death. Under severe infections; liver damage is often

observed, weight loss, unthriftiness, dehydration and diarrhoea. Young animals and female animals that are soon to give birth are most vulnerable to this parasite infection (Machen *et al.* 2002).

Toxascaris leonina infections are mostly asymptomatic, i.e. infected host do not become sick and do not show any clinical signs. However, in special cases few clinical signs are associated with the infections in felids such as diarrhoea is common in young animals, mucus faeces and the infection can also cause vomiting, with worms at times. These worms absorb nutrients from the host, which can interfere with digestion and can also damage the lining of the intestine. Other signs include digestive disturbance, allotriophagia and unthriftiness appears, but none of these signs have been observed in adult animals (AAVP 2013). Under heavy infestation an infected animal may develop enteritis (inflammation of the small intestine). An infected host may also shows signs of inflated belly and a dull hair coat (Bowman *et al*. 2002).

During infection with *Toxocara cati*, infected kittens show no clinical signs (asymptomatic). Under heavy infestation, *T. cati* can result in the inflammation of the small intestines (enteritis). *Toxocara cati* is said to be capable of displaying signs similar to those of *T. canis*, such as potbellied appearance, failure to thrive. Fatalities can occur due to intestinal obstruction and even rupturing of the intestines. Aoki *et al*. (1990) reported on a domesticated cat that had anorexia, vomiting and enlarged abdomen, laparotomy revealed the presence of *T. cati* in the abdominal cavity and a gastric ulcer that had perforated the stomach wall.

Diagnosis

The diagnosis of the nematodes is simply based on the observation of eggs in the faecal matter of the infected host by microscopy and also to determine faecal concentration of eggs. The microscopic differentiation between *Trichostrongylus* sp*.* eggs and hookworm eggs is difficult and time consuming as the eggs are similar morphologically (Ralph *et al*. 2006) therefore it is difficult to identify various *Trichostrongylus* sp. from the eggs and to distinguish the eggs from those of the hookworm therefore to overcome such limitations, molecular approaches have been conducted such as PCR which can be applied to distinguish between *Trichostrongylus* sp. and other species with similar morphological characteristics (Yong *et al*. 2007).

Trichostrongylus sp. infections can also be laboratory-acquired, in some cases through mouth-pipetting techniques (Ralph *et al*. 2006).

The following parameters are useful in the diagnostic problem of Ostertagiasis (Myers & Taylor 1989):

- History of the animal, this includes previous parasite infection, previous grazing pasture and the age of the infected animal
- Routine examination of abomasum of the ruminants, as well as routine parasitologic examination for necropsy
- Knowledge of *Ostertagia ostertagi* epidemiology in the area of study or of farming, location where infections occur and weather conditions in the area.

During *Haemoncus contortus* infections clinical diagnosis is primarily based on the clinical signs and confirmation is only after the detection of eggs in the faeces. Young animals can become sick before the larvae develop into adults, i.e. before the onset of egg production. Because anemia is leading problem in haemonchosis, monitoring the mucous membrane is very important and has proven to be effective as a diagnostic approach. The FAMACHA system uses a scale to compare with ocular mucous membrane colour, which correlates with packed cell volume in sheep (Bath & van Wyk 2001). Although egg floatation is a valuable diagnostic tool to assess gastro-intestinal parasitism, differentiation cannot be made easily from other strongylids (Zajac 2006).

Faecal examination for egg detection of *Toxocara cati* and *Toxoscaris leonina* includes floatation techniques. Under heavy infestations full grown worms or larvae can be found in vomit, morphological features of the larvae can be used for identification and therefore diagnosis (Pawar *et al*. 2012). Pawar *et al*. (2012) was able to detect *T. leonina* and *T. cati* eggs in captive Asiatic lions based on microscopic analysis and molecular analysis was based on PCR amplification of the ITS-2 region of ribosomal DNA.

Prevention and control

In humans, the use of herbivores manure as fertilizer is common route leading to infection; therefore it is important to clean vegetable and fruits before eating to prevent infection (Garcia 2007). Treatment with pyrantel pamoate has been recommended,

alternative drugs mebendazole and albendazole have been used (The Medical Letter 2004), there has been reports on successful treatment with ivermectin (Ralph *et al.* 2006). In ruminants, *Trichostrongylus* sp. worms occur mainly in mixed infection with other gastro-intestinal parasites such as *Haemonchus contortus, Cooperia* sp. and *Ostertagia* sp. Livestock exposed to hairworms may develop natural resistance and recover spontaneously and therefore need not to be treated as they would not become sick if they would be re-infected. Effective anthelminthic include benzimidazoles, levamisole, macrocyclic lactones. Tetrahydropyrimidines is also effective, but only against adult worms but not so much on the larvae. There have been reports which confirm resistance of several *Trichostrongylus* sp. to most used anthelmintic in livestock, sheep, goats and cattle, so far no reports on resistance on horses and or poultry (Junquera 2015)

The excrements should be eliminated regularly to avoid cross contamination. There are a number of different anthelminthic products which could effective for *T. leonina* and *T. cati*; however there are no true vaccines available. There are no reports on resistance on *T. cati* to anthelmintics, therefore failure for the anthelmintics to achieve expected efficacy would be because of incorrect use and not because of resistance. Albendazole is the treatment of choice for toxocariasis (Despommier 2003). In humans a dose of 400 mg of albendazole twice a day for 5 days is the currently recommended therapy, because the alternative mebendazole is poorly absorbed outside the gastro-intestinal tract (Despommier 2003).

Resistance

There have been reports confirming resistance of *Ostertagia* sp. to common anthelmintics e.g. Benzimidazole, lactones, levamisole etc. The problem is not as severe as in *Haemonchus* species but it spreading. Therefore if anthelminthic treatment fails to achieve expected efficiency, it could be because of anthelminthic resistance. In a study by Mungube *et al*. 2015, it was found that although Valbazen® (albendazole 10%), Nilzan® plus (levamisole 1.5% and oxyclozanide 3%) and Noromectin® (ivermectin 1%) were used to treat effectively against nematodes in cattle, *Ostertagia* sp. persisted among cattle treated with the three drugs.

1.2.2 Trematodes

They are multicellular eukaryotic helminths, with a unique life cycle involving sexual reproduction in mammalian or other vertebrate definitive hosts and asexual reproduction in snail intermediate hosts (Doughty 1996). Trematode infections such as schistomiasis have emerged as important tropical infections. An estimated 200 million people in the tropical belts of the world may have schistosomal infection (Mutapi *et al*. 2016). Making *Schistosoma* infection the second most prevalent tropical disease in areas such as sub-saharan Africa after malaria (Hotez *et al*. 2006). There are four groups of the trematodes, grouping is primarily based on human host: 1) hermaphroditic liver flukes which reside in the bile ducts and infect humans on ingestion of watercress or raw fish; 2) the hermaphroditic lung fluke which infects human on ingestion of raw crabs; 3) the hermaphroditic intestinal fluke which infects the host on ingestion of water chestnuts and 4) the bisexual blood fluke which live in the intestinal or vesical venule and infect humans by direct penetration through skin (Doughty 1996). Trematodes do not multiply directly in their definitive host, but instead mate and produce large numbers of eggs that pass out of the body in the feces, urine, or sputum. Thus, the intensity of human infection is related largely to the rate of exposure to infective larvae (Doughty 1996).

Anatomy

Trematodes are flattened oval or worm-like animals, their most distinctive external feature is the presence of two suckers, one close to the mouth and other on the under side of the animal (Barnes 1982). The body is made up of tough syncytial tegument which protects the internal orgarns and it is also used for surface gas exchange since these helminths have no respiratory organs (Barnes 1982). Like other flatworms, trematodes have an incomplete digestive system with only one opening; mouth. Excretion occurs mostly through the tegument. Most trematodes are simultaneous hermaphrodites, having both male and female organs.

Life cycle

The trematodes have a unique lifecycle involving sexual reproduction in mammalian hosts or other vertebrate host and asexual reproduction in the intermediate hosts i.e. snail. Trematodes have a variety of different lifecycle stages; egg, miracidium,

sporocyst, redia, cercaria and adult (Figure 2). The eggs are passed out with the faeces in the environment, as well as open water in the form of pond. In the water, under favourable conditions, the egg hatch into a miracidium (Colley *et al*. 2014). A miracidium is a free swimming ciliated form that settles in the snail to become sporocyst. The sporocyst then moult into the redia, a larval form with oral sucker. A cercaria is a free-swimming larva that emerges from the snail. The body and tail are greatly varied in form and specialized function is adapted to particular lifecycle demands of each species (Doughty 1996). This results in an embryonic amplification in which hundreds or even thousands of cercariae are produced. Thus the snails serve as an incubator for embryonic amplification. The cercariae then leave the snail in response to environmental stimuli (e.g. light and or temperature) and swim in search for intermediate host, and it is in the second intermediate host where the cercariae form cysts (metacercariae). If such second intermediate host is ingested by the final host, the metacercariae excyst to form adult trematode worms (Colley *et al*. 2014).

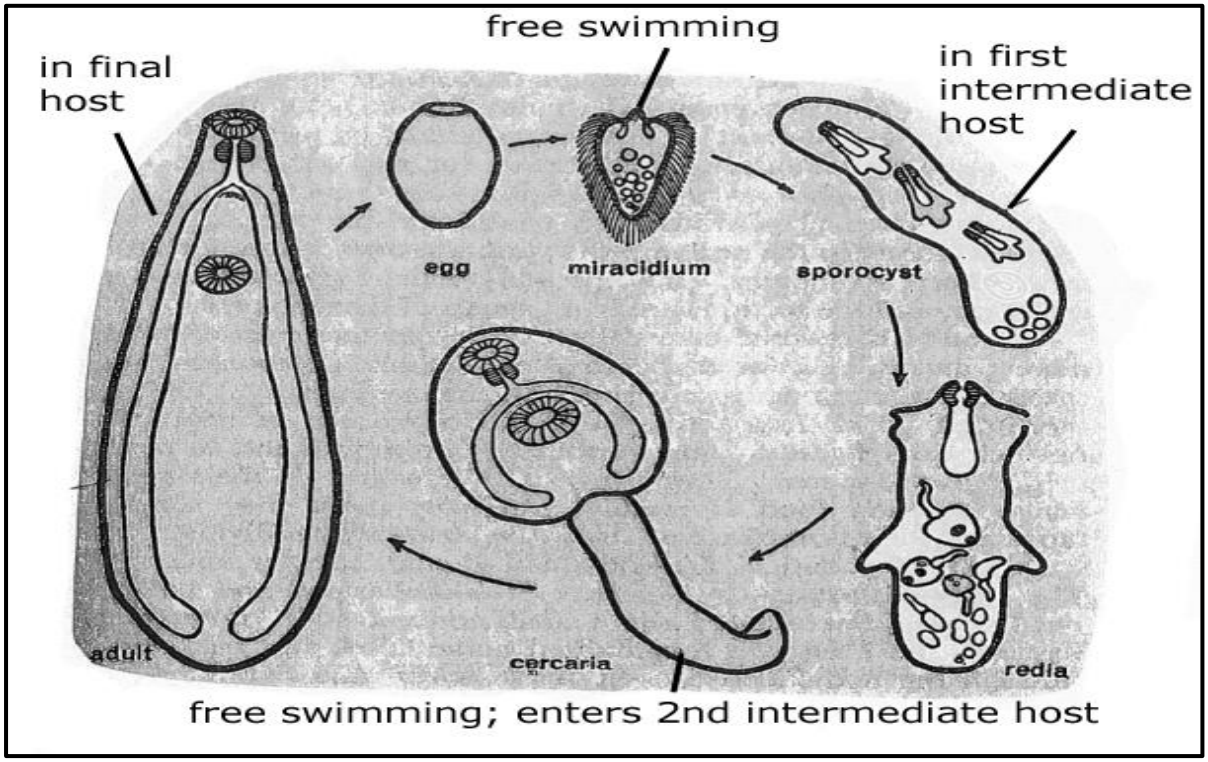


Figure 2: Lifecycle of the trematodes (https://quizlet.com/25021930/parasitology-module-3-wvu-global-medicine-trematodes-flash-cards/)

Signs and symptoms

Different symptoms are observed for different type of infection. Most intestinal fluke infections are asymptomatic. Heavy infections may associated with fever, weight loss, abdominal pain, diarrhea and obstruction. During lung fluke infections, predominant symtoms include chronic cough and production of brown sputum. Other symptoms include chest pains and shortness of breath. During fascioliasis, acute phase may last for a few months and occur within few weeks of infections, symptoms include abdominal pain, cough, urticaria and fever (Doughty 1996).

Diagnosis is suggested by clinical manifestations, geographic history and exposure to infective larvae. The diagnosis is confirmed by the presence of parasite eggs in excreta (Doughty 1996). In diagnosing schistosomiasis, a history of significant contact with fresh water is of diagnostic value.

As a control measure, exposure to parasite larvae in water and food should be prevented. Primary treatment for trematodal infections is praziquantel. Another approach is to control snail populations, largely by the use of molluscicides. Treatment of all trematodal infections, other than fascioliasis is accomplished by a one day course of the oral drug. During *Fasciola* infections, treatment of choice is bithional, given orally for 15 days (Doughty 1996)

1.2.3 Cestodes

Tapeworms are ribbon-shaped, multisegmented flatworms that inhabit the small intestine of their vertebrate host (Heyneman 1996). The larval form lodge in the skin, liver, muscle and other various organs. Their life cycles involve a specialized pattern of survival and transfer to specific intermediate hosts, which they are transmitted to another definitive host. *Taenia* sp. has a cosmopolitian distribution, but are more common in developing countries where hygiene is poor and the inhabitants have a tendency of eating raw or insufficiently cooked meat. More than 60 million people are infected with *Taenia saginata* world wide and about 4 million are infected with *T. solium*.

Anatomy

The most distinctive part of an adult tapeworm is the scolex, which the worm use for attachment in the intestine of the defifntive host. In some species, the scolex is dominated by bothria, or sucking grooves that function like a suction cups. Cerebral ganglion in the scolex is the main nerve centre in the cestodes. Their motor and sensory innervation depends on the number of nerves in and complexity of the scolex. The body is made up multiple segments called proglottids. These are continually produced by the neck region of the scolex. Mature proglottids are released from the tapeworm's end segment and leave the host in faeces. Mature proglottids are essentially bags for eggs, each of which is infective tot the proper intermediate host (Heyneman 1996).

Life cycle

All tapeworms, *Moniezia* spp. have an indirect lifecycle that requires two hosts (Figure 3), a mite as an intermediate host and a ruminant as definitive host (Denegri *et al*. 1998). The eggs are passed out along with gravid proglottids with the faeces when the animal defecates. The eggs are sticky and adhere to the vegetation or soil particles. These can survive for months in the environment and can even withstand cold winters. When they eggs are ingested by the oribatid mites in the soil, they have a timeframe of a day to reach the gut of the mite, or they are desiccated. However, chances of development is good as soil mites can be numerous on the pasture that even if only 3% are infected, a grazing ruminant may ingest over 2000 cysticercoids per kilogram of grass. In the intestine of the mites, the eggs hatch and the oncosphere, a six-hooked larva, penetrates the blood cavity of the mites and develop into cysticercoid stage. This stage may take up to 4 months. While grazing, the ruminant may ingest the infected mite and become infected. The mature cysticercoids are digested out of the mite and develop into mature tapeworms in the in the small intestine within 5 – 6 weeks, they attach to the wall of the intestines. The adult worm can live for up to 18 months inside the definitive host (Barriga 1994).

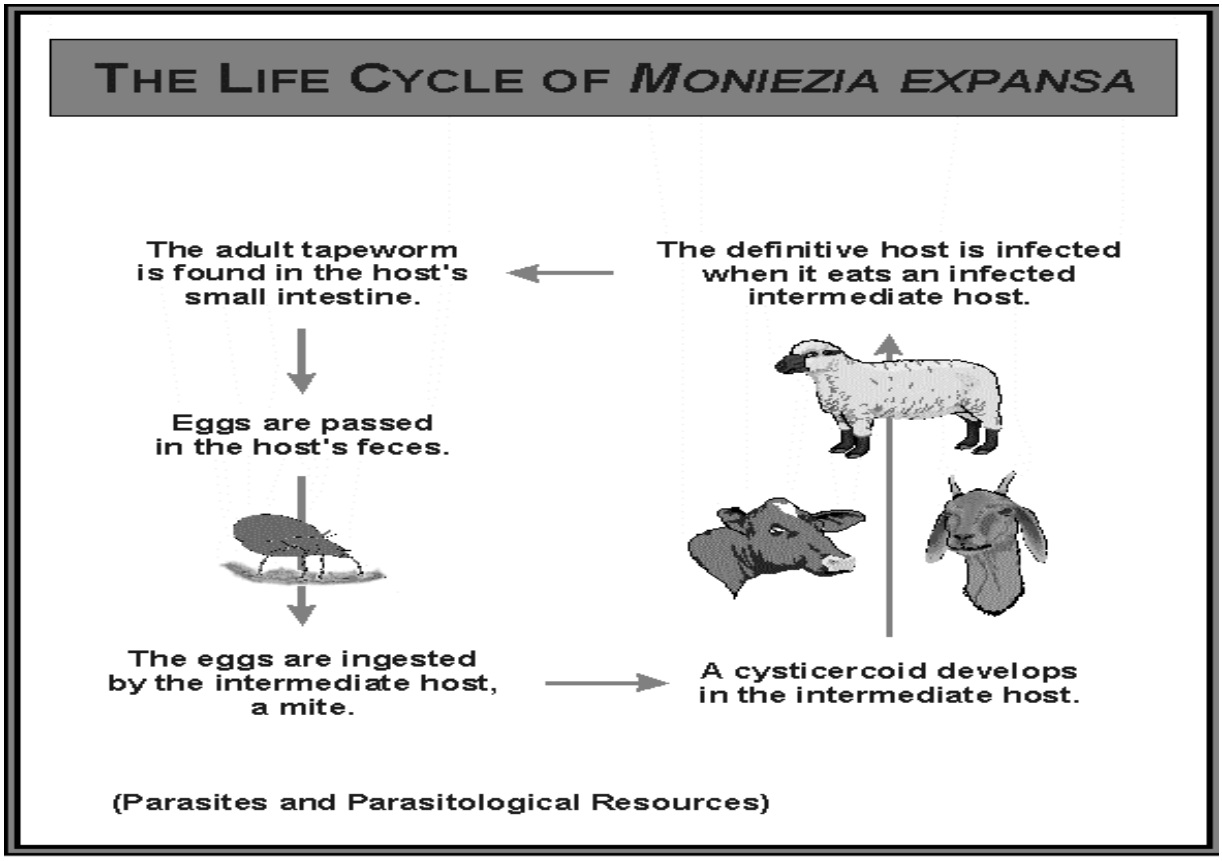


Figure 3: Lifecycle of *Moniezia* sp. (Milk tapeworm) in ruminants (https://www.google.co.za/search?q=moniezia+expansa+life+cycle&espv).

Signs and symptoms

In humans, infections may be asymptomatic although some people may experience upper abdominal discomfort, diarrhea and loss of apetite. In rare cases, the worms may migrate to the brain causing severe headaches, seizures and other neurological problems (Zimmer 2012). Young animals are more affected by tapeworms as they start licking grass at an early age. The symptoms of infection are potbellied, dullness or poor growth and diarrhea.

Diagnosis

Infections can be diagnosed by identifying segments of proglottids in the faeces of the suspected host. The species of *Taenia* sp. can be identified only by the segments, because their eggs are identical (Heyneman 1996)

1.2.4 Eimeria sp.

They are protozoan unicellular organisms of the phylum Apicomplexa that parasitize vertebrates as well as invertebrates, the most common affecting vertebrates is the genus *Eimeria* (Helke *et al*. 2006), and a worldwide distribution (Dong *et al*. 2012). *Eimeria* sp. has been observed in almost all areas where cattle are raised and cause disease in calves (Dong *et al*. 2012). It is responsible for several of the severe diseases known in animals and man, in domestic animals, *Eimeria tenella* is responsible for decreased growth and development of domestic poultry flocks by damage caused by intestinal lining during infections, and economic loss exceed $1.5 US billion annually (Sharman *et al*. 2010). Eucoccidia has been described in all classes of vertebrate such as fish, bird, reptile and mammals including humans. Although *Coccidia* infections in nature are self-limiting, in captive environments coccidiosis can cause morbidity and mortality (Helke *et al*. 2006).

Life cycle

The *Eimeria* and *Isopora* species, commonly called coccidia have a complex but direct lifecycle, which requires no intermediate hosts (Dong *et al*. 2007). There are three different lifecycle stages namely; sporogony, merogony and gametogony (Figure 4). The first two stages are asexual, while sexual reproduction occurs in the third stage. The sporogony is an asexual stage in the lifecycle of the parasite. It is the reproduction of spores, sporulation can only take place when the environmental conditions are favourable; high humidity, temperature that averages 27°C and good oxygenation (Dong *et al*. 2007). The nucleus divides and forms sporocysts, after division the conical bodies formed around the nucleus will form a sporoblast. The sporoblast creates a wall and simultaneously protoplasm forms sporozoites within the wall. Under suitable conditions, this process can take 2 to 4 hours; it may take longer if the conditions are not favourable. The sporogony is followed by merogony; an asexual reproduction of the parasite, it replicates its own nucleus inside the host cell. The ingested sporozoites are released from the oocyst and activated by serine protease (trypsin) before migrating to the intestinal epithelial cells. Once sporozoites are in the epithelial cells, they become trophozoites (active & feeding stage) and then divide into merozoites. Once merozoites have matured, it breaks open allowing the merozoites to re-infect the epithelial cells or progress to sexual reproduction; gametogony. After re-infection, the

merozoites differentiate into gametocytes. The females, also known as the macrogametocytes increase in size to fill the host cell, while the males known as microgametocytes divide into large numbers of small flagellated cells. Once the microgametocytes are released from the ruptured cell, they penetrate the macrogametocytes and the nuclei of cell fuses. The new oocyst formed is protected by a wall, and remains in this stage until release from the host when the animal defecates. The entire lifecycle is completed in 1–2 weeks depending on the environmental conditions (Dong et al. 2007).

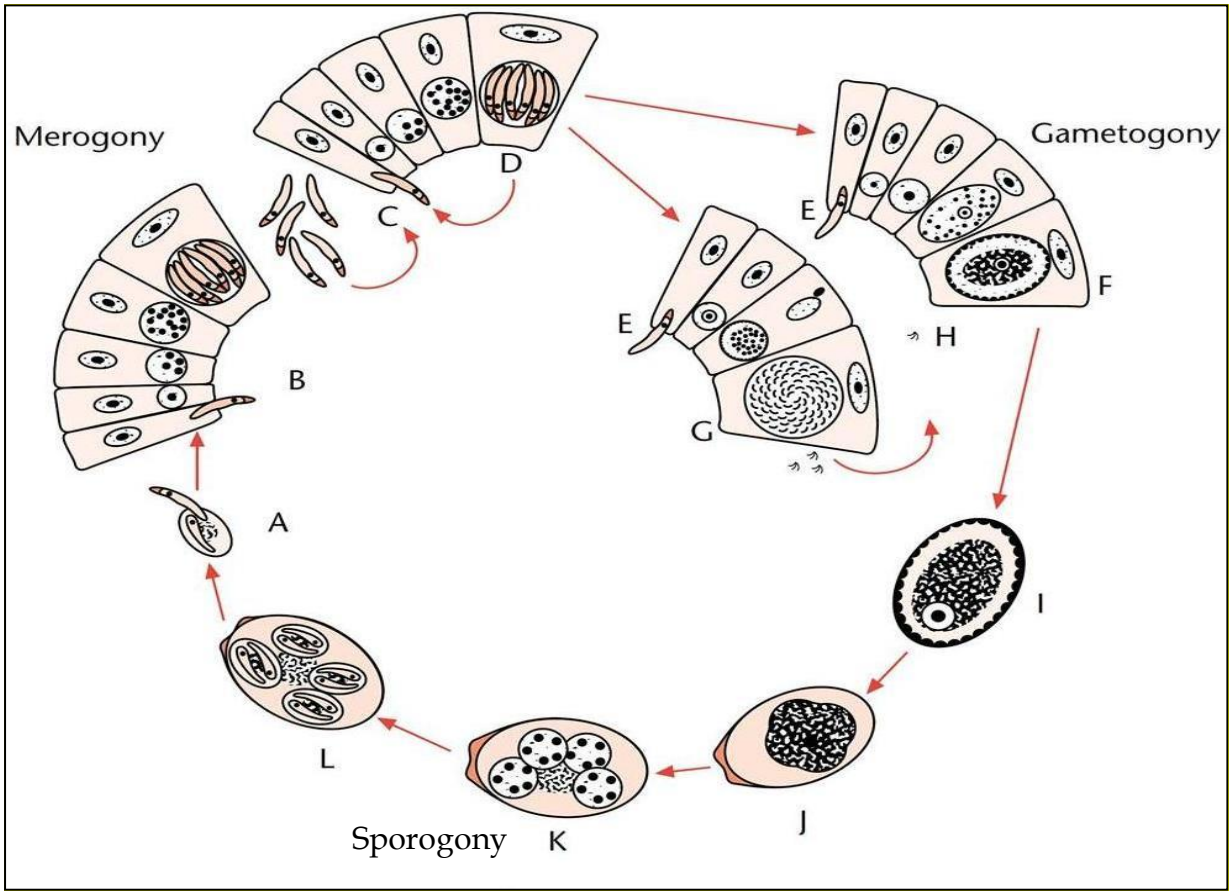


Figure 4: Lifecycle of *Eimeria* and Isopora species (http://www.uoguelph.ca/omafra_partnership/ktt/en/johnbarta/Life-Cycle.asp?_mid_=26491)

Signs and symptoms

In cattle coccidia infection is usually asymptomatic, signs of infections are often not apparent until 3 - 8 weeks after initial infection (Fox 1985). In mild infections, the animal may have diarrhoea with little or no blood in the faeces and may be anaemic (Dong *et*

al. 2002). In severe infections, the faeces are fluid and bloody and may contain mucus and strands of intestinal mucosa. Infected animals may show signs dehydration, weight loss, reduced weight gain and loss of appetite (Dong *et al*. 2002). Death may occur in severe cases due to the coccidiosis or due to secondary infections especially bacterial enteritis or pneumonia

Diagnosis

Symptoms alone are not reliable indicators of clinical disease, because GIT parasites have similar clinical signs. Coccidiosis can exist concurrently with any disease such as colibacillosis, chronic bovine viral diarrhoea, malnutrition or gastro-intestinal helminthosis (Oetjen 1993). Diagnosis of coccidiosis should be based on the history of the animal population, presence of clinical signs, level of parasite load and the occurrence of intestinal lesions at necropsy (Helke *et al*. 2006).

Prevention and control

Coccidiosis is treated through the administration of coccidiostats, this medication stop coccidia from reproducing. In pets, sulfa-based antibiotics are commonly administered. Once reproduction stops, the animal can recover within a few weeks depending on the severity of the infection (http://www.marvistavet.com/coccidia.pml).

1.3 Seasonal abundance of gastro-intestinal parasites in captive wildlife

Wild animals in captive environments harbour a variety of gastro-intestinal parasites and although infections may not always show signs and symptoms, their presence have a negative impact on the host (Colditz 2008b; Fagiolini *et al*. 2010). Gastro-intestinal parasites in wild animals is influenced by a number of factors such as season, climate, age of the host, breeding and immune status and stocking density (Turner & Getz 2010). In the wild, ungulates carry out selective grazing and defecation as natural antiparasitic behaviour; however, captivity inhibits such and may increase the burdens of internal and external parasites (Ezenwa 2004).

Seasonal environmental changes can influence transmission of gastro-intestinal tract (GIT) parasites by affecting the development and survival of the parasite in the external environment and host contact with infectious free-living parasites (Turner & Getz 2010). The prevalence of parasites in host population may increase or decrease depending on factors such as weather conditions and quality and quantity of forage (Ličina 2014). Seasonal dynamics of nematode infections are the consequence of complex inter-relationships between the captive animals, their husbandry and the prevailing climate. The development of the parasitic helminths depends largely on weather conditions; more especially when the eggs are in the external environment and changes in humidity and temperature play a major role on the outcomes of development and survival of parasites. Long dry season may limit development and survival of the parasite stage in the environment, eliminating host contact and parasite transmission (Turner & Getz 2010). It is believed that there is strong relationship between parasite prevalence or intensity and season. Humidity is primarily required for successful development and survival of parasite stages and movement of larval round helminths in the environment (Nielsen *et al.* 2007). Lack of humidity may be a limiting factor some nematode transmission especially in subtropical and semiarid environments.

Coccidiosis is particularly a problem of confined animals, and affects over 50% of cattle, sheep and goats and is considered the fifth most important bovine disease in USA (van Veen 1986). In a study by Pap *et al*. 2015, there was a significant seasonal variation in coccidian infestation, their results indicated that natural level of chronic coccidian infection have a limited effect on the seasonal change of physiological traits.

In a survey study by Nalubamba *et al*. (2012), the occurrence of coccidia oocysts were only seen in captive wild impala antelope during the rainy season.

Dreyer *et al*. 1999 found that calves in communal farm in Botshabelo harboured GIT parasites; the numbers GIT parasites fluctuated, however remained low throughout the four seasons of the year, with the most observed parasites were *Trichostrongylus axei*, *Haemonchus placei*, *Cooperia punctate* and *Cooperia pectinata.* Weather parameters were important factors causing seasonal fluctuation in parasite load. Decreased parasite load was due to low rainfall and low mean minimum atmospheric temperature during cool winter months. Tsotetsi & Mbati (2003) collected faecal samples from 682 cattle, 501 sheep and 300 goats for over a period of 14 months, in the eastern Free State namely Harrismith, Kestell and QwaQwa and observed that samples were infected with different GIT parasites such as *Cooperia* sp.*, Haemonchus* sp.*, Oesophagostomum* sp.*, Ostertagia* sp.*, Trichostrongylus* sp. and *Nematodirus* sp. *Haemonchus* sp. and *Oesophagostomum* sp. were the dominant nematode genera found to be infecting the animals. The EPG counts where higher in small stock than those of cattle

1.4 Economic importance of GIT parasites in livestock and wildlife

It is known that GIT parasitic infections are common in both livestock and wildlife and results in enormous economic losses since they cause morbidity and sometimes mortality (Swai *et al*. 2013). Economic losses are primarily caused by decreased production, cost of prevention, cost of treatment and death of the infected animal (Chattopadhyay & Bandyopadhyay 2013). Gastro-intestinal parasites are highly prevalent in India and have accounted for significant economic loss in various livestock species. One of the reasons for the high prevalence is the prevailing weather conditions which facilitate the development of parasite and therefore increasing the parasitic infections in animals (Bandyopadhyay *et al*. 2010). These parasites cause economic loss to both domestic livestock and game animals worldwide not only because the infection cause debilitating diseases but also because they cause malnutrition and therefore leads to decreased animal productivity (Egbetade *et al*. 2014). In the past, GIT helminths were not considered a production problem especially in Canada, this was because the winter weather conditions kept parasites at a minimum level (Mederos *et al*. 2007).

The annual cost associated with parasitic diseases in Australia has been estimated at 1 billion dollars and it is believed to be tens of billions of dollars worldwide (Roeber *et al.* 2013). There is a major economic gain to be made by agriculture by enhancing the control of parasitic infections. Parasitic diseases of livestock are controlled mainly through anthelmintics treatment. Even with correct administration of treatment, it is expensive manner of controlling parasitic nematode diseases (Roeber *et al*. 2013). Additionally, it has been observed from previous studies that frequent use of anthelmintics in animals often leads to development of resistance strains. Furthermore, the nutritional status of captive animals can also have an effect and or diminish their resistance to parasitic diseases (Lim *et al*. 2008). There is a need for development of improved means of controlling gastro-intestinal parasites.

It was reported in India that there was 4 – 18% increase in milk production in anthelminthic treated animals as compared to those who were not on treatment (Kumar *et al*. 2005). This show how important the anthelminthic drugs are to the animals, and how valuable they are to the economy, however anthelminthics that are not properly administered may lead to resistance of the GIT parasites. The effects of

infection by GIT parasites is decided by a number of factors such as the pathogenicity of the parasite species; the host/parasite interaction; susceptibility of the host species and the infective dose of the parasite (Gul & Tak 2014). Because some of the GIT parasites are zoonotic, they pose a health risk to the human, this further has an impact on the cost of treatment to man, this therefore causes public health including severe infections in humans which sometimes results in mortalities. Economic losses are caused by GIT parasites in a variety of ways; they cause losses through lowered fertility, reduced work capacity, a reduction in food intake and lower weight gains, treatment cost and mortality in heavy parasitized animals. (Gul & Tak 2014)

In Kenya, the infection of the parasitic round worm (*Haemonchus* sp.) causes a loss of US $26 million in sheep and goats, loss due to GIT parasite can be categorized as direct and indirect. The direct loss is due to acute illness and death, premature slaughter and rejection of some parts at meat inspection. Indirect loss is the most important because it results in economic loss; it includes diminution of productive potential such as decreased growth rate, weight loss in growing young animals and late maturity of slaughter stock (Maichomo *et al*. 2004; Swai *et al*. 2006).

In the USA, 2-3 million cattle are treated every year for clinical coccidiosis. Mortality rate can be as high as one in five (Fox 1985). Cost of coccidia infection to cattle rancher mounts up to $400 million annually in lost profits due to reduced feed efficiency, slow weight gain and increased susceptibility to other diseases (Matjila & Penzhorn 2002). Economic loss related to coccidia infections due to mortality, poor performance, cost of treatment and prevention is remarkable, especially in farms and calf-rearing systems (Thomas 1994).

The cost of parasitic infection arises in that some of the helminths can be transmitted to man. A lot of parasitic infections can infact be transmitted between vertebrate animals and man (i.e. zoonotic potential) and about 20 species are of public health importance causing severe to fatal infections. In many countries of Africa parasitic helminths are responsible for enormous economic losses, hampering rural development programmes and reducing the pace of economic growth (Tisdell *et al*. 1999).

Mukaratirwa & Singh (2010) conducted a study on stray dogs impounded by the SPCA in Durban, where there was a prevalence of 82.5% GIT parasites, 93.1% helminths

parasites and 6.9% protozoan parasites. The following parasites and their prevalence were observed; *Ancylostoma* sp. (53.8 %), *Trichuris vulpis* (7.9 %), *Spirocerca lupi* (5.4 %), *Toxocara canis* (7.9 %), *Toxascaris leonina* (0.4 %), *Giardia intestinalis* (5.6 %) and *Isospora* sp. (1.3 %). Dogs harbouring a single parasite species were more common (41.7 %) than those harbouring two (15 %) or multiple (2.1 %) species.

Boomker *et al*. (1991) described *Ostertagia harrisi* as the most prevalent nematode and was the most common GIT parasites in nyala from Umfolozi, Mkuzi and False Bay, while *Cooperia rotundispiculum* was the most common in nyala from Ndumu. There were no clear-cut trends in the seasonal abundance that could be discerned for any of the worms species recovered in the study.

Appleton *et al*. (1994) examined GIT parasites of non-human primates, where they found that five protozoan and six helminths species inhabited the GIT of Samango monkeys in Natal. In subspecies of Samango monkey, *Cercopithecus mitis labiatus* it was found that most of the adult worms occurred in the caecum and the colon; the gut regions also contained the highest volatile fatty acids levels. In another subspecies *C. m. erythrarchus*, nine helminths eggs were recovered; however, protozoans were not looked for in these samples.

1.4.1 GIT parasites affecting ungulates

Trichostrongylus species

Also known as hairworms are parasitic worms from the family Strongylidae. The *Trichostrongylus* species are parasites of either the abomasum or the small intestine of herbivores, although sporadic human infections have been reported in many countries (Souza *et al*. 2013), they are generally uncommon, they suck gastric fluids and causes necrosis of the mucosa and therefore a dangerous parasite in large numbers. They are zoonotic nematode parasites and common among herders of sheep and goats (Ralph *et al*. 2006). They affect a whole range of hosts including cattle, sheep, goats and wild ruminants as well as pigs, horses and poultry. *Trichostrongylus* species can also infect wildlife (antelope, deer, zebra etc.) and remains as one of the most important zoonotic nematodes as they are considered as one of GIT parasites of veterinary importance due to their impact on livestock health and production (Ghasemikhah *et al*. 2011).

Epidemiological studies indicate a worldwide distribution of *Trichostrongylus* sp. infections in humans, and highest prevalence observed in individuals in areas with poor sanitary conditions as well as in rural areas (Adams *et al*. 2005). The parasite has a worldwide distribution (Ghasemikhah *et al*. 2011). This parasitic worm is mostly found in mixed infections with other gastro-intestinal parasitic roundworms such as *Haemonchus contortus*, *Cooperia* sp. and *Ostertagia* sp. Trichostrongyles' closes relatives are the hookworms; they are related in terms of how they are transmitted, their morphology and pathophysiology (Yong *et al*. 2007). Trichostrongylosis is a disease caused by more than 30 species of *Trichostrongylus* sp. Most of them are parasitic in herbivores, although 10 species have also been reported from humans. Humans and herbivores become infected with *Trichostrongylus* spp. mainly by ingesting infective-stage larvae (Gholam *et al*. 2015). Information on cross-transmission of these nematodes among different animal host and human is lacking (Ghasemikhah *et al*. 2011).

Ostertagia sp.

These are parasitic roundworms that infect cattle, sheep, goats and other wild ruminants. Also known as the brown stomach worms, their closest relative are the *Teladorsagia* species. It is probably the most studied and prevalent parasite of livestock. In their larval stage, these parasites invade and destroy the gastric glands. Under severe infections, parasite can significantly reduce digestion efficiency. At the larval stage, they undergo a period of inhibition and remain in the glands for months before migrating to the lumen where they develop into adult worms. The *Ostertagia* sp. has a worldwide distribution, but are more abundant in regions with temperate and cool climate. It is also important in subtropical regions with winter rainfall (Myers & Taylor 1989). These roundworms are considered the most damaging gastro-intestinal parasite worms in ruminants especially in moderate and cool climates. *Ostertagia ostertagii* infects mainly cattle and other wild ruminants, theseparasitic worms however do not affect dogs and the cats. Myers & Taylor (1989) reported an annual cost of gastro-intestinal nematodiasis in the United States mounted to $250 million whereby Ostertagiasis accounted for a large portion of it.

Trichuris ovis

Often referred to as the whip-worms; these parasitic roundworms are short and have a broad posterior end and very long and narrow whip-like anterior end (hence the name), they are soil-transmitted helminths and are the third most common roundworm worldwide (CFSPH 2005; CDC 2013), they are rare in arid, very hot, or very cold regions. They are known to infest the lower intestines of the ruminants irrespective of age, gender and breed of the host (Kuchai *et al*. 2013). These parasitic roundworms have a worldwide distribution (Kaufmann 1996) but are more abundant in regions with tropical or subtropical climate and poor sanitation practices and among children. An estimated number of persons infected with whipworm in 2002 were 1 billion, and studies indicate that *Trichuris* sp. infections also occur in the southern United States (CDC 2013). According to a review by Pfukenyi & Mukaratirwa (2013) prevalence of infection of *Trichuris* sp. in cattle is generally low, with *Trichuris globulosa* being frequently encountered. There are several *Trichuris* sp. that are of veterinary and medical importance, these are a few that affect both wild and domestic ruminants *Trichuris discolor, T. globulosa, T. ovis* etc. *Trichuris* species are considered harmless except in very heavy infections (for example, large soil intake by grazing animals in drought) in which case there may be a sub-acute typhlocolitis, diarrhoea and illthrift (Robles *et al*. 2014).

Haemonchus contortus

One of the most common gastro-intestinal nematode in both captive and free ranging wildlife is the *Haemonchus contortus*. Also known as the barber's pole worm, this cylindrically shaped worm, tapered at both ends has a complete digestive system, and a worldwide distribution however more prevalent in sub-tropical and tropical regions under warm and wet weather conditions (Qamar *et al*. 2009). *Haemonchus contortus* remains a great threat to goat industry in the south-eastern region of the United States (Machen *et al*. 2002). *Haemonchus contortus* sucks blood of the host and cause significant production losses due to morbidity and mortality (Rahman & Hamid 2007). It is estimated that each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Qamar *et al*. 2009). These parasitic worms cause significant economic loss especially in domestic animals, specifically sheep, cattle and goat. Because *H. contortus* is blood sucker, it causes anaemia and edema in infected

animals, the proteins released by the *H. contortus* can cause intestinal disturbances. It is known to adapt well to unfavourable conditions, which makes it difficult to eradicate it in the environment (Sendow 2003).

1.4.2 GIT parasites affecting felids

Toxascaris leonina

An ascarid nematode which inhabits the small intestines of both of domestic and wild canids and felids. These parasitic worms are zoonotic and therefore can infect humans (Minnaar *et al*. 2002), but does not affect cattle, sheep, pigs and any other livestock but rather the cats and dogs. It has a worldwide distribution, but most prevalent in subtropical regions, which facilitates development of the egg in the external environment. As a general rule, they are more abundant in rural regions where wild carnivores serve as a reservoir, previous study determine that 60% of the foxes in rural areas were infected with *T. leonina*, compared with only 8% of the foxes in the urban areas. The rodents (mice and rats) serve as paratenic hosts of *T. leonina*, infections occur only in the definitive host, when they consume an infected rodent. The *T. leonina* occurs in both canids and felids but more frequent in felids (Sheng *et al*. 2012). Several studies indicate co-occurrence of both *Toxocara cati* and *T. leonina* in wild and domestic felids, and *Toxocara canis* and *T. leonina* in canids (Okulewicz *et al*. 2012), a good example is when a study was conducted and it revealed *T. canis* and *T. leonina* co-occurred in 14 of the red foxes population of Geneva, Switzerland (Reperant *et al*. 2007).

Toxocara cati

This particular parasitic roundworm, also known as the cat round worm is very common in domestic cats and other wild felids, can infect humans and results in toxocariasis. It is believed to be the most encountered gastro-intestinal parasite in cats. *Toxocara cati* is a close relative of the *Toxocara canis* which infects the dogs. Although *T. cati* is very common round worm it does not affect livestock such as cattle, sheep, goats and horses. *Toxocara cati* may feed on body fluids depending on development stage and location of infestation. Infection can occur by host infesting viable, embryonated eggs from contaminated sources or can acquire infection from transplacentally from the infected mother when they ingest infective eggs (Despommier 2003). This parasitic roundworm has a worldwide distribution

(Despommier 2003), and the eggs and larvae can survive up to 12 months in the environment. *Toxocara cati* is a zoonotic pathogen, and its importance as a zoonotic pathogen should not be overlooked since toxocarosis is one of the most encountered zoonotic helminth infections worldwide (Magnaval *et al*. 2001). First human infection with *Toxocara* sp. was described in 1950 by Wilder; where he identified nematode larva of unknown species within a retinal granuloma of a child. This child presented clinical signs of high circulating eosinophilia, and suffered from severe, long-term, multisystem disease (Despommier 2003). In humans, *T. cati* larvae may infect any organ where they finally get encysted. Experiential data indicated that *T. cati* migrates more slowly to other organs including the central nervous system and or the eye as compared to *T. canis* (Strube *et al*. 2013).

1.4.3 GIT parasites affecting avian species

Capillaria species

Birds of prey commonly parasitized by a wide variety of helminths, including numerous species of nematodes, trematodes, cestodes and acanthocephalans. Infections usually cause little or no distress to healthy individuals in the wild, however parasites can become significant problem in captive environments, as well as in birds that have recently come into captivity that are stressed by illness, injury or acclimatization to new surroundings (Smith 1993). Helminthiasis is considered as one of the most significant constraints on poultry production in humid tropical climatic conditions of India which are favourable for faster propagation and development of the larval stages of helminths parasites (Katoch *et al.* 2012). *Capillaria* sp. has been reported to occur in domesticated and wild birds, and is cosmopolitan in their distribution. However, there has been no report on avian *Capillaria* sp. infection in Korea (Park & Shin 2010). Capillarid worms of birds are known as hairworms because of their extreme thinness in size, are divided into two groups: those that burrow into the epithelium of the upper digestive tract and those that burrow into the epithelium of the lower digestive tract (Park & Shin 2010). *Capillaria* sp. is rarely found and apparently does not affect the host's health (Mawson 1985). India has 498 million poultry population with an average growth rate of 8 – 10% per annum (Katoch *et al*. 2012).

Ascaridia species

These are parasitic nematodes which primarily infect many species of birds. These parasitic worms may cause serious and frequently fatal diseases in farm and wild birds kept in captivity, including parrots (Kajerova *et al.* 2004), they are most common parasites found in birds that are maintained in enclosures with access to the ground (González-Hein *et al*. 2012). The disease that is caused by *Ascaridia* worms is called ascaridiasis. There are three well known species *A. galli* which is found in chickens, *A. dissimilis* which infects the turkey and *A. columbae* which infects the pigeons. The less known species are *A. hermaphrodita, A. sergiomeirai, A. ornata, A. nicobarensis* and *A. platyceri*. *Ascaridia* species all have a worldwide distribution (Mehlhorn2015); in many birds species both farmed and wild, but very common in chicken. *Ascaridia* sp. are much more abundant in farming with outdoor run than in industrial facilities. These worms do not however infect the dogs, cats, cattle, sheep, goats, horse and pigs. . *Ascaridia columbae* (Gmelin) is a common parasite of domestic pigeons *Columba livia* all over the world and is also found, to a lesser extent, in captive parrots (Mawson 1985). Ascaridia platyceri was first described from cage parrots in a German zoo, and has since been identified from parrots in aviaries in New Zealand, Queensland, New South Wales, Victoria, Tasmania and South Australia (Mawson 1985)

1.5 Identification and characterization of the gastro-intestinal parasites

Gastro-intestinal tract (GIT) parasites are a serious problem throughout the world, especially in small ruminants and results in significant production loss in animals, these parasites also contribute largely in reduction of production such as meat, milk and wool in animals. Control of GIT parasites is primarily through administration of anthelminthic treatment, and this is done after identification and quantitation of the parasite load in an individual host by microscopy (Ahmed *et al*. 2011). Whilst the lifecycle of most strongyloid eggs are very similar, there are physiological differences in the lifecycle which leads to differences in their pathogenicity and economic impact. With the widespread resistance of gastro-intestinal helminths to different anthelminthics compounds has led to the need to develop unique technologies to prevent and control parasitic diseases in both livestock and wildlife. The developments of new pesticides focus the attention to those with low toxic substances, low

environmental impact, and low residues in animal by-products (Wu *et al*. 2015). Identification of GIT parasites is done through microscopic method by identifying specific morphological characteristics of egg and stage 3 larvae. Since most of parasite eggs are having similar morphological characteristics, identification up to species level through microscopy is not always possible; however molecular techniques are viable alternative for identification of eggs up to species level. The ideal diagnostic test for GIT nematodes infections has the following characteristics (Windon 1996; Colditz 2008a):

- Reliable in terms of accuracy and repeatability
- Ease to measurement
- Cost effective
- The ability to be used on farms

1.5.1 Identification by microscopy

For identification of GIT parasite, microscopic examination of the eggs and the third stage larvae (L_3) cultured are considered to be gold standard tests (Ahmed *et al*. 2011).

Morphological features used to identify species of helminths eggs are:

- Size, depending on different eggs, the size ranges from 10 to 200 µm.
- Shape
- Stage of development when the egg is passed in the faeces
- Thickness of the eggshell
- Colour of the egg
- Presence of structures such as operculum, spine or plugs.

The structural characteristics used in identification include according to Levine (1985):

- Oocysts shape and size
- The presence of a micropyle of micropylar cap
- The number of layers in the oocysts wall
- Size, shape and arrangement of sporocysts
- The presence or absence and the size and appearance of oocysts or sporocyst residua

Morphological features used to identify L_3 parasitic nematodes

Identification of L_3 of parasitic nematodes is based primarily on inspection of the of the caudal and cranial extremities, however other features such as length and shape of the oesophagus or cranial refractile spots are important for identification up to genus level. Important features include the length of sheath tail extension (STE) and the proportion of the sheath tail extension (STE) in comparison with a filament. The length of STE is an important criterion used for identification and to assist with the identification, *Trichostrongylus colubriformis* and *Trichostrongylus axei* are used as standard measurement (±33µm). The proportion of the length of STE is usually estimated and there is no need for measurement. It should be noted that there is no exact point of transition from the shealth filament to the cranial portion of the STE per genus and or species (Van Wyk & Mayhew 2013).

1.5.2 Identification by molecular techniques

Anthelmintic resistance is a serious concern to the effective control of gastro-intestinal parasites and has fuelled the focus to design and promote sustainable control of practices of parasite control. Diagnostics are required to determine anthelmintic efficacies, for targeted treatment programs and selection of animals for parasite resistant breeding (Preston *et al*. 2014). There have been significant advances in diagnosing nematode infections using molecular techniques. Three major characteristics of DNA molecule which make it a useful tool for molecular species identification; (a) DNA is a stable biological material which can be recovered even after extreme conditions. (b) DNA is found in all biological material and has nucleated cell which enables analysis from all sorts of biological substrates (faeces, blood, hair, urine, *etc*). (c) DNA can provide more information about a specimen than proteins due to non-degeneracy of genetic code (Ahmed *et al*. 2011). Polymerase Chain Reaction (PCR) is a technique used in molecular biology to amplify a single copy of DNA into thousands to millions of copies (Newton & Graham 1997). It is reliable, relatively inexpensive and most sensitive of the existing rapid methods to detect microorganism pathogens in a specimen (Belgrader *et al*. 1999; Yamamoto 2002). PCR-based methods using specific genetic markers in the internal transcribed spacers of nuclear rDNA have provided enhanced epidemiological tools. Recent studies have

demonstrated that real-time PCR and multiplexed-tandem PCR assays can replace the time-consuming method of larval culture (Admed *et al*. 2011).

CHAPTER 2

STATEMENT OF THE PROBLEM

2.1 Justification of study

Zoos are open to the public and are home to variety of animal species which are confined within enclosures. These facilities are a form of an *ex-situ* conservation which involves protecting endangered species outside their natural habitat for aesthetic, educational, research and recreational purposes (Adeniyi *et al*. 2015). In their natural habitat, wild animals might have resistance against parasitic infections or may have a mutualistic relationship with their parasite (Borkovcova & Kopriva, 2005). On the other hand, captivity may influence the animal and may increase susceptibility to parasitic infections (Singh *et al*. 2006). Some animals may not be indigenous to the geographic location of the zoo which would therefore provide opportunities for infection by parasites that these animals would not normally come in contact with in the wild (Adeniyi *et al*. 2015). Occurrence of parasitic infections in animals housed in zoological gardens might vary according to the type of husbandry practices, disease prophylaxis and treatment (Lim *et al*. 2008). Parasitic diseases serve as one of the major problems in zoological gardens resulting in high rates of mortality (Varadharajan & Kandasamy, 2000); therefore, the effects of these parasitic infections range from subclinical to death (Otegbade & Morenikeji, 2014).

Information on the gastro-intestinal (GIT) parasites that occur in captive wildlife management systems in South Africa is lacking. It is important to have such information as it contributes to the conservation of the rich and diverse wildlife. If animals in captivity are not monitored for various pathogens and parasites, especially GIT parasites it becomes costly to treat. The parasites may become resistant to drugs, there is reduced reproduction rate in infected animals and lastly death is sometimes the result. The information on occurrence of disease causing agents is crucial to the management and control of pathogens including GIT parasites in South Africa especially in captive animals.

The National Zoological Gardens of South Africa (NZG) houses 3117 animal specimens (http://www.nzg.ac.za/aboutus/index.php), many of which are prone to GIT parasite infections because zoo animals are confined to enclosures and there is no

change of location thus this increases the risk of GIT parasitic infections in animals. Although the GIT parasites at the NZG are monitored regularly, there are no scientific research studies done on this subject, let alone DNA-based studies. Little work has been done to understand the epidemiology of different parasitic diseases in wild animals kept at NZG and there is a conspicuous lack of molecular data focusing on GIT parasites in captive wildlife at NZG.

This study seeks to provide information on the prevalence of gastro-intestinal parasite infections in selected captive wildlife at the National Zoological Gardens of South Africa (NZG), including their seasonal abundance, difference in infection levels between selected ungulates, felids and avian species, and sequence PCR positive amplicons for genetic confirmation. The findings of the study will facilitate a better understanding of gastro-intestinal tract (GIT) parasites of wildlife in captivity and assist in prevention of spread of the infectious parasitic diseases among animals within the zoo.

2.2 Objectives

2.2.1 General objective

The objective of the study was to determine the seasonal prevalence of gastro-intestinal parasites infecting selected ungulates, felids and avian species at NZG using both microscopic and molecular techniques.

2.2.2 Specific objectives

- To determine the distribution and seasonal prevalence of gastro-intestinal parasites in selected animals by egg floatation techniques through microscopy
- To use polymerase chain reaction (PCR) to detect and confirm identity of GIT parasites present in felids, avian and ungulate species
- To sequence PCR positive amplicons for further confirmation of species using nucleotide basic local alignment search tool (BLASTn) for identical matches in the NCBI database

2.3 Research hypothesis

- Abiotic factors such as rainfall, temperature and/or humidity will have a positive effect on GIT parasite distribution in ungulate, felid and avian species sampled at the NZG during this study.
- The level of GIT parasitic infections will be higher in ungulates than felids and avian species.

2.4 Outline of dissertation

Chapter 1 – Introduction and literature review: It gives background of gastro-intestinal parasites, their relation with animals in captivity, their life cyles, economic importance, identification and various diagnostic techniques.

Chapter 2 – Statement of the problem: It includes justification of the study, aims, objectives and hypothesis.

Chapter 3 – Materials and methods: Describes in detail the study approach including, description of the study area, materials used and methods applied, as well as how data was analysed.

Chapter 4 – Results: Is a full presentation of data obtained in this study.

Chapter 5 – Discussion, conclusions and recommendations: Is the interpretation of data with conclusions showing the aims and objectives of the study have been achieved as well as suggections of further actions and studies that needs to be undertaken with reference to data obtained from this study.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study period

The investigation was carried for a period of thirteen months, from October 2015 to October 2016. The sampling period covered the four climatic seasons of South Africa namely, spring (October 2015 to November 2015), summer (December 2015 to February 2016), autumn (March 2016 to May 2016), winter (June 2016 to August 2016) and spring (September 2016 to October 2016).

3.2 Study area

The study was conducted at the National Zoological Gardens of South Africa (NZG, Figure 5 and 6), located in Pretoria, South Africa. The 850 000 m^2 zoo is located at S25˚ 44’ 18’’ E28˚ 11’ 21’’. It is home to about 9000 animals from about 700 species of mammals, birds, reptiles, amphibians, fish and invertebrates. It is the largest zoo in the country with over 600 000 visitors annually and the only one with national status. The NZG also has excellent research facilities, a veterinary hospital, an aquarium, reptile-park and the third largest collection of exotic trees (http://www.nzg.ac.za/aboutus/index.php). Half of the zoo is situated on flat ground, while the other half is on a sloping area and the two are separated by Apies River flowing through the zoo. Enclosures at the NZG replicate natural habitats and include both single and mixed exhibits e.g. the Waterhole is a mixed exhibit which caters for Cape eland (*Taurotragus oryx*), Gemsbok (*Oryx gazella*), Springbok (*Antidorcas marsupialis*), Ostriches (*Struthio camelus*) and free roaming birds.

Figure 5: Satellite map of the National Zoological Gardens of South Africa (Google Maps, S25˚ 44’ 18’’ E28˚ 11’ 21’’).Yellow lines indicate the boundary of the zoo.

Figure 6: NZG map showing location of animal enclosures (NZG pamphlet).

3.2.1 Climatic conditions

Pretoria has a humid subtropical climate with long hot rainy summers and short cold dry winters. The annual mean temperature in Pretoria is 17.8°C and precipitation averages 679 mm. The least amount of rainfall is experienced in June, in January the precipitation reaches its peak, with an average of 110 mm. The temperatures are highest on average in January, Maximum mean temperature of 27.5°C in summer, and at minimum mean temperature of 5°C on average in July, which is the coldest month of the year. (http://www.saexplorer.co.za/south-africa/climate/pretoria_climate.asp).

For this study, Climate conditions were checked daily between 08:00 – 09:00, on the South African Weather Services website; www.weathersa.co.za. Weather parameters such as temperature, humidity and rainfall were recorded daily for the duration of the study.

3.3 Study design

In this study, both microscopic and nucleic acid-based methods were used to determine prevalence and seasonal distribution of GIT parasites in selected captive animals at the NZG. Briefly, samples were collected and examined macroscopically. Thereafter aliquots were used for larval cultures and microscopic analysis. Nucleic acids were extracted from positive samples (both larval cultures and faecal samples) for detection of GIT parasite DNA by PCR. Lastly, Sanger sequencing was conducted to confirm species using nucleotide basic local alignment search tool (BLASTn) (Figure 7).

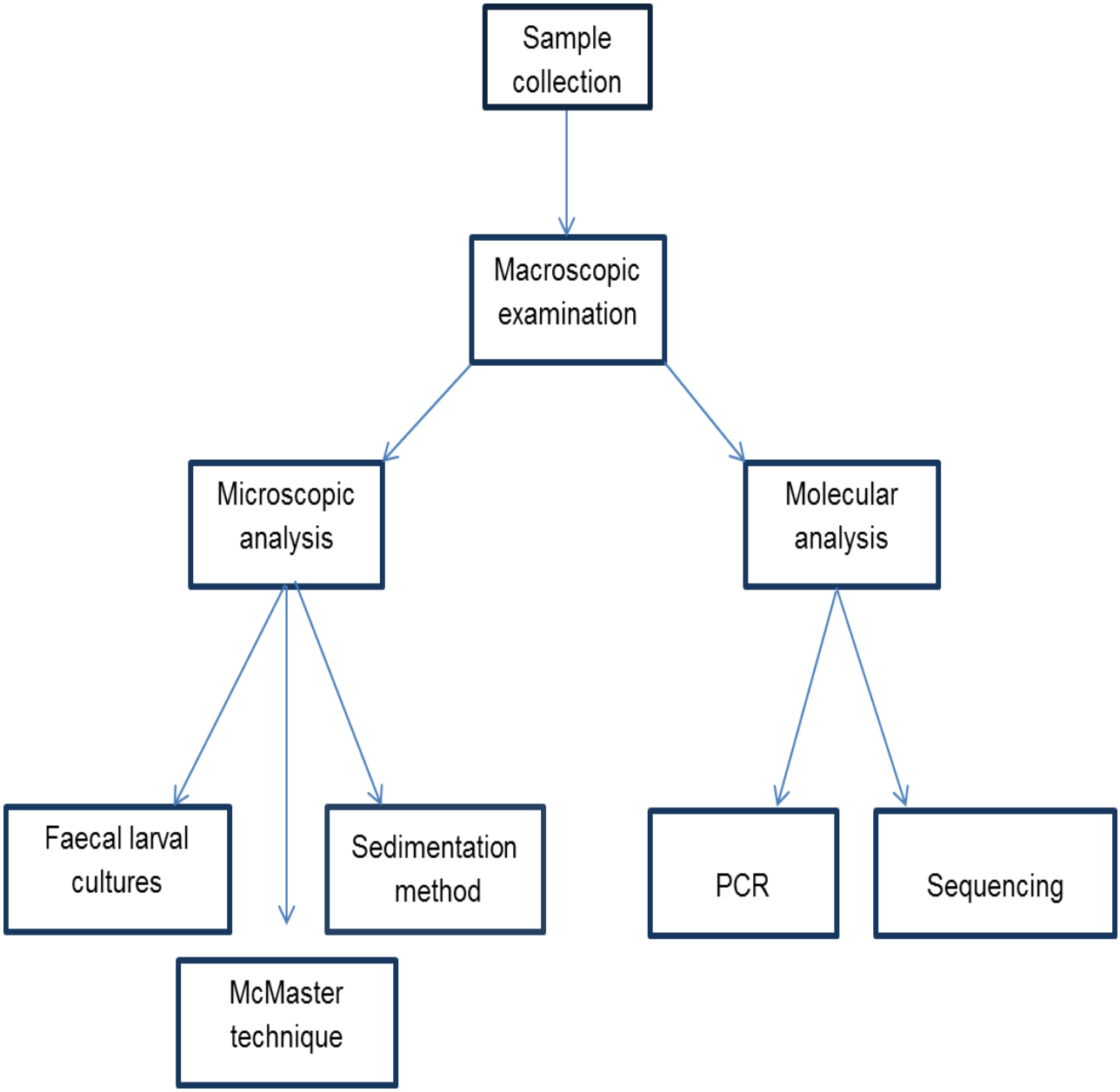


Figure 7: Flow chart showing research outline

3.4 Selection of animals

The study covered selected ungulates, felids and avian species housed at the National Zoological Gardens of South Africa. For the present study, faecal samples were collected from a total of 772 animals. Of these, faecal samples were collected from 406 ungulates, 97 felids and 269 avian species (Table 1).

Collected samples were from two types of enclosure set up; mixed and single exhibit. Mixed exhibits house different animal species in the same enclosure, for example the (D2/195-6) BuiteKamp enclosure mimics the savannah biome and houses different antelopes including blesbok, springbok, cape eland and southern lechwe, free roaming birds including peafowl and guineafowl have easy access to the enclosure. Single exhibit enclosures house a single type of animal species, for example the sable antelope enclosure.

Table 1: List of animals (ungulates, felids and birds) which were sampled at the NZG in the present study.

Common name	Scientific name	No. of samples	Enclosure number	Type of sample	Gender	Type of enclosure
Ungulates						
Arabian oryx	*Oryx leucoryx*	39	D2/161	Individual	Female	Single
Blesbok	*Damaliscus pygargus phillipsi*	3	Waterhole	Individual	Female	Mixed
Cape eland	*Taurotragus oryx*	65	Waterhole	Individual	Male & females	Mixed
Addax	*Addax nasomaculatus*	14	D2/164	Individual	Male & female	Single
Gemsbok	*Oryx gazella*	22	Waterhole	Individual	Female	Mixed
Kalahari red goat	*Capra aegagrus hircus*	10	Farmyard	Individual	Male & female	Single
Lechwe	*Kobus leche*	74	D2/196	Individual	Males & Females	Mixed
Nubian ibex	*Capra nubiana*	12	D2/193	Individual	Females	Single
Nyala	*Tragelaphus angasii*	11	D2/165B	Individual	Male & females	Single
Okapi	*Okapia johnstoni*	3	D2/165A	Individual	Male	Single
Red forest buffalo	*Syncerus caffer nanus*	7	D2/162	Individual	Male & females	Single
Sable antelope	*Hippotragus niger*	85	D2/193	Individual	Male & females	Single
Scimitar horned oryx	*Oryx dammah*	4	D2	Individual		Single
Springbok	*Antidorcas marsupialis*	45	Waterhole	Individual	Male & females	Mixed
Urial	*Ovis orientalis vignei*	8	D2/192	Individual	Male & females	Single

Felids						
African leopard	*Panthera pardus*	19	E2/57	Individual	Male	Single
African lion (cubs)	*Panthera leo*	26	E2/100	Pooled	Males	Single
African lion	*Panthera leo*	10	E2/191	Pooled	Females	Single
Bengal tiger	*Panthera tigris tigris*	10	E2/100	Individual	Male	Single
Cheetah	*Acinonyx jubatus*	32	E2/102	Pooled	Males	Single
Birds						
African goshawk	*Accipiter tachiro*	1	C4/89	Individual	Unknown	Single
Bald ibis	*Geronticus eremita*	10	C1/120-122	Pooled	Unknown	Mixed
Blue & yellow macaw	*Ara ararauna*	1	C1/216	Individual	Unknown	Single
Blue fronted amazon	*Amazona aestiva*	7	C2/212	Individual	Unknown	Mixed
Blyth hornbill	*Rhyticeros plicatus*	1	C2/30	Individual	Unknown	Single
Brown snake eagle	*Circaetus cinereus*	2	C2/94	Individual	Unknown	Single
Cape vulture	*Gyps coprotheres*	28	C2/46	Pooled	Unknown	Single
Chilean flamingo	*Phoenicopterus chilensis*	31	C2/89	Pooled	Unknown	Mixed
Common ostrich	*Struthio camelus*	5	Waterhole	Pooled	Unknown	Mixed
Domestic chicken	*Gallus gallus domesticus*	35	Farmyard	Pooled	Unknown	Single
Domestic/ Chinese goose	*Anser cygnoides*	32	Farmyard	Pooled	Unknown	Mixed
East African grey crown crane	*Balearica regulorum*	11	C3/12C	Individual	Unknown	Single

Gymnogene	*Polyboroides typus*	1	C2/93	Individual	Unknown	Single
Hadeda ibis	*Bostrychia hagedash*	1	Quarantine	Individual	Unknown	Single
Illigers macaw	*Primolius maracana*	2	C2/22	Individual	Unknown	Single
Jackal-buzzard	*Buteo rufofuscus*	1	C2/88	Individual	Unknown	Single
King vulture	*Sarcoramphus papa*	2	C2/86	Pooled	Unknown	Single
Lanner falcon	*Falco biarmicus*	1	C2/199	Individual	Unknown	Single
Olive thrush	*Turdus olivaceus*	1	Quarantine	Individual	Unknown	Single
Orange winged amazon	*Amazona amazonica*	1	C2/37	Individual	Unknown	Single
Red crown crane	*Grus japonensis*	7	C2/13D	Individual	Unknown	Single
Rosy flamingo	*Phoenicopterus ruber*	32	C2/42	Pooled	Unknown	Mixed
Scarlet ibis	*Eudocimus ruber*	3	C2/11	Individual	Unknown	Single
Southern ground hornbill	*Bucorvus leadbeateri*	2	C2/195	Individual	Unknown	Single
Steppe buzzard	*Buteo buteo*	1	C2/86	Individual	Unknown	Single
Sulphur crested cockatoo	*Cacatua galerita*	1	C2/255	Individual	Unknown	Single
Sun conure	*Aratinga solstitialis*	6	C2/24	Individual	Unknown	Mixed
Wattled crane	*Grus carunculata*	39	C2/114	Individual	Unknown	Single
Yellow billed kite	*Milvus aegyptius aegyptius*	1	C2/87	Individual	Unknown	Single

3.5 Faecal sample collection

Fresh faecal samples were collected from the enclosure grounds in the morning between 8 am to 10 am soon after the animals (Table 1) had defecated (Figure 8) to minimize the chance of drying out from direct sunlight. Pooled samples were collected from most of the animals except for a few such as the leopard, all the ungulates and some avian species which had individual enclosures. Proper care was taken to prevent environmental contamination of faecal samples when these were collected from the ground. The samples were clearly labelled with the identification of the animal from which the faeces were collected, the enclosure of collection and sample collection date. Samples were packed in a cool box and transported to the Parasitology laboratory at the NZG's Center for Wildlife Health for further analysis. Samples were stored in a refrigerator at 4°C until they were processed. The samples were analysed microscopically within 24 hours of collection as recommended by Reinecke (1983).

Figure 8: Collection of Cape eland faecal samples from the Waterhole enclosure which is a mixed display exhibit

3.6 Examination of faecal sample condition

Faecal samples were examined macroscopically for presence of blood, mucus, tapeworm proglottids and larval roundworms or any other unusual substance/s and notes were taken.

3.7 Microscopic analysis

3.7.1 Faecalyser

Samples were first qualitatively analysed by the egg floatation method using an egg floatation kit (Figure 9). At least 2 g of faecal material was added to a cubic cm cup, a small narrow tube was pushed into the cup. Egg floatation fluid ($NaNO_3$; specific gravity (SG) = 1.22, Kyron, South Africa) was added to the tube (approximately half-full) and thoroughly mixed with the faeces to break faecal pellets. Thereafter the strainer was put in the tube to push down all the faecal debris and coarse faecal material, and the tube filled with egg floatation fluid until it made a convex meniscus. Air bubbles were removed and a cover slip was placed over the top of the tube. The solution was left to stand for 5 – 10 minutes; the cover slip was then transferred to a glass slide and examined under a light microscope (ZEISS Axiovert 25 CFL, Germany) at 10× and/or 40× magnification. The whole area beneath the coverslip was systematically examined for presence of parasite eggs which were then identified using the identification guide by Thienpont *et al*. 1979. Parasite pictures were taken on Nikon Eclipse DS-Fi2 light microscope (Nikon, South Africa) using the Nikon DS-L3 camera (Nikon, South Africa). Thereafter, approximately 1.5 ml of faecal mixture (egg floatation fluid and faeces) was transferred to a 1.5 ml eppendorf tube and stored at -20°C until used for DNA extraction

Figure 9: Faecal analysis apparatus used for faecalyser method

3.7.2 McMaster technique

Faecal samples were analysed using the McMaster technique for quantification of gastro-intestinal (GIT) parasite load according to the procedure described by Reinecke (1983). Briefly, 4 g of faeces was mixed thoroughly with 56 ml of 40% sugar solution ensuring that the faecal pellets were disrupted. A Pasteur pipette was used to transfer aliquots of the mixture into the three chambers of a McMaster slide. The slide was left to stand for 5 – 10 minutes to allow the eggs to float to the surface of the medium. The slide was examined using the ZEISS Axiovert 25 CFL microscope (Zeiss, South Africa) at 10× and/or 40× magnification, as described above. Parasite eggs were counted in each chamber of the McMaster slide and the eggs per gram (EPG) was calculated using the following formula;

$$EPG = \frac{(a) \times (b)}{(c) \times (d) \times (e)}$$

where

(a) = (total number of eggs counted);

(b) = 60 (total volume of faecal suspension);

(c) = 3 (the number of chambers counted);

(d) = 4 (grams of faeces) and

(e) = 0.15ml (standard volume of the chamber).

The level of infection in samples which were positive for *coccidia*, was graded into three categories based on Oocyst per gram (OPG) of faeces. Infection levels were recorded as +1 (for low level infections below 50 OPG), +2 (medium level infections between 51 to 100 OPG) or +3 (for high level infections of more than 101 OPG).

3.7.3 Direct faecal smear

The direct faecal smear method was used when there was a small quantity of faecal sample available (e.g. bird samples), thus faecalyser or McMaster techniques were not possible to conduct, method conducted per Bush 1975. Briefly; A small quantity (about 2 mg) of faeces was placed on the centre of the microscope slide. Using pasture pipette, one to two drops of water were added on the faecal matter and mixed thoroughly using mounted needle or scalpel blade. All the large pieces of faecal matter were removed from the suspension with a scalpel blade. A coverslip was carefully placed on the microscope slide ensuring that there were no bubbles trapped. The slide was examined using the ZEISS Axiovert 25 CFL microscope (Zeiss, South Africa) at 10× magnification and parasites identified as described above (Section 3.7.1).

3.7.4 Sedimentation method

Two methods of sedimentation were used (Reinecke 1985; Thawait *et al*. 2014)
In a 100 ml glass beaker, 5 g of faecal sample was mixed with water by a means of tongue depressor. The suspension was strained through a piece of cloth. The filtrate was left for 20 minutes and the supernatant was decanted. The step was repeated 4 – 5 times until the filtrate was clear. The sediment was stirred with a tongue depressor. With a Pasteur pipette, a few drops of the sediment were transferred to microscope slides. A coverslip was carefully placed on the microscope slide ensuring that there were no bubbles trapped. The slide was examined using the ZEISS Axiovert 25 CFL microscope (Zeiss, South Africa) at 10× magnification and parasites identified as described above (Section 3.7.1).

The second sedimentation method (Thawait *et al*. 2014) was used to detect eggs of trematodes and cestodes. A small amount of faeces was mixed with water and mixture was strained to remove faecal debris. The filtrate was poured into an eppendorf tube

and centrifuged at 2000 rpm for 3 min. The supernatant was discarded and refilled with water. This step was repeated three to four times until the supernatant was clear. A Pasteur pipette was used to transfer a drop of sediment to a clean, dry glass slide and examined under the ZEISS Axiovert 25 CFL microscope (Zeiss, South Africa) at 10× magnification and parasites identified as described above (Section 3.7.1).

3.7.5 Larval culture preparation

Faecal cultures were prepared to identify the third stage nematode larvae using molecular techniques (van Wyk & Mayhew 2013). Approximately 10 g of fresh faeces were added to a container, crushed and thoroughly mixed with an equal amount of vermiculite (SA Vermiculite (PTY) LTD). The vermiculite improves air circulation in the culture and facilitates maximum hatching of eggs. A stick was placed in the center at an upright position and the faeces were added and pressed down using the second stick. The stick was removed, leaving a hole in the center which facilitated aeration. The exterior surface of the container was cleaned. The cultures were moistened by adding water using a water bottle. A lid was lightly screwed back onto the containers and incubated at 27°C for seven to ten days. To harvest the culture, the inner surface of the container and the cultures were moistened and washed. The larvae were collected by rinsing the sides of the container into a petri-dish and thereafter examined under a light microscope at 10× magnification. The larvae were stored at 4°C fridge until used for DNA extraction. DNA was extracted directly from the larvae as described below (section 3.4.1.2).

3.8 Molecular identification of GIT parasites by PCR and sequencing

3.8.1 DNA extraction

3.8.1.1 DNA extraction from faeces

DNA was extracted from previously frozen faecal samples which were stored in 1.5 ml of egg floatation fluid at -20°C (as described above, section 3.3.1) using the ZR Fecal DNA MiniPrep™ extraction Kit (Zymo Research, California, USA). The DNA extraction procedure was conducted according to the manufacturer's instructions as follows. Before starting: Zymo-Spin™ IV-HRC Spin Filters (green tops) were prepared by

snapping off the base, inserted into a collection tube and spun in a microcentrifuge at 8 000 rpm for 3 minutes. Five hundred microliters of faecal solution and 750 µl Lysis solution were added to a ZR BashingBead™ Lysis Tube. The tubes were secured in a bead beater and processed at maximum speed for 5 minutes. The samples were centrifuged in the ZR BashingBead™ Lysis Tubes in a microcentrifuge at 10 000 rpm for 1 minute. Thereafter, 650 µl of supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange tops) with a collection tube and centrifuged at 7000 rpm for 1 minute. One thousand two hundred microlitres of Fecal DNA Binding Buffer was added to the filtrate in the collection tube, 800 µl of that mixture was transferred to Zymo-Spin™ IIC Column in a Collection tube and centrifuged at 10 000 rpm for 1 minute. The flow through was discarded again 800 µl of the mixture was transferred to Zymo-Spin™ IIC Column in a Collection tube and centrifuged at 10 000 rpm for 1 minute. Two hundred microlitres of DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10 000 rpm for 1 minute. Five hundred microliters of Fecal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and Centrifuged at 10 000 rpm for 1 minute. Zymo-Spin™ IIC Column was transferred to a clean 1.5 ml eppendorf tube and 100 µl of DNA Elution Buffer was added directly to the column matrix, and centrifuged at 10 000 rpm for 30 seconds to elute the DNA directly. The previously eluted DNA was transferred to a prepared Zymo-Spin ™ IV-HRC Spin Filter (green tops) in a clean 1.5 ml eppendorf tube and centrifuged at 8 000 rpm for 1 minute. The extracted DNA concentration and quality (OD260/280 ratios) was determined using the NanoDrop spectrophotometer (ND1000, Thermo Fisher Scientific) and then stored in a -20°C freezer for future use.

3.8.1.2 DNA extraction from third stage (L_3) larval cultures

DNA was extracted from larval cultures with the ZR Tissue DNA MiniPrep™ (Zymo Research, California, USA) extraction kit, according to the manufacturer's instructions, as follows;

Harvested larvae were centrifuged at 5000 rpm for 2 minutes and the supernatant was carefully removed with a pipette ensuring that the pellet at the bottom was not disturbed. Thereafter 95 µl nuclease free H_2O, 95 µl of 2× Digestion buffer and 10 µl of Proteinase K were added to the eppendorf tube mixed and incubated at 55°C until tissue was completely digested. After digestion, 700µl of Genomic Lysis Buffer was

added to the tube, mixed thoroughly by vortexing and then centrifuged at 10000 rpm for 1 minute to remove insoluble debris. The supernatant was transferred to a Zymo-Spin™ IIC Column in a collection tube and centrifuged at 10 000 rpm for 1 minute. The spin column was transferred to a new collection tube and 200µl of DNA Pre-wash Buffer was added to the spin column and centrifuged at 10 000 rpm for 1 minute. After centrifugation, the flow through was discarded, 400µl of g-DNA Wash Buffer was added to the spin column and centrifuged at 10 000 rpm for 1 minute. The spin column was transferred to a clean eppendorf tube; 100µl of DNA Elution buffer was added to the spin column, incubated for 3 minutes at room temperature and centrifuged at top speed for 30 sec to elute the DNA. Extracted DNA concentration and quality was then measured on the NanoDrop spectrophotometer (ND1000, Thermo Fisher Scientific) and stored at -20°C for future use. DNA concentrations for all samples were standardized to a final working concentration of 10ng/µl.

3.8.2 Polymerase Chain Reaction (PCR)

In this study, several PCR assays were used to evaluate and confirm results obtained for GIT parasites identification in ungulates, felids and avian species by egg floatation techniques through microscopy. The PCR assays for the GIT parasites were performed at the Center for Conservation Science laboratories at the NZG using the Multigene OptiMAx Thermal Cycler (Life Technologies Corporation, USA). PCR was performed on DNA extracted from faecal and larval culture samples isolated from the various ungulates, felids and avian species that were sampled at the NZG. Master mixes for PCR assays were prepared as indicated in Table 2.

Table 2: Standard reaction composition for a single PCR assay

Reagent	Volume (in µl)
Kapa HiFi Hotstart mastermix	12.5
dd H_2O	8.5
Forward primer [25µM]	1
Reverse primer [25µM]	1
DNA template [10ng/µl]	2
Total volume	25

3.8.3 Optimization of PCR assay

All PCRs were setup to the volume of 25 µl. For all PCR assays prepared, published primers were used. Initially, previously published PCR conditions were used for detection of *Trichostrongylus, Trichuris ovis, Ostertagia ostertagii, Haemonchus contortus* and *Nematodirus spathiger*. Detection of *H. contortus* via PCR was performed according to previously published conditions (Bott *et al*. 2009). Further adjustments were done on all the other assays and later standard Kapa HiFi Hotstart master mix PCR conditions were used and adjusted accordingly by modifying the annealing temperatures (55°C - 58°C) and annealing time (15 seconds – 1 minute), based on the length of the expected fragment size.

For the felids, previously published PCR conditions were used to detect *Toxocara cati* and *Toxascaris leonina*. To determine a suitable annealing temperature for *Toxascaris leonina* and *Toxocara cati using T. leo*1*, T.cat*1 and NC2 primers, PCR was carried out in a Multigene OptiMAx Thermal Cycler (Life Technologies Corporation, U.S.A.) wherein primer sets were tested using gradient PCR at annealing temperatures from 55°C - 62°C.

3.8.4 PCR detection of GIT parasites in ungulates, felids and avian species at NZG

The PCR experiments were performed at the National Zoological Gardens, Center for Conservation Science laboratories. Polymerase Chain Reaction (PCR) was performed on DNA extracted from positive ungulate (faecal and larval cultures; n =250), felid (n=35) and bird (n=15) faecal samples collected in this study using previously published primers (Table 3) for detection of *Haemonchus contortus*, *Ostertagia ostertagii*, *Nematodirus spathiger*, *Trichuris*, *Trichostrongylus, Toxocara cati* and *Toxascaris leonina*. The reactions were prepared as shown in Table 2. The PCR assays were performed under the conditions shown in Table 3.

Table 3: List of primers used for the detection of the GIT parasites in the present study

GIT parasite	Primer name	Sequence	PCR conditions	Number of cycles	Fragment size	Target gene	Reference
Haemonchus contortus	Hae-F	CAA ATG GCA TTT GTC TTT TAG	94°C – 5 minutes 94°C – 30 seconds 55°C – 15 seconds 72°C – 45 seconds 72°C – 7 minutes 4°C - ∞	40	265bp	18S	Bott *et al*. 2009
	NC2-R	TTA GTT TCT TTT CCT CCG CT					
Trichuris sp.	IST2-F	GCG GCA GTG TGG ATC TGG CTG	95°C – 4 minutes 95°C – 15 seconds 75°C - 30 seconds 72°C – 30 seconds 72°C – 7 minutes 4°C - ∞	30	Species dependent	5.8S	Demeler *et al*. 2013
	IST2-R	GTG ATC CGC CGT TCG GAA					
Ostertagia ostertagii	Oo-F	ATG AAA CTC TAC AGT GTG GCT AGTT	95°C – 5 minutes 98°C – 20 seconds	35	92 bp	18S	Schnieder *et al*. 1999

	Oo-R	TTC TTG AAC TGG AAT GGG AAT TAT	55°C – 15 seconds 72°C – 15 seconds 72°C - 5 minutes 4°C - ∞				
Nematodirus spathiger	NESPRVI-F	GTA GGT GAA CCT GCG GAA GGA TCAT	94°C – 3 minutes 94°C – 1 minute 55°C – 1 minute 72°C – 1 minute 72°C – 10 minute 4°C - ∞	40	860bp	18S	Oliver *et al*. 2014
	NC2-R	TTA GTT TCT TTT CCT CCG CT					
Trichostrongylus sp.	jhTsp-F	TTA TGT GCC ACA AAT GAA GA	94°C – 5 minutes 94°C – 30 seconds 58°C – 15 seconds 72°C – 45 seconds 72°C – 5 minutes 4°C - ∞	35	475bp	5.8S	Yong *et al*. 2007
	NC2-R	TTA GTT TCT TTT CCT CCG CT					

Toxascaris leonina	*Tleo*1-F	CGA ACG CTC ATA TAA CGG CAT ACTC	95°C – 5 minutes 94°C – 20 seconds 55°C – 30 seconds 50°C – 30 seconds 72°C – 5 minutes 4°C - ∞	30	300bp	5.8S	Pawar *et al*. 2012
	NC2-R	TTA GTT TCT TTT CCT CCG CT					
Toxocara cati	*Tcat*1-F	GGA GAA GTA AGA TCG TGG CAC GCGT	98°C – 5 minutes 94°C – 20 seconds 55°C – 30 seconds 72°C – 30 seconds 72°C – 5 minutes 4°C - ∞	30	370bp	5.8S	Pawar *et al*. 2012
	NC2-R	TTA GTT TCT TTT CCT CCG CT					

3.9 Agarose gel electrophoresis

PCR products were visualized by agarose gel electrophoresis. Agarose gels (2%) were prepared by mixing 2 g of agarose powder (Lasec, South Africa) with 100 ml 1x TBE Buffer (Thermo Fischer Scientific) and heated for 2 minutes or until all the agarose had dissolved. Once cool, 8 µl of SYBR Safe DNA Gel Stain (Invitrogen, Thermo Fischer Scientific) was added to the agarose gel mix. Individual PCR products (8 µl) were mixed with 2 µl loading dye, loaded on the agarose gel, and ran at 140 V for 40 min. Thereafter the gels were visualized and examined using Gel Doc Ez Imager system (Bio-Rad) to detect amplification products. The size (molecular weight) of PCR amplification products were determined by comparison to a 100 bp DNA ladder (Thermo Fischer Scientific).

3.10 Purification and sequencing of positive PCR amplicons

Positive PCR products were excised from the gels using sterile scalpel blades and DNA purification was done using the Zymoclean™ Gel DNA Recovery Kit as follows. Before starting, 24 ml of 100% ethanol was added to 6 ml of DNA wash buffer concentrate to obtain the final DNA wash buffer solution. The DNA fragment was cut from the agarose gel using a scalpel blade and transferred to a sterile 1.5 ml microcentrifuge tube. Three volumes of Agarose Dissolving Buffer (ADB) were added to 1 volume of agarose cut from the gel and incubated at 55°C for 10 minutes until the gel slice was completely dissolved. The melted agarose gel solution was transferred to a Zymo-Spin™ Column in a collection tube. The tube was centrifuged at 10 000 rpm for 60 seconds, the flow through was discarded. Thereafter, 200 µl of wash buffer was added to the column and centrifuged at 10 000 rpm for 30 seconds. The flow through was discarded and the wash step repeated. Purified DNA was eluted by adding 6µl of nuclease free water directly to the column matrix. The column was placed into a 1.5 ml microcentrifuge tube and spun down at 10 000 rpm for 30 seconds to elute DNA.

Selected positive PCR products were cycle sequenced on ABI PRISM 3100 Genetic Analyzer (Applied Biosystem USA) at North-West University, Potchefstroom campus. All sequencing PCRs were setup to the volume of 10 µl. One microliter of purified PCR product, 1 µl (10 µM) primer forward or reverse and 0.5 µl Big Dye (Applied Biosystem USA), plus 2 µl of sequencing buffer (80 mM Tris, pH 9.0, 2mM $MgCl_2$) in PCR tubes.

The total volume 10 µl was adjusted by adding double distilled water. PCR for sequencing was conducted with the following conditions: 96°C for 1 minute followed by 30 cycles at 96°C for 30 seconds, 50°C for 5 seconds, 60°C for 1 minute and held at 4°C.

Ten microliters of sequencing PCR product, 2 µl of 125 mM EDTA, 2 µl of 3 mM NaOAc, 50 µl of 100% EtOH and 10 µl of ddH_2O were all added to PCR tube and mixed well. The samples were centrifuged at maximum speed for 30 minutes at room temperature. Ethanol was removed by adding 70 µl of 70% EtOH and centrifuged at maximum speed for 15 minutes at room temperature. The samples were then dried up followed by 15 µl of HiDi formamide that was added and heated at 98°C for 3 minutes. The samples were placed on ice for 2 minutes then transferred to the wells of the sequencer plates. The resulting sequences were identified using basic local alignment search tool.

3.11 Sequence confirmation

DNA sequences were initially edited by first converting from AB1 format to FASTA format and the mixed bases were also converted to their appropriate base pairs (A, C, G and T) and subjected to BLAST sequence similarity search (National Center for Biotechnology Information; NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify similar sequences.

3.12 Ethical approval

The study was conducted after the approval of the National Zoological Gardens of South Africa's Research Ethics and Scientific Committee (Project number: P15/11; Appendix 1). The study was also approved by the scientific committee of the Integrated Pest Management sub-program of the Unit for Environmental Sciences and Management of the North-West University, Potchefstroom Campus.

3.13 Statistical analysis

Parasite prevalence is a population level measure of parasitism. It is calculated from the number of the host infected divided by the number of all animals examined, expressed as a percentage. We tested the prevalence of eggs of strongyles, *Trichuris*

sp., *Nematodirus* sp., *Moniezia* sp., *Strongyloides* sp., *Toxocara* sp., *Toxascaris* sp., *Heterakis* sp., and *Capillaria* sp.

CHAPTER 4

RESULTS

4.1 Climate data

The mean monthly rainfall ranged from 124 mm in March 2016 to 0 mm rainfall during the dry months from June to November 2016. Mean monthly maximum temperatures ranged from 20.3°C in June in the cold dry months to 34.89°C in December in warm months (Figure 10).

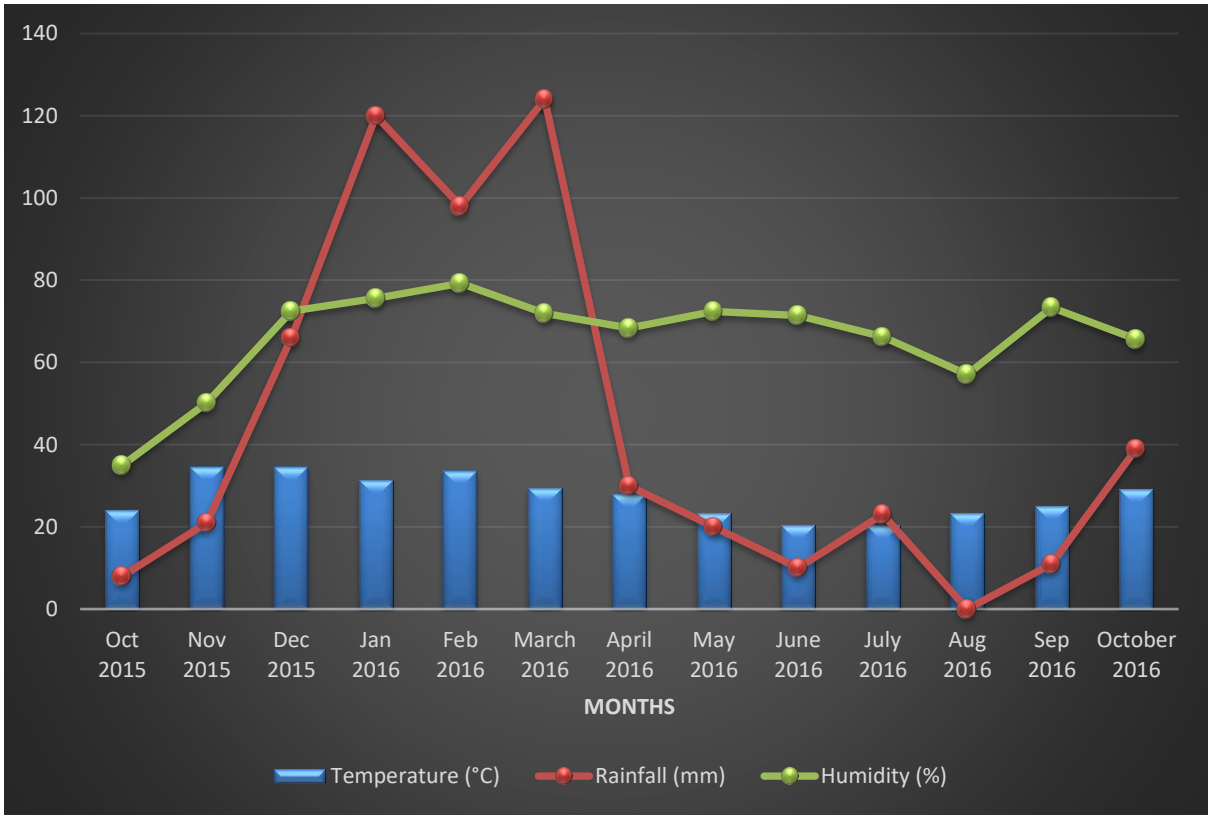


Figure 10: Monthly rainfall, mean humidity and mean temperatures in Pretoria (NZG) between October 2015 and October 2016.

4.2 Macroscopic examination of faecal samples

During the entire study period, all animals visually examined at the NZG were of good physical condition with no animal exhibiting any overt clinical signs of helminths infections, such as poor body condition, rough coat or lethargy. The faecal samples from animals were examined macroscopically for presence of any macroscopic organisms in the samples. Faeces of the three animal groups varied, from soft faeces in the ungulates to watery in some avian species, the faeces of the felids were always hard and had bone fragments from their diet. Plant debris was always noticed, particularly grass blades in the cheetah faecal samples. The consistency and viscosity of the faeces varied in animals from pronounced constipation to diarrhoea. The colour of the faeces varied throughout the course of the study, depending on season and the type of diet the animals had to adapt to. Some of the observations in the faeces were full grown helminths with mucous in the faeces of cheetah and lion cub and cysts from sable antelope faeces samples (Figure 11).

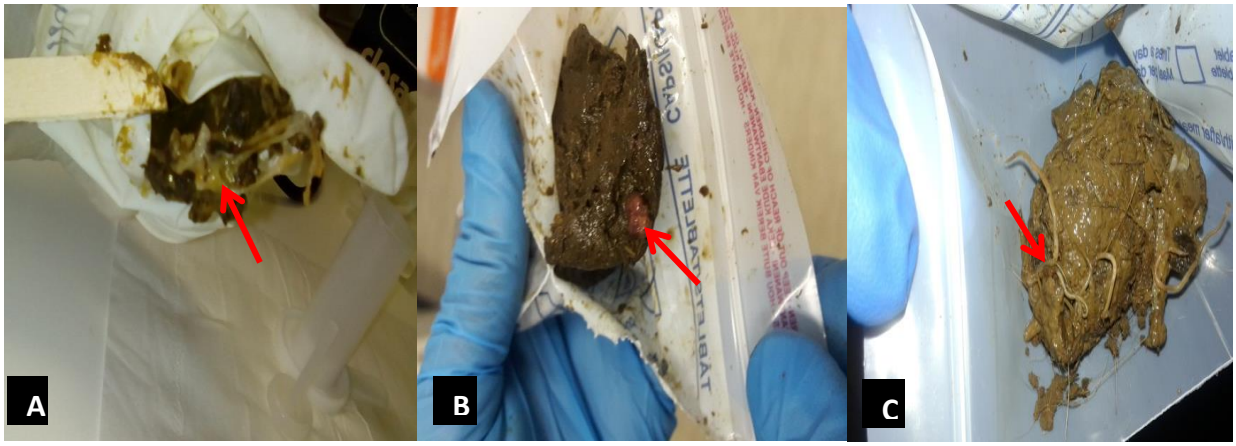


Figure 11: Macroscopic examination of faecal sample after collection before microscopic analysis (arrow). A) Cheetah faecal sample with full grown worms and mucous (arrow). B) Sable faecal sample with cyst. C) Lion cub faecal sample with full grown worms (arrow)

4.3 Prevalence of GIT parasites in selected ungulate, felid and avian species at the NZG

A total of 772 (406 ungulate, 97 felid and 269 avian) faecal samples were collected from animal enclosures at the NZG and analysed. Of the 772 faecal samples analysed, 319 samples were positive for one or more GIT parasites by microscopy with the overall prevalence of 42.19%. The prevalence of GIT parasites across the study area (in different animal groups) varied from 0 to 100%, depending on animal group. The prevalence of gastro-intestinal parasite infections in different selected captive wildlife is presented in Table 4.

Table 4: Prevalence of gastro-intestinal parasites detected in selected captive wildlife at the National Zoological Gardens of South Africa from October 2015 to October 2016

Animal group	No. of tested samples	No. of positive samples	Prevalence (%)
Ungulates	406	258	63.55
Felids	97	38	39.18
Avian species	269	17	6.32
Total	**772**	**313**	**40.54**

Collected samples were from two types of enclosure set up; mixed exhibit and single exhibit. Mixed exhibits house different animal species in the same enclosure, for example the (D2/195-6) BuiteKamp enclosure mimics the savannah biome and houses different antelopes including blesbok, springbok, cape eland and southern lechwe, free roaming birds including peafowl and guineafowl have easy access to the enclosure. Single exhibit enclosures house a single type of animal species, for example the sable antelope enclosure. Faecal samples were collected from 49 enclosures in the zoo, 12 (24.49%) mixed exhibit, 37 (75.51%) single exhibit. More animals were from single exhibit enclosures than mixed exhibit in this study. By observation, mixed exhibit enclosures are bigger and therefore house more animal

species, particularly in mammals (antelopes) as compared to the single exhibit enclosures.

4.3.1 Microscopy

Microscopic examination of the eggs collected from the selected captive wildlife samples revealed the presence of eggs of nematodes, trematodes, cestodes and protozoan parasites. Helminths from different genera were detected, including four nematodes (strongyle-type eggs, *Strongyloides* spp., *Toxocara cati, Toxoscaris leonina*, and *Nematodirus* sp. *Trichuris* sp. *Capillaria* sp. and *Heterakis* sp.). Only one species of cestode was observed, *Moniezia* sp. which was recovered from springbok, and one species of trematode, *Calicophoron* spp. was recovered from Cape eland (Figure 12-15).

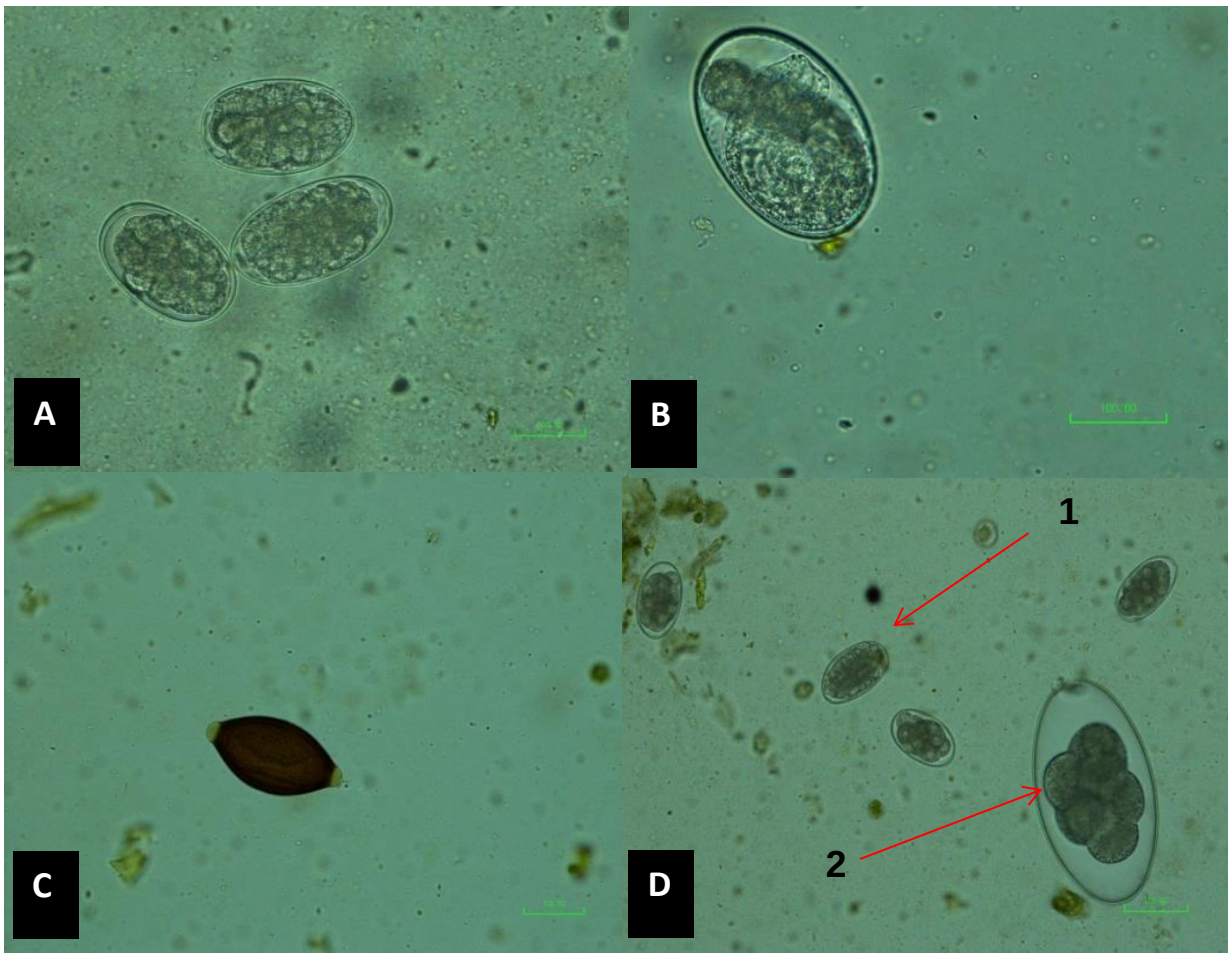


Figure 12: Micrograph of different gastro-intestinal parasite eggs obtained from captive ungulates at the NZG, Pretoria. Micrograph was taken at 20x magnification unless stated. A) Single infection of strongyles-type egg (*Haemonchus* sp.) from springbok. B) Single infection of *Strongyloides* sp. from Arabian oryx. C) Single infection from *Trichuris* sp. from Kalahari red goat. D) Mixed infection of (1) strongyle-type eggs (*Haemonchus* sp.) and (2) *Nematodirus* sp. from sable antelope. Scale bar 100.00µm

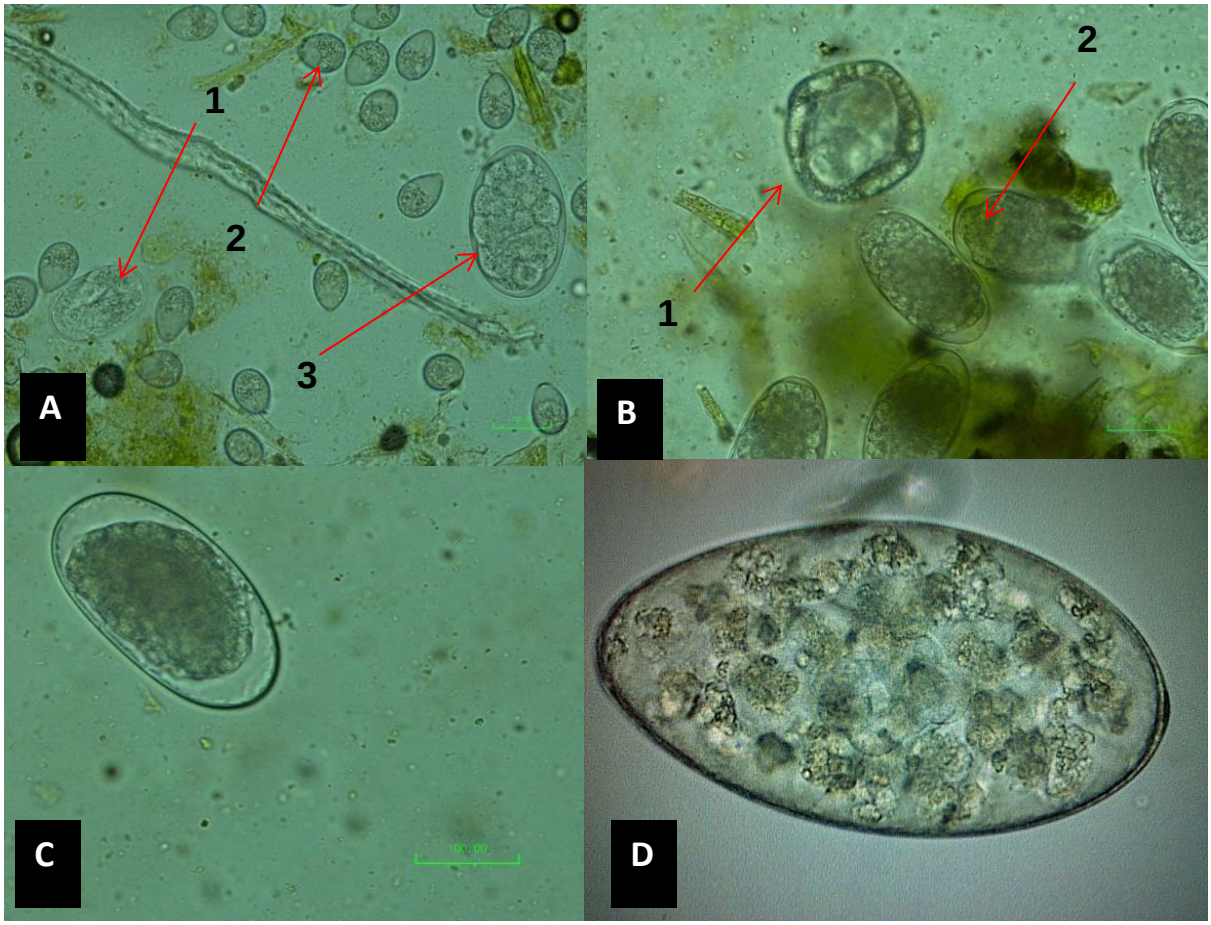


Figure 13: Micrograph of different gastro-intestinal parasite eggs obtained from captive ungulates at the NZG, Pretoria. Micrograph was taken at 20x magnification unless stated. A) Mixed infection of (1) *Strongyloides* sp., (2) coccidia from Arabian oryx and (3) strongyles-type egg (*Haemonchus* sp.). C) Mixed infection of (1) *Moniezia* sp. and (2) strongyle eggs (*Ostertagia* sp.) from Cape eland. C) Single infection of strongyle egg (*Trichostrongylus* sp.) from Scimitar horned oryx. D) Single infection of *Calicophoron* sp. from Cape eland at 40x magnification. Scale bar 100.00µm.

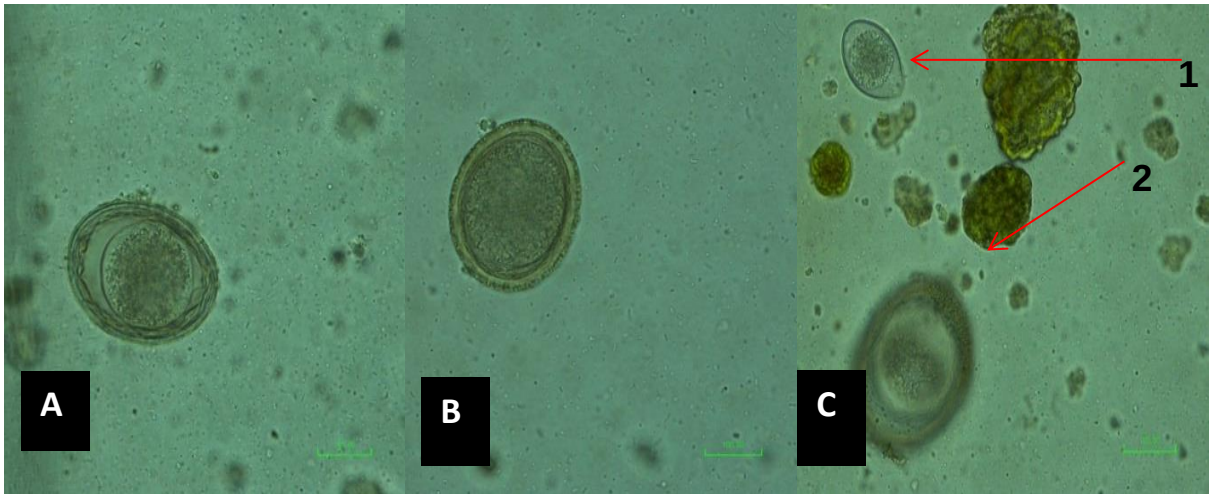


Figure 14: Micrograph of gastro-intestinal parasite eggs from captive felids at the NZG. Micrograph was taken at 20x magnification unless stated. A) Single infection of *Toxascaris leonina* obtained from Lion. B) Single infection of *Toxocara cati* obtained from lion cubs. C) Mixed infection of (1) coccidia and (2) *Toxascaris leonina* obtained from cheetah. Scale bar 100µm.

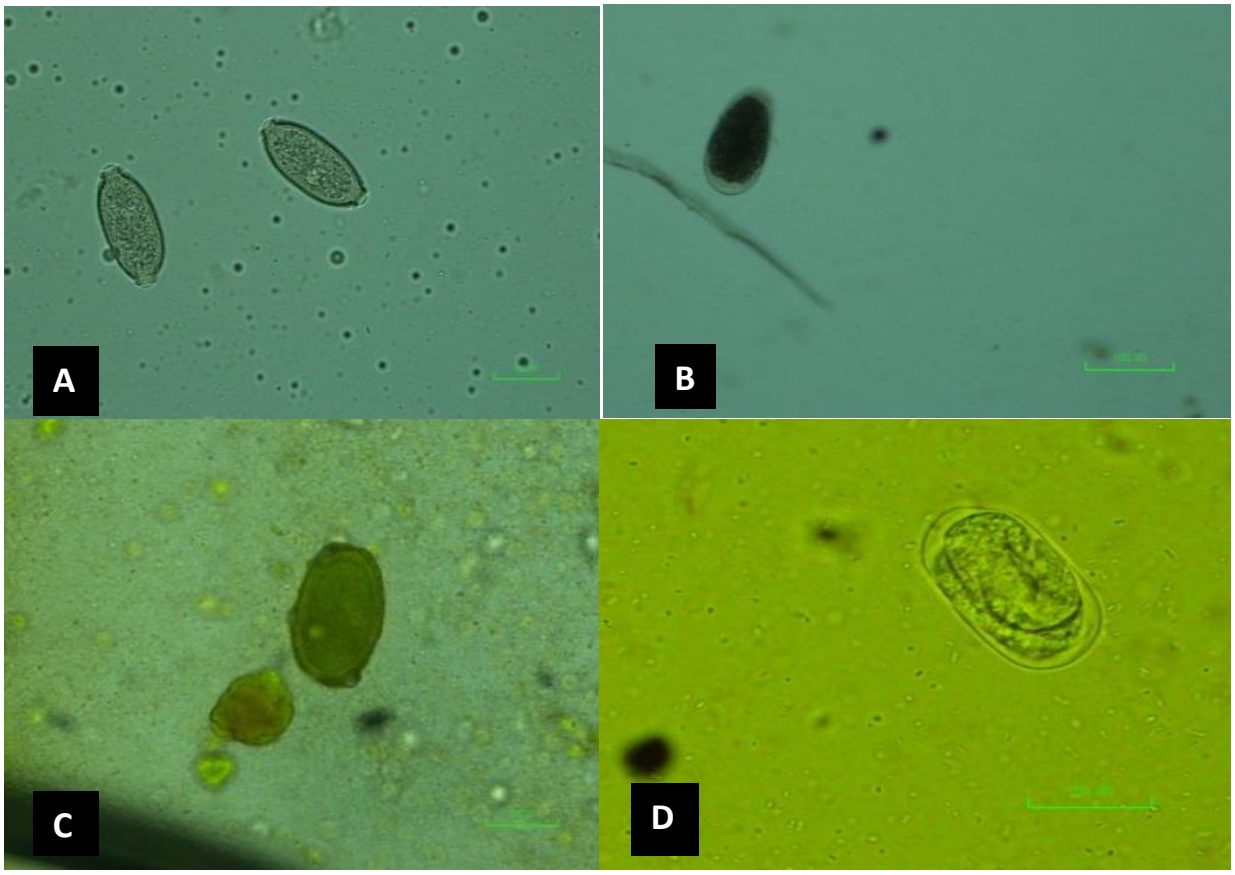


Figure 15: Micrograph of different gastro-intestinal parasite eggs obtained from captive avian species at NZG, Pretoria. Micrograph was taken at 40x magnification unless stated. A) Single infection of *Capillaria* spp. from Sun conure. B) Single infection of strongyles egg (*Trichostrongylus* sp.*) from* scarlet ibis. C) Single infection of *Heterakis* sp. infection from Hadeda Ibis. D) Single infection of *Strongyloides* sp. from Scarlet ibis. Scale bars are 100µm

4.3.2 Overview of GIT parasite prevalence and levels at the NZG

The results obtained indicated that parasitic infections were highest in ungulates followed by felids and avian species. In general, ungulates were infected, with at least one or more intestinal parasite, followed by felids and avian species (Table 5 – 7). Results indicated that GIT helminth infections are more common as compared to GIT protozoal infections in both ungulates and felids. It was demonstrated that infections were highest in ungulates followed by felids and avian species. The prevalence of gastro-intestinal parasite infections in different selected captive wildlife is presented in Table 5-7. Mixed infections were observed in over 90% of the ungulates such as sable (strongyle-type egg, *Nematodirus* sp. and *Trichuris* sp), Arabian oryx (*Strongyloides* sp., *Trichuris* sp. and strongyles-type egg), Springbok (Coccidia, strongyles-type eggs and *Moniezia* sp.). Lion cubs (*Toxocara* sp*., Toxascaris* sp*.* and coccidia) and cheetah (*Toxascaris* sp*.* and *Toxocara* sp.).

Table 5: Gastro-intestinal parasites detected in selected captive ungulates at the National Zoological Gardens of South Africa from October 2015 to October 2016

	Strongyle-type egg	Coccidia (ungulates)	*Trichuris* sp.	*Cooperia* sp.	*Strongyloides* sp.	*Moniezia* sp.	*Calicophoron* sp.
Arabian oryx	+	+	-	-	-	-	-
Blesbok	+	+	-	-	-	-	-
Cape eland	+	+	+	-	+	+	+
Gemsbok	+	+	-	-	-	-	-
Kalahari red goat	+	-	+	-	+	-	-
Lechwe	+	+	+	+	+	-	-
Nubian ibex	+	+	-	-	-	-	-
Nyala	-	+	-	-	-	-	-
Okapi	+	-	-	-	-	-	-
Red forest buffalo	-	-	-	-	-	-	-
Sable antelope	+	+	+	-	-	-	-
Scimitar-horned oryx	+	-	-	-	-	-	-
Springbok	+	+	+	+	+	-	-
Urial	+	+	+	-	-	-	-
Addax	+	-	+	-	-	-	-

Strongyle-type egg: Strongyles – helminths eggs coming from different genera such as the Haemonchus, Trichostrongylus, Oesophagostomum and Ostertagia were grouped together in this table, and recorded separately as strongyles eggs.

+: positive

-: negative

In felids, all animals sampled were infected at one point during the course of the study with the exception of Bengal tiger. Infection levels were found to be lower (Table 6) as compared to the ungulates, higher as compared to the avian species. Cheetahs had the highest egg.

Table 6: Gastro-intestinal parasites detected in selected captive felids at the National Zoological Gardens of South Africa from October 2015 to October 2016

	Toxascaris sp	*Toxocara* sp.	Coccidia
African Lion (cub)	+	+	+
African leopard	-	+	-
Cheetah	+	+	+
African lion	-	+	-
Bengal tiger	-	-	-

+: positive
-: negative

The results indicated single infections in the avian species (Table 7). And the most encountered GIT parasite was *Capillaria* sp. However, no occurrences of protozoan parasites were observed in avian species, only helminth infections were encountered.

Table 7: Gastro-intestinal parasites and their direct egg counts detected in selected captive avian species at the National Zoological Gardens of South Africa from October 2015 to October 2016

	*C*apillaria sp.	*Heterakis* sp.	*Trichostrongylus* sp.	*Unknown egg*
Ground hornbill	-	-	-	-
Sun conure	+	-	-	-
Orange winged amazon	+	-	-	-
Scarlet ibis	-	-	+	-
Gymnogene	-	-	-	-
African goshawk	-	-	-	-
Yellow billed kit	-	-	-	-
Jackal-buzzard	-	-	-	-
Steppe buzzard	-	-	-	-
Lanner falcon	-	-	-	-
Sulphur crested cockatoo	-	-	-	-
Blyth hornbill	-	+	-	-
Illigers macaw	+	+	-	+
Blue and Yellow macow	+	+	-	-
East African Grey Crown Crane	-	-	-	-
Red crown crane	-	-	-	-
Wattled crane	-	-	+	-
Bald ibis	+	-	-	-
Common ostrich	-	-	-	-
Cape vulture	-	-	-	-
King vulture	-	-	-	-
Brown snake eagle	-	-	-	-
Chilean flamingo	-	-	-	-
Rosy flamingo	-	-	-	-
Domestic chicken	-	-	-	+
Domestic/Chinese goose	-	-	-	-
Olive thrush	-	-	-	-

+: positive

-: negative

4.4 Distribution of GIT parasites in across selected in selected captive ungulates at the NZG

Distribution of GIT parasites at the NZG was continuous throughout the year with highest prevalence recorded in July 2016 and the lowest in September 2016 (Figure 16).

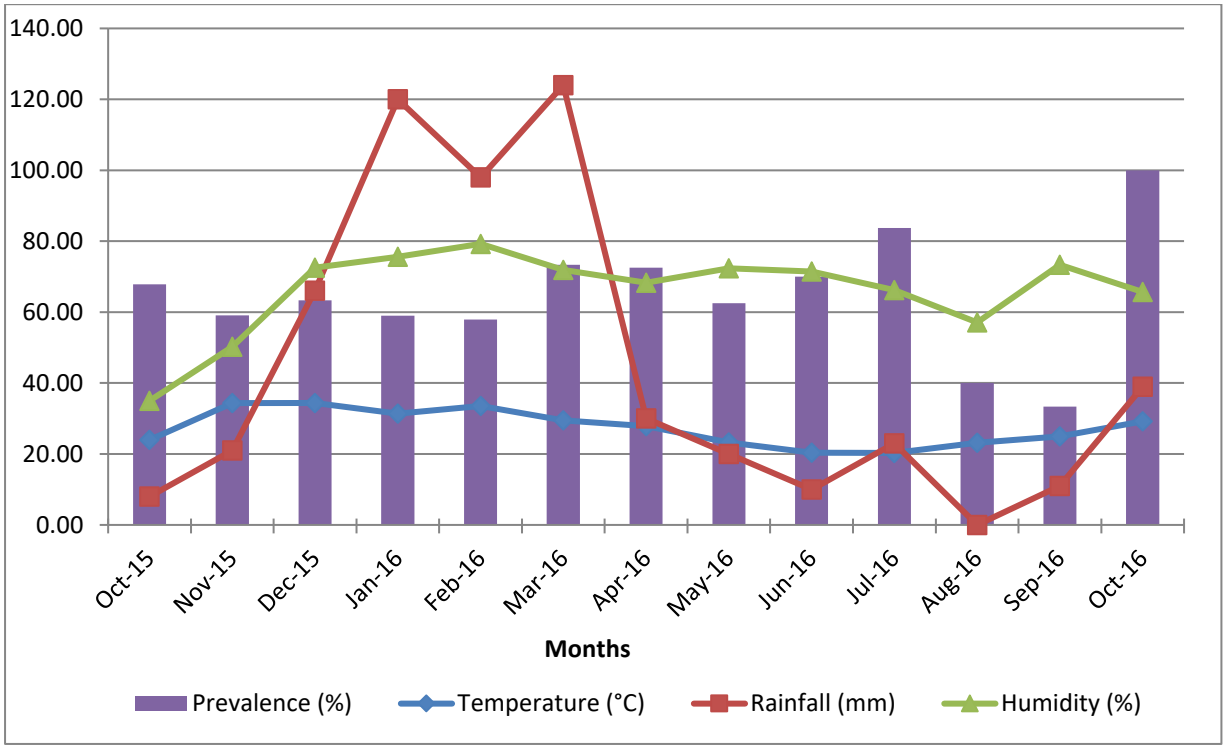


Figure 14: Seasonal prevalence of GIT parasites in ungulates at the NZG

Strongyle-type eggs (*Haemonchus* sp.) were most common and were detected throughout the collection period in the ungulates, with the highest peak observed in November (EPG 1386), although the prevalence (32%) was relatively low (Figure 24), the high infection was soon after the first rain of the season and again in May (EPG: 864) (Figure 17). Throughout the study, *Haemonchus* had the highest egg counts as compared to other GIT parasites; however between January and February, *Oesophagostomum* sp. took the lead, with highest egg count. From June to September, the EPG count of *Haemonchus* sp. declined to a point were other GIT parasites such as *Trichuris* sp., *Nematodirus* sp. and *Ostertagia* sp. were dominant.

Trichostrongylus sp. was isolated in the present study, with the highest record (EPG: 650) being in November 2015, however the prevalence of the parasite was low (16%), this means, although the number of animals infected with *Trichostrongylus* sp. was small, the parasite load (EPG) was high, and this could be a serious problem. Throughout the study, the egg count for *Trichostrongylus* sp. decreased (Figure 17), reaching its lowest record in September 2016 and October 2016, where no eggs were isolated at all. The prevalence of *Trichostrongylus* on the other hand remained stable throughout the study. In November 2015, the egg count for *Trichostrongylu*s sp. was second highest after, *Haemonchus* sp. While in December 2015, the egg count was higher (EPG: 416) than all the other GIT parasites, including the dominant *Haemonchus* sp. It was followed by *Ostertagia* sp. and then *Haemonchus* sp.

Nematodirus sp. was recovered from the ungulates throughout the study, with the exception of January 2016, were no eggs of *Nematodirus* sp. were observed in all the ungulates. The highest peak of Nematodirus (EPG: 600) in July 2016, followed by August 2016 (EPG: 383) and November 2015 (EPG: 350) (Figure 17). The prevalence of *Nematodirus* sp. infection and the average EPG indicated as similar pattern. When there was an increase in prevalence of *Nematodirus* sp. in ungulates, the same increase was observed in the average EPG, with the exception of December 2015, where there was a decrease in the parasite load while the prevalence remained constant. The increase in infection of *Nematodirus* in July was higher than all the other GIT parasites, including the common strongyle-type egg *Haemonchus* sp.

Trichuris sp. parasite was not continuous and was barely seen between November to March, with small peak in January 2016 (EPG 67), there were two high peaks in April and July with the EPG of 333 and 529 respectively (Figure 17). The increase in *Trichuris* sp. in April was higher than all the other GIT parasites, with the exception of the strongyle-type egg *Haemonchus* sp. The prevalence remained constant with no obvious variations. The increase in infections of *Trichuris* sp. in July was higher than all the other GIT parasites, except *Nematodirus* sp. It was noted that, the pattern of *Trichuris* sp. and *Nematodirus* sp. was similar, except in November 2015, when no sign was observed, while there was an increase in *Nematodirus* sp.

Moniezia sp. on other hand was only recovered in March and May with an EPG of 33 and 267 respectively and therefore can be classified as an occasional parasite, whilst *Calicophoron* sp. was only recovered during December 2015 and January 2016, during the first rainy months and the highest EPG recorded was 250 and this was December (Figure 17).

The highest record of *Strongyloides* sp. was in October 2015 (EPG: 815), thereafter the EPG count for *Strongyloides* sp. remained under 200 count for the rest of the study. The parasite load and prevalence patterns for *Strongyloides* sp. infections correlated (Figure 17).

Oesophagostomum sp., a strongyle-type egg was isolated in the present study. Although *Oesophagostomum* was not serious parasite in the current study, considering its EPG count and prevalence (Figure 17), however between January (EPG: 950) and February 2016 the parasite load (EPG: 1480) was extremely higher than all the other GIT parasites, including the common strongyle-type egg of *Haemonchus* sp.

Cooperia sp. was the least detected strongyle-type egg parasite in the study, in terms of severity and prevalence of infection. First isolated in February, the highest infection (EPG: 350) recorded for *Cooperia* sp. in the study, the parasite remained unseen until June 2016, July 2016 and August 2016 were they minor infections, with of EPG 267, 166 and 150 respectively (Figure 17).

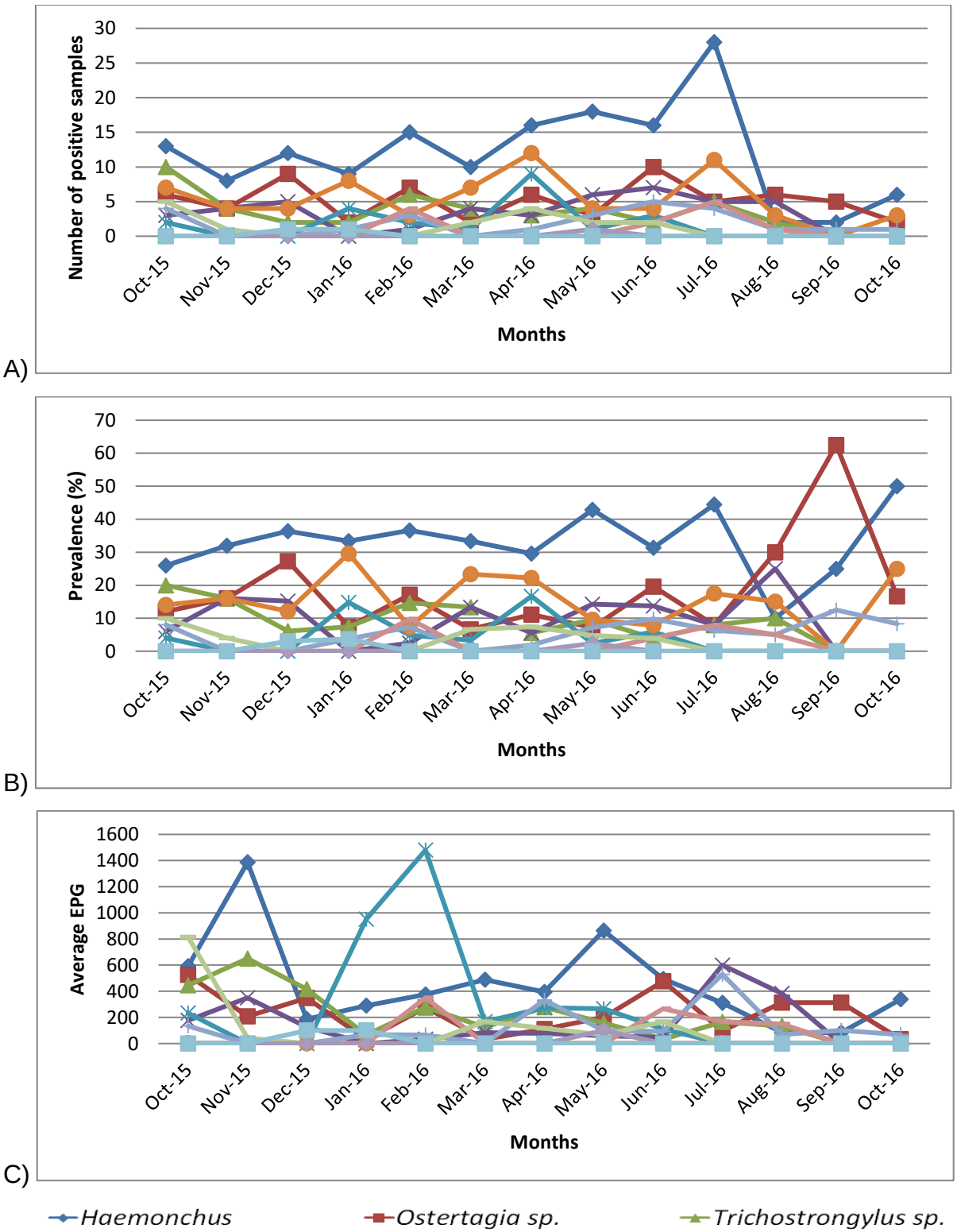


Figure 15: Distribution of GIT parasites from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites. (B) Percentage prevalence of GIT parasite infections. (C) Average eggs per gram (EPG) of GIT parasites.

4.4.1 *Haemonchus* species

One of the most common gastro-intestinal nematode in both captive and free ranging wildlife is the *Haemonchus contortus*. Also known as the barber's pole worm, this cylindrically shaped worm, tapered at both ends has a complete digestive system, and a worldwide distribution however more prevalent in sub-tropical and tropical regions under warm and wet weather conditions (Qamar *et al*. 2009). In this study, *Haemonchus* sp. was one of the most common parasites (Figures 18).

Haemonchus sp. was the most important GIT parasite in the ungulates, and was isolated in over 73.33% of the ungulates samples in this study. Overall, the prevalence of the ungulates was above 40% in all the positive samples. The sable antelopes and cape eland had infections more or less throughout. In terms of average EPG, November 2015, May 2016 and June 2016 was an important months for *Haemonchus* sp. During the month of November 2015, sable antelope, cape eland and blesbok where infected with *Haemonchus* sp. and two of these animals' EPGs was well above the threshold of EPG for ungulates. In May, three of the five ungulates infected with GIT parasites had EPG above the threshold, Arabian oryx EPG was the highest in May 2016.

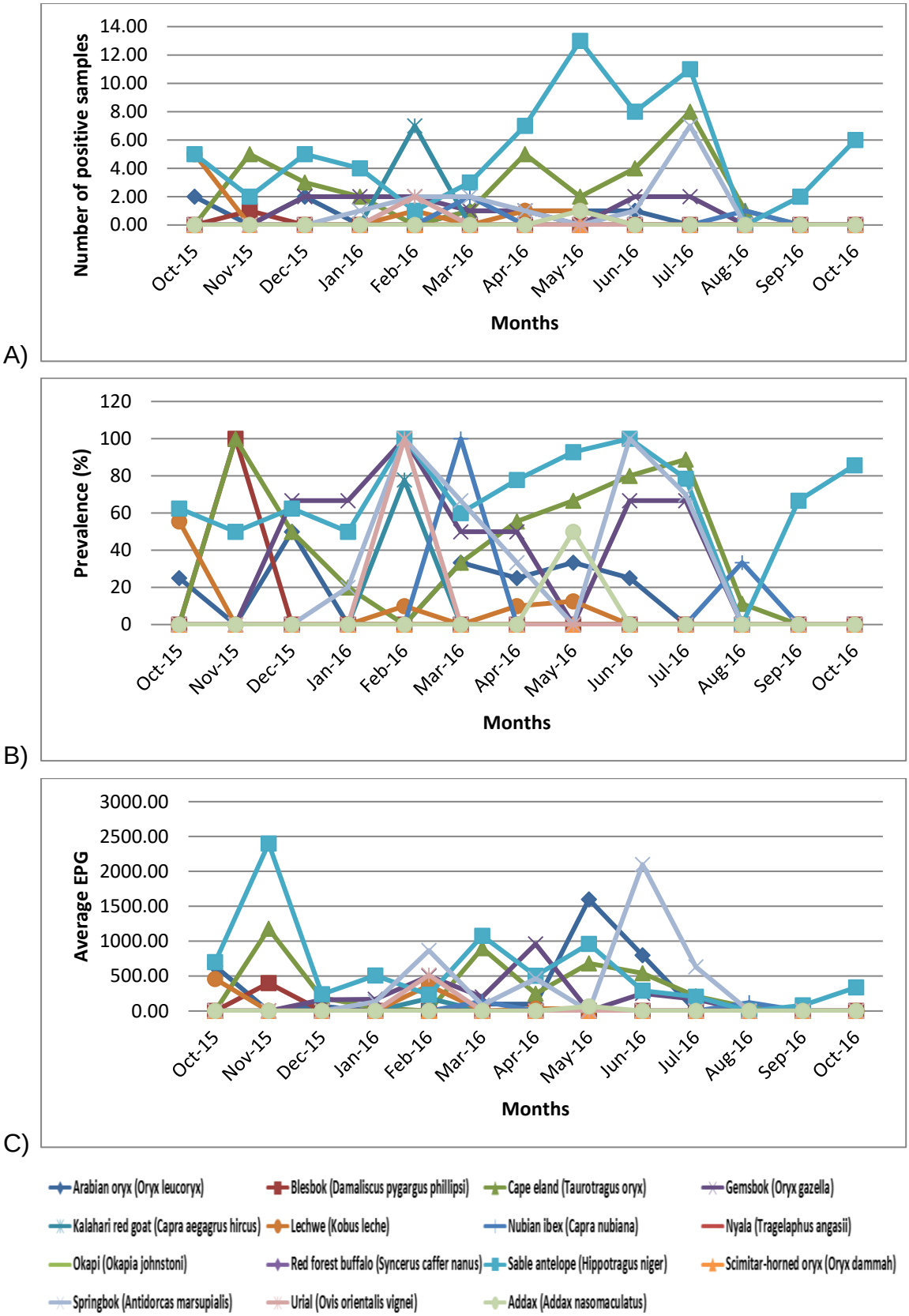


Figure 16: Distribution of *Haemonchus* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Haemonchus* samples. (B) Percentage prevalence of *Haemonchus* sp. infections. (C) Average eggs per gram (EPG) of *Haemonchus* sp.

4.4.2 *Ostertagia* species

Ostertagiasis, a parasitic gastric caused by the nematode *Ostertagia ostertagii* is an important GIT parasites infections of cattle. *Ostertagia* species is one of the most economically significant parasites of cattle and other ruminants. The annual cost of gastro-intestinal nematodiasis in the United States has conservatively been estimated in excess of $250 million, and *Ostertagia* species infection accounts for a major portion of that loss (Myers & Taylor 1989). Affected animals lose weight and can die of overwhelming clinical Ostertagiasis, characterized by severe diarrhoea, edema and weight loss.

Throughout the study, there were at least one or more *Ostertagia* sp. infections in at ungulates (Figure 19). *Ostertagia* sp. did not follow any trend throughout the study. Overall, the prevalence of *Ostertagia* sp. was above 50% throughout the study, with only a few exceptions. Although a common parasite in the ugnulates, there were only a few months (October 2015, December 2015, June 2016 and August 2016) where the average EPG was on or above the threshold

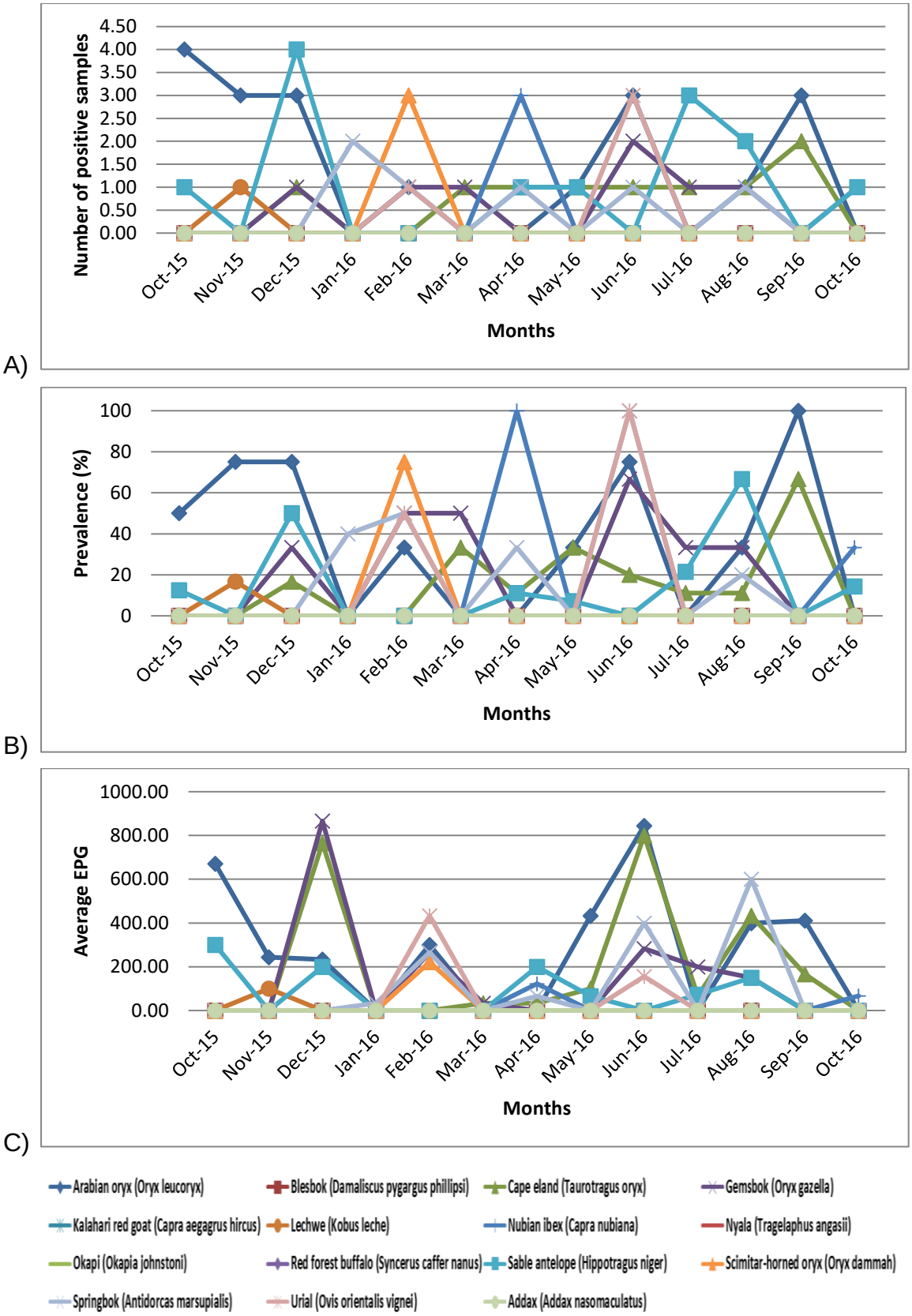


Figure 17: Distribution of *Ostertagia* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Ostertagia* sp. samples. (B) Percentage prevalence of *Ostertagia* sp. infections. (C) Average eggs per gram (EPG) of *Ostertagia* sp.

4.4.3 *Trichostrongylus* species

Trichostrongylus species parasites of domestic animals and specially ruminants are a most important cause of economic loss throughout the world. The economic impact of these parasites include weight loss, impaired wool and milk production and poor reproductive performance (Shahbazi *et al*. 2012). *Trichostrongylus* sp. was an important GIT parasite in captive ruminants at NZG (Figure 20).

The overall prevalence of *Trichostrongylus* in ungulates was above 40% for most animal species (Figure 20). Prevalence of 100% was observed in Urial and Okapi, in February 2016 and March 2016. February 2016 was an important month for *Trichostrongylus* sp. as more animals (Urial, Gemsbok, Arabian oryx, Scimitah-horned oryx and Addax) were infected with *Trichostrongylus* sp. Although the average EPG were below 200 for most, for Urial it was at 583.50, which is the borderline of the threshold (600 EPG) and therefore the animal would require treatment. On the other hand, January 2016 and September 2016 were the least important months. In September 2016 and October 2016, there were no *Trichostrongylus* sp. infections on all the samples tested. In January 2016 only two samples were positive (both from Cape eland) therefore 20% prevalence of *Trichostrongylus* sp., infection with an average EPG: 66.50.

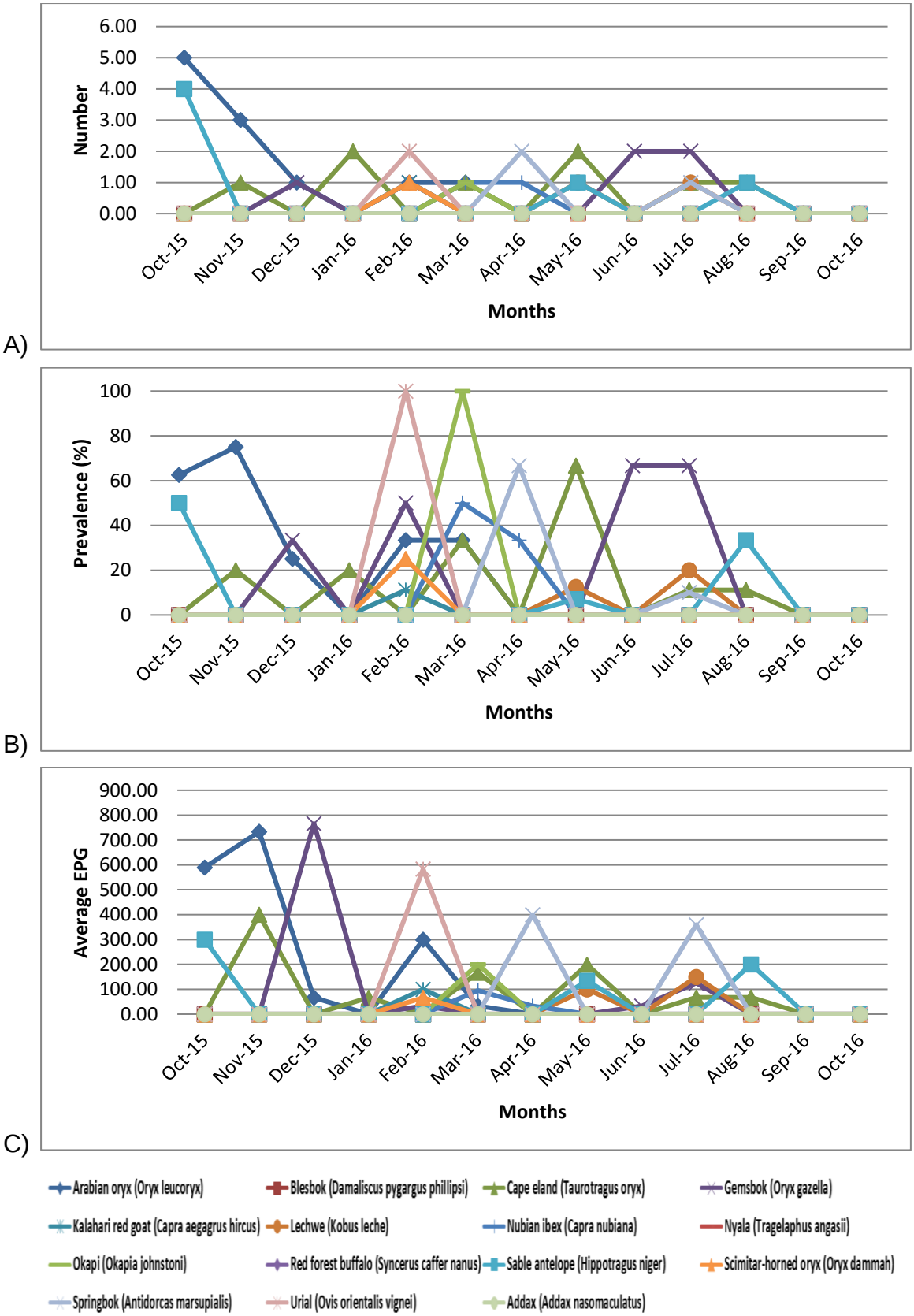


Figure 18: Distribution of *Trichostrongylus* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Trichostrongylus* sp. samples. (B) Percentage prevalence of *Trichostrongylus* sp. infections. (C) Average eggs per gram (EPG) of *Trichostrongylus* sp.

4.4.4 *Nematodirus* species

Nematode parasites belonging to the genus *Nematodirus* cause disease in domesticated and wild ruminants. Two species, *N. spathiger* and *N. filicollis* are recognised as important parasites of lambs in New Zealand in that both species are common and they are pathogenic in comparatively low numbers.

In the present study, *Nematodirus* sp. was common in Sable antelope where it was detected in nine of the 13 months of the study (Figure 21). The number of samples positive for this parasite ranged from 0 – 6 across all animal species in the ungulates. Although most of the infections were not important (average EPG: 200 throughout the study), there was some exceptions. In July 2016 and August 2016 there was an increase average EPG of *Nematodirus* sp, and these were observed in the Sable antelopes. Although the number of positive samples decreased, prevalence increase and as well as the parasite load. This means that although a few samples were positive for *Nematodirus* sp. infections, they were high loads of parasitic infections.

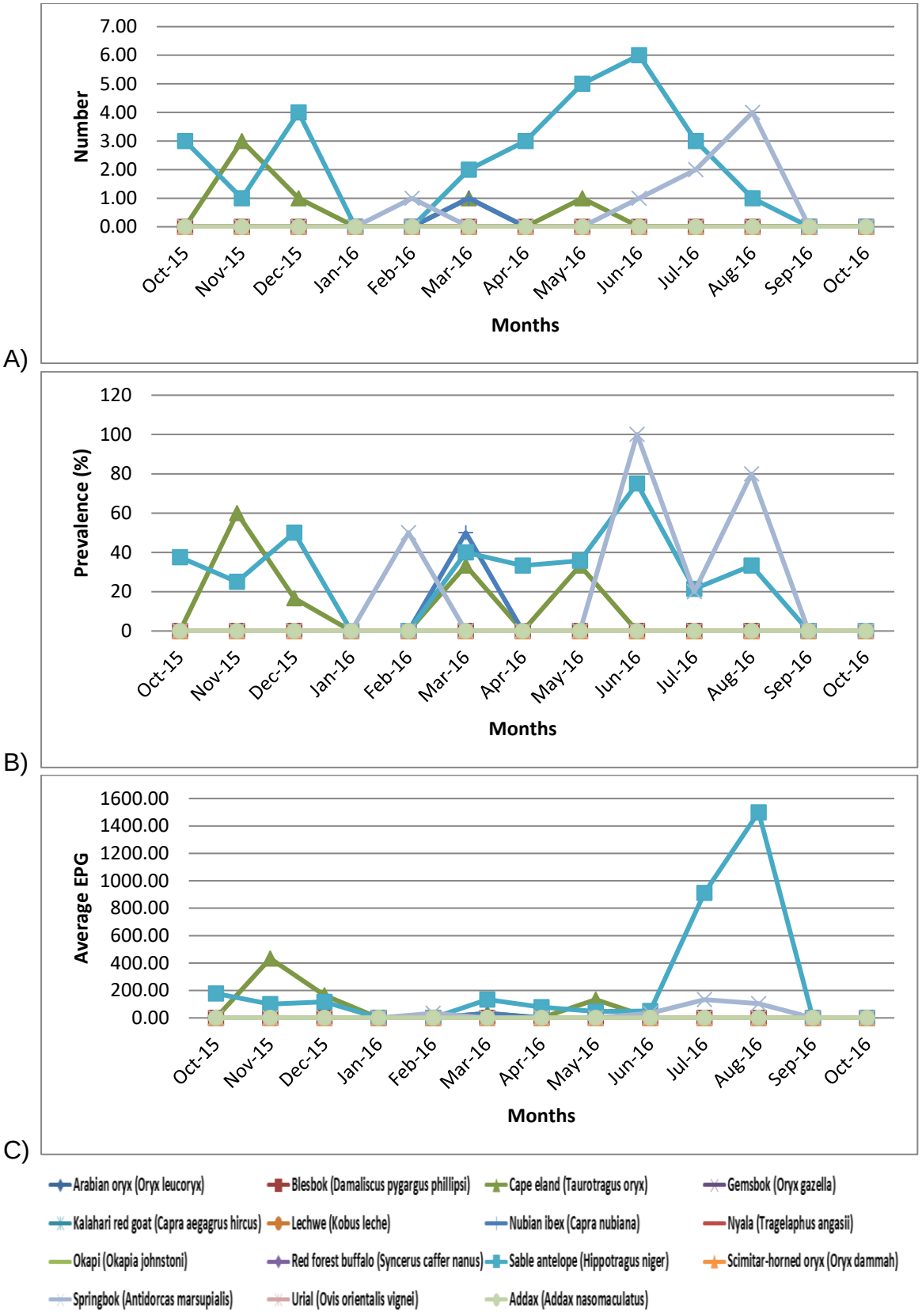


Figure 219: Distribution of *Nematodirus* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Nematodirus*** sp. samples. (B) Percentage prevalence of ***Nematodirus*** sp. infections. (C) Average eggs per gram (EPG) of ***Nematodirus*** sp.

4.4.5 *Oesophagostomum* species

Oesophagostomum sp. is a parasite of cattle and is distributed throughout South Africa from the arid north-west to the subtropical north and north-east, the coastal regions of KwaZulu Natal in the east and the non-seasonal rainfall regions of the south (Horak *et al*. 2004). The disease they cause, oesophagostomiasis, is known for the nodule formation it causes in the intestines of its infected host. It has a worldwide distribution, however more prevalent in warm and humid climates in tropical and subtropical regions. As a general rule, within the mixed infections with gastrointestinal roundworms *Oesophagostomum* helminths are usually not a predominant species. In the study, the parasite did not follow any trend (Figure 22).

April 2016 was an important month for Oesophagostomum sp. at NZG, where five animal species (springbok, sable antelope, gemsbok, Cape eland and Arabian oryx) were infected with this parasite. This was only month where more than three animal species were infected at the same time. It is also important to note that, although more animal species were infected in April 2016, the average parasite load was below EPG: 400. January 2016 and February 2016 had extremely high parasite load, 1244.67 and 1479.50 respectively. Such high parasite had never been seen before and they were never seen again in this study.

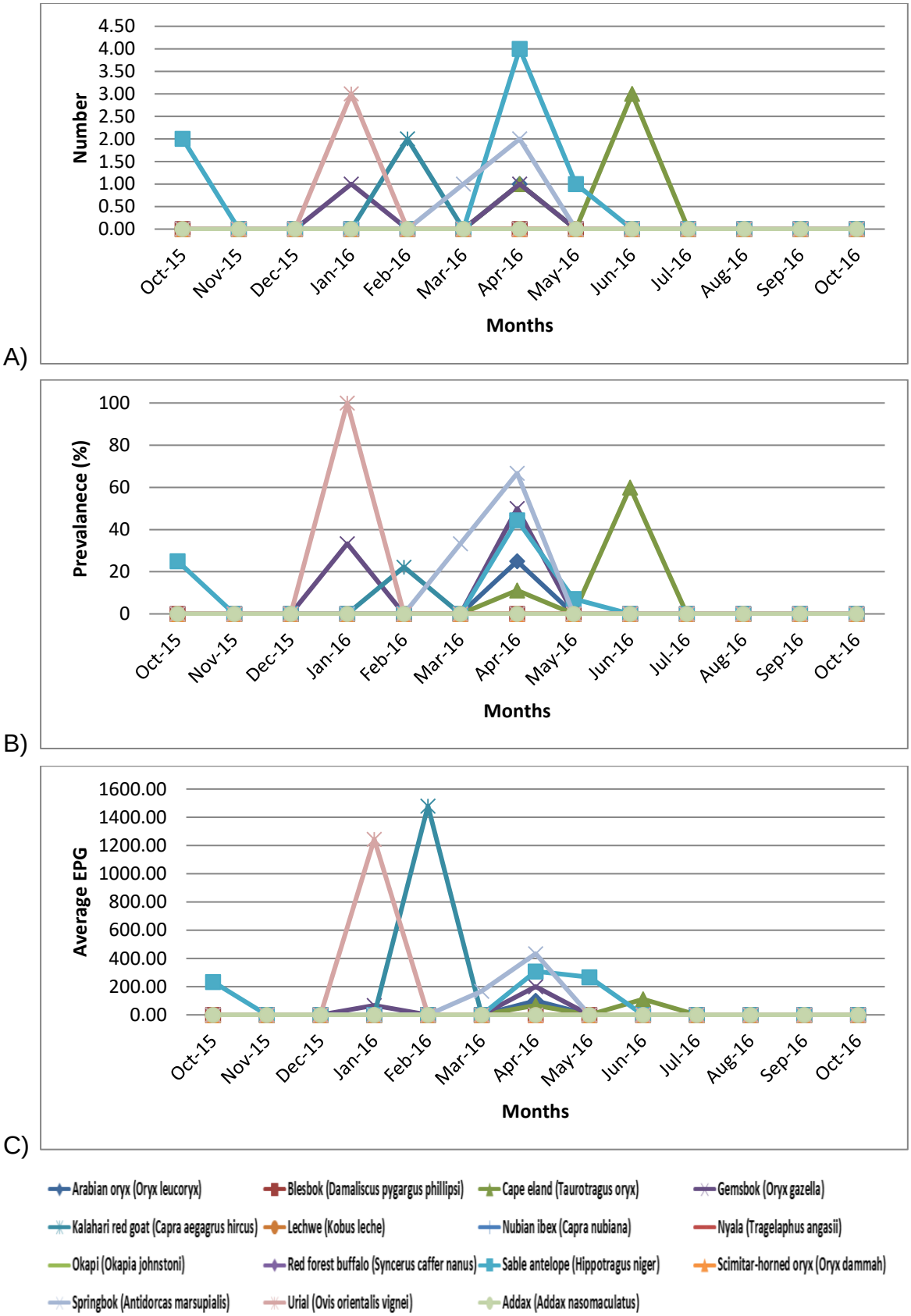


Figure 20: Distribution of *Oesophagostomum* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Oesophagostomum*** sp. samples. (B) Percentage prevalence of ***Oesophagostomum*** sp. infections. (C) Average eggs per gram (EPG) of ***Oesophagostomum*** sp.

4.4.6 *Trichuris* species

Small ruminant helminthiasis is found to be an important problem and Trichuriasis is among one such problem caused by *Trichuris* sp. *Trichuris* sp. prefer the caecum and the first section of the colon up to the beginning of the disk-like section (Gul & Tak 2014). *Trichuris ovis* is among the most prevalent nematode parasite that infects ruminants irrespective of age, gender and breed of the host worldwide leading to considerable loss in variety of ways (Kuchai *et al*. 2013). *Trichuris* sp. has been reported in cattle worldwide. In the present study, *Trichuris* sp. was not a common parasite (Figure 23).

Overall prevalence of *Trichuris* sp. in ungulates ranged from 0 – 50%. No infections of *Trichuris* sp. were observed in November 2015 and December 2015. More infections were observed from April 2016 – July 2016 where at least three animal species were infected with *Trichuris* sp. The highest parasite loads where observed in July 2016, three animals were infected with the parasite and the average parasite load was EPG: 650, which is higher than the threshold and would therefore mean the animal had to have been treated.

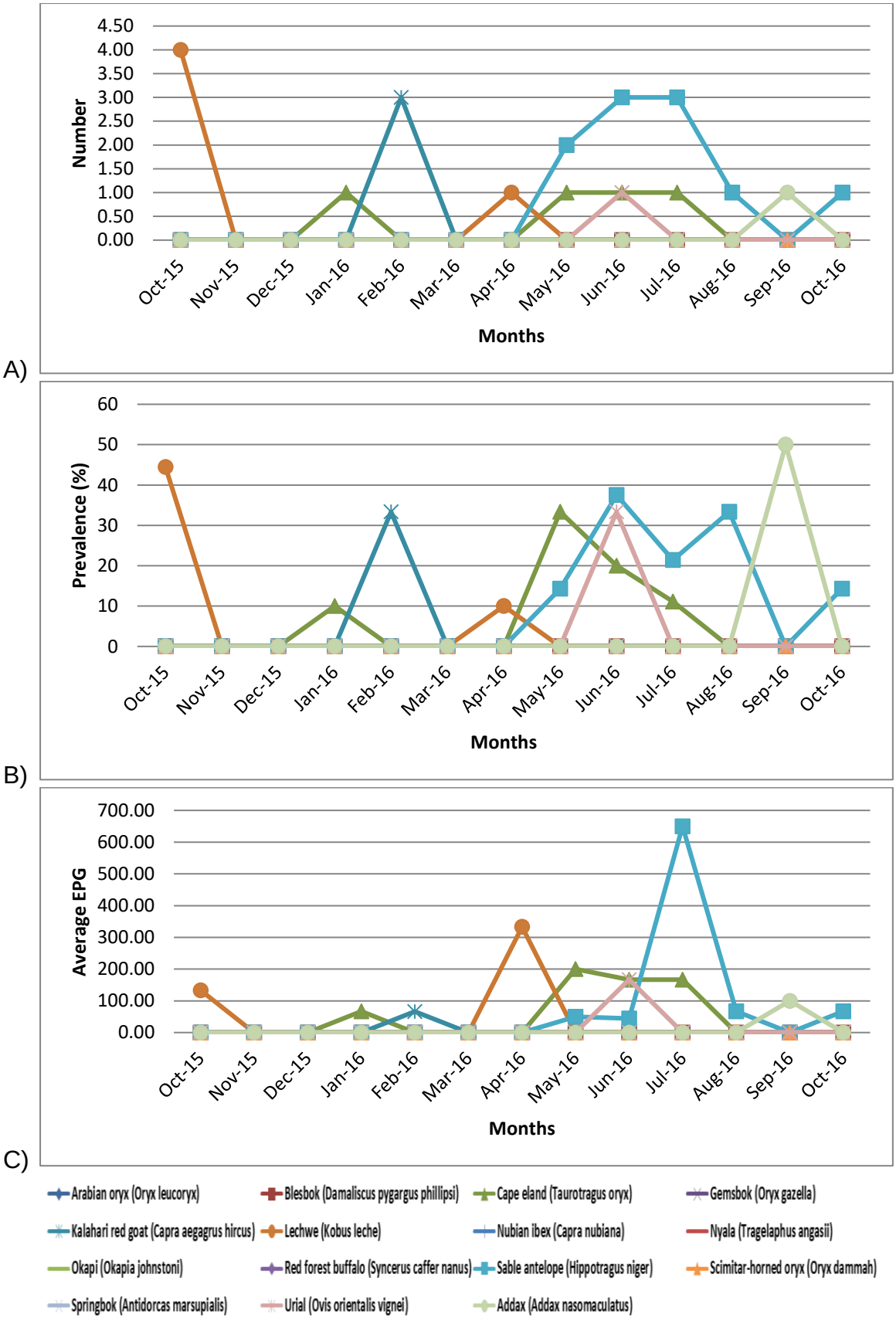


Figure 21: Distribution of *Trichuris* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Trichuris*** sp. samples. (B) Percentage prevalence of ***Trichuris*** sp. infections. (C) Average eggs per gram (EPG) of ***Trichuris*** sp.

4.4.7 *Cooperia* species

Cooperia sp. is parasites of the both domestic and wild ruminants and parasitizes the small intestines. They are considered mild pathogens however there are a few species that are of greater veterinary importance. *Cooperia* sp. have become the among the most prevalent nematode parasite in the United States cow/calf operations as observed in the USDA NAHMS Beef Cow/Calf survey in 2008 (Stromberg *et al*. 2012). *Cooperia* sp. was rarely seen in the current study, and was seen only three times (Figure 24).

Cooperia sp. was observed in the springbok and lechwe. In February 2016 both springbok and lechwe were infected with *Cooperia* sp. A 100% and 20% prevalence was noted for springbok and lechwe respectively, the average parasite load of lechwe was however higher than that of the springbok. Two samples were positive for both animals; however the parsite load was higher and therefore

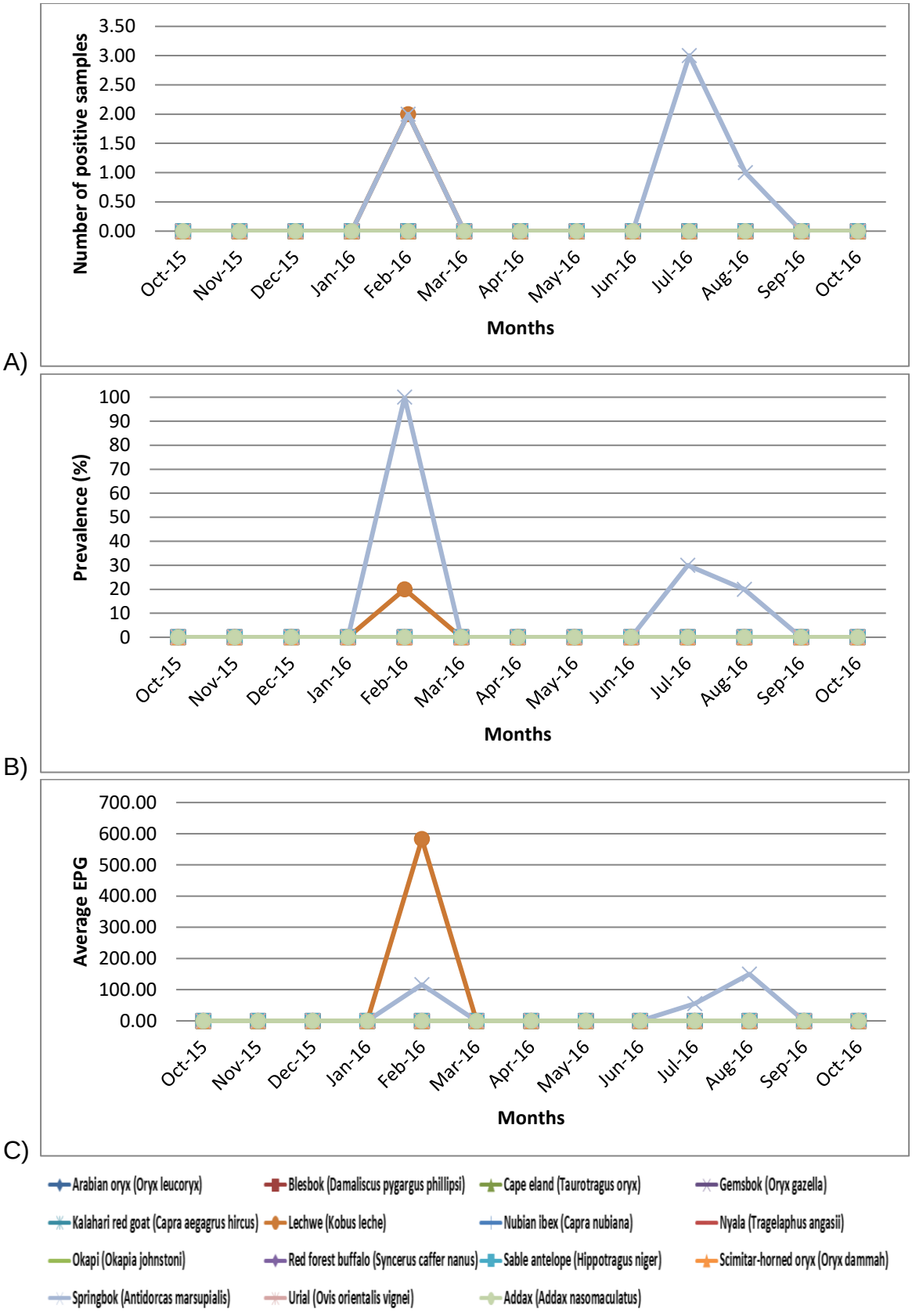


Figure 22: Distribution of *Cooperia* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Cooperia*** sp. samples. (B) Percentage prevalence of ***Cooperia*** sp. infections. (C) Average eggs per gram (EPG) of ***Cooperia*** sp.

4.4.8 *Strongyloides* species

Strongyloidiasis a helminthic disease caused by nematodes or roundworms, in the genus Strongyloides, they form infective, filariform third-stage larvae that infect a new host by skin penetration and develop to parthenogenesis females, like their mothers. Alternatively, the eggs can develop into rhabditiform third-stage larvae and give rise to free living generation that consist of females and males. *Strongyloides* sp. is the most common in the tropical or subtropical climates (Eberhardt *et al.* 2007). In the present study, *Strongyloides* species was not an important parasite. It was occasionaly seen (Figure 25).

In April 2016, three animal species (lechwe, springbok and cape eland) of the ungulates were infected with the parasite; this was highest number of infections noted for the current study. However, the parasite loads in these animals for that particular month was not high (below 300). In October 2015, five Arabian oryx were infected with Strongyloides sp., with 62.5% prevalence and EPG: 800. October 2015 was the only month were the parasite load of Strongyloides sp. infection was above the threshold.

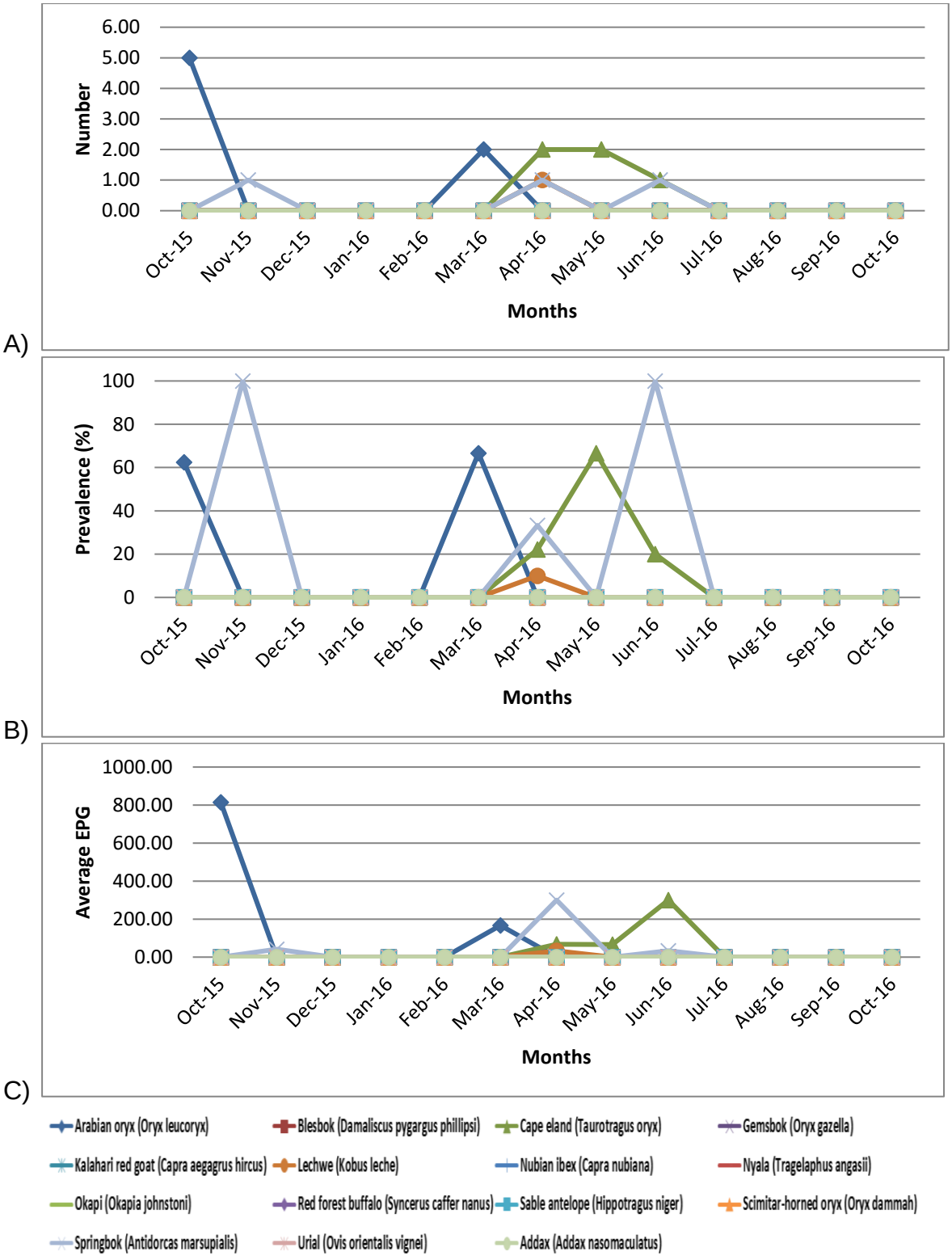


Figure 23: Distribution of *Strongyloides* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Strongyloides*** sp. samples. (B) Percentage prevalence of ***Strongyloides*** sp. infections. (C) Average eggs per gram (EPG) of ***Strongyloides*** sp.

4.4.9 *Moniezia* and *Calicophoron* species

Moniezia species is a parasitic tapeworm that affects both domestic and wild ruminants as final hosts. There is one report on *Moniezia* sp. found in pigs. *Moniezia* sp. has a worldwide distribution, with a variable incidence. In endemic regions more than 50% of the herds may be infected. Infections of *Moniezia* sp. in adult are benign and usually do not cause clinical signs. Young animals are more susceptible and massive infections can cause diarrhoea, reduced weight gain and intestinal obstruction (Gilfillan 2010).

Calicophoron sp. are small conical fluke that inhabit the small intestines of the host. Outbreaks are common in livestock that graze in marshy areas, and they occur mid-autumn and continue throughout winter. Clinical signs of conical fluke infestation are anorexia, diarrhoea and weight loss (Gilfillan 2010).

Both *Moniezia* sp. and *Calicophoron* sp. were occasional parasites in this study (Figure 26 and Figure 27), rarely seen and when seen, the parasite load was not important. It is important to note that both these parasite were detected in the same animal species (cape eland), although in different months.

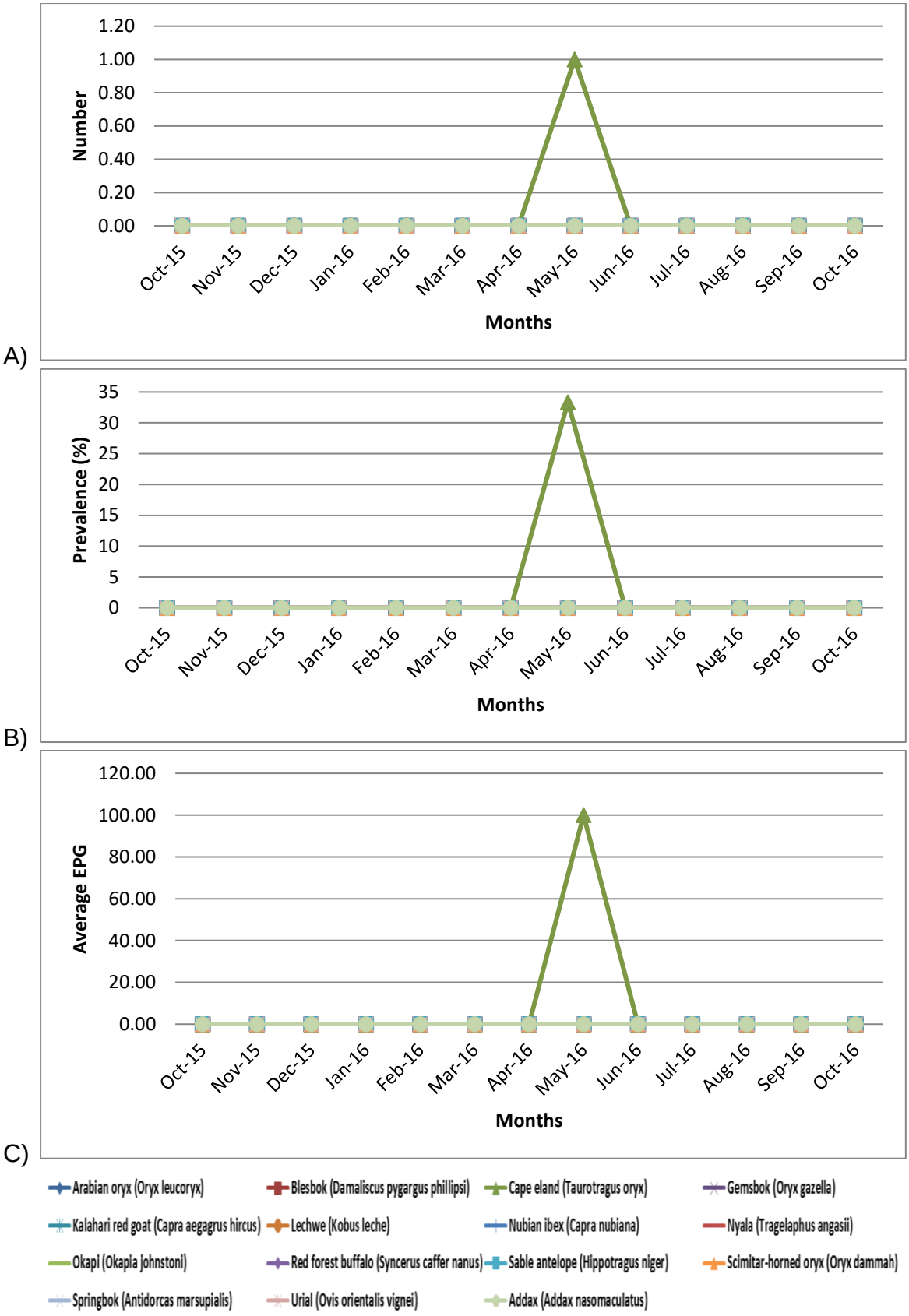


Figure 24: Distribution of *Moniezia* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive ***Moniezia*** sp. samples. (B) Percentage prevalence of ***Moniezia*** sp. infections. (C) Average eggs per gram (EPG) of ***Moniezia*** sp.

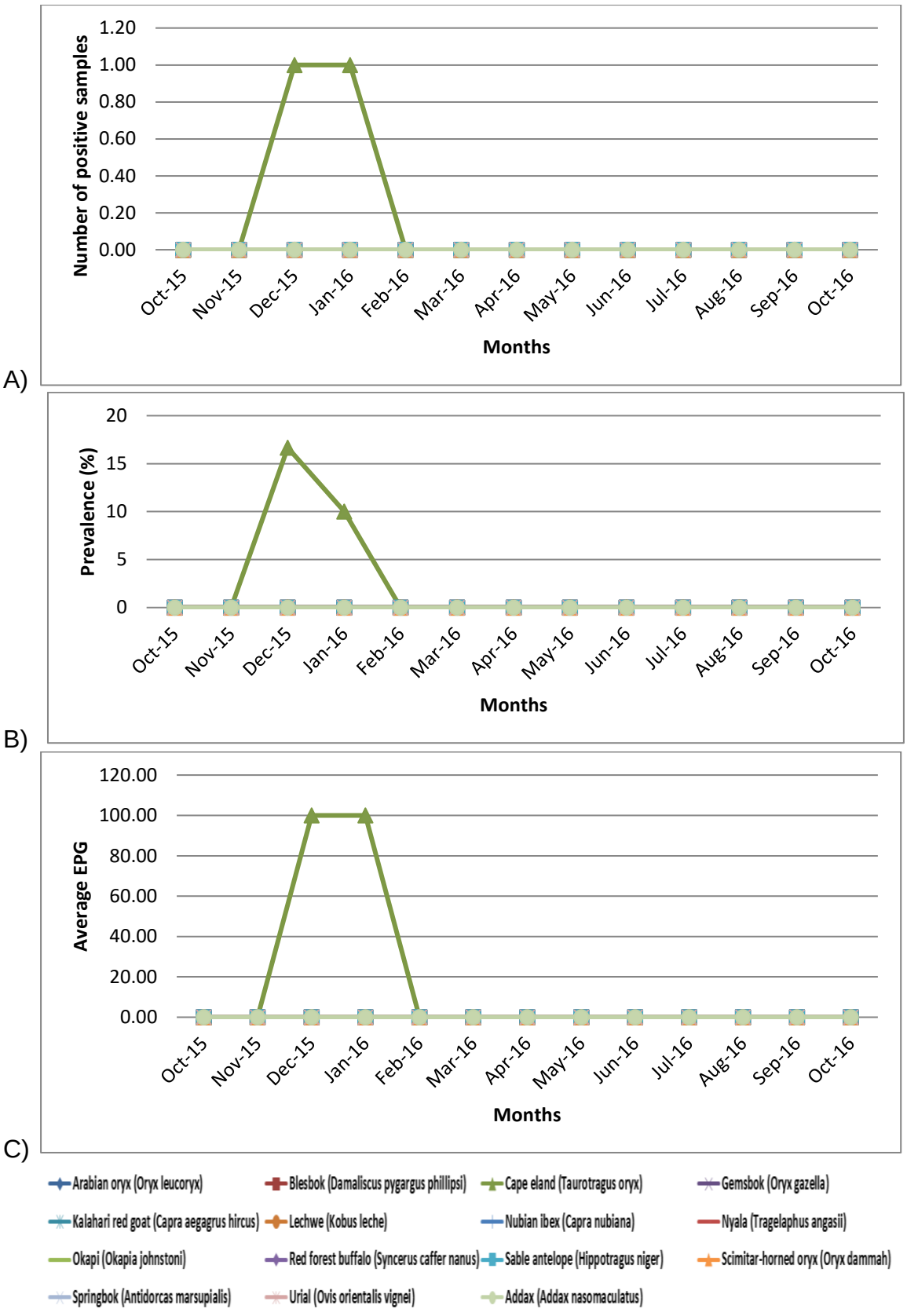


Figure 25: Distribution of *Calicophoron* sp. from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Calicophoron* sp. samples. (B) Percentage prevalence of *Calicophoron* sp. infections. (C) Average eggs per gram (EPG) of *Calicophoron* sp.

4.4.10 Coccidia

They are protozoan unicellular organisms of the phylum Apicomplexa that parasitize vertebrates as well as invertebrates, the most common affecting vertebrates is the genus *Eimeria* (Helke *et al*. 2006), and a worldwide distribution (Dong *et al*. 2012). *Eimeria* sp. has been observed in almost all areas where cattle are raised and cause disease in calves (Dong *et al*. 2012).

The only protozoal parasite detected in this study, coccidia was very common especially in the ungulates (Figure 28). It was recovered throughout the present study in different animal species of ungulates. Coccidia did not follow any trend; there were however more animals infected between March 2016 and April 2016.

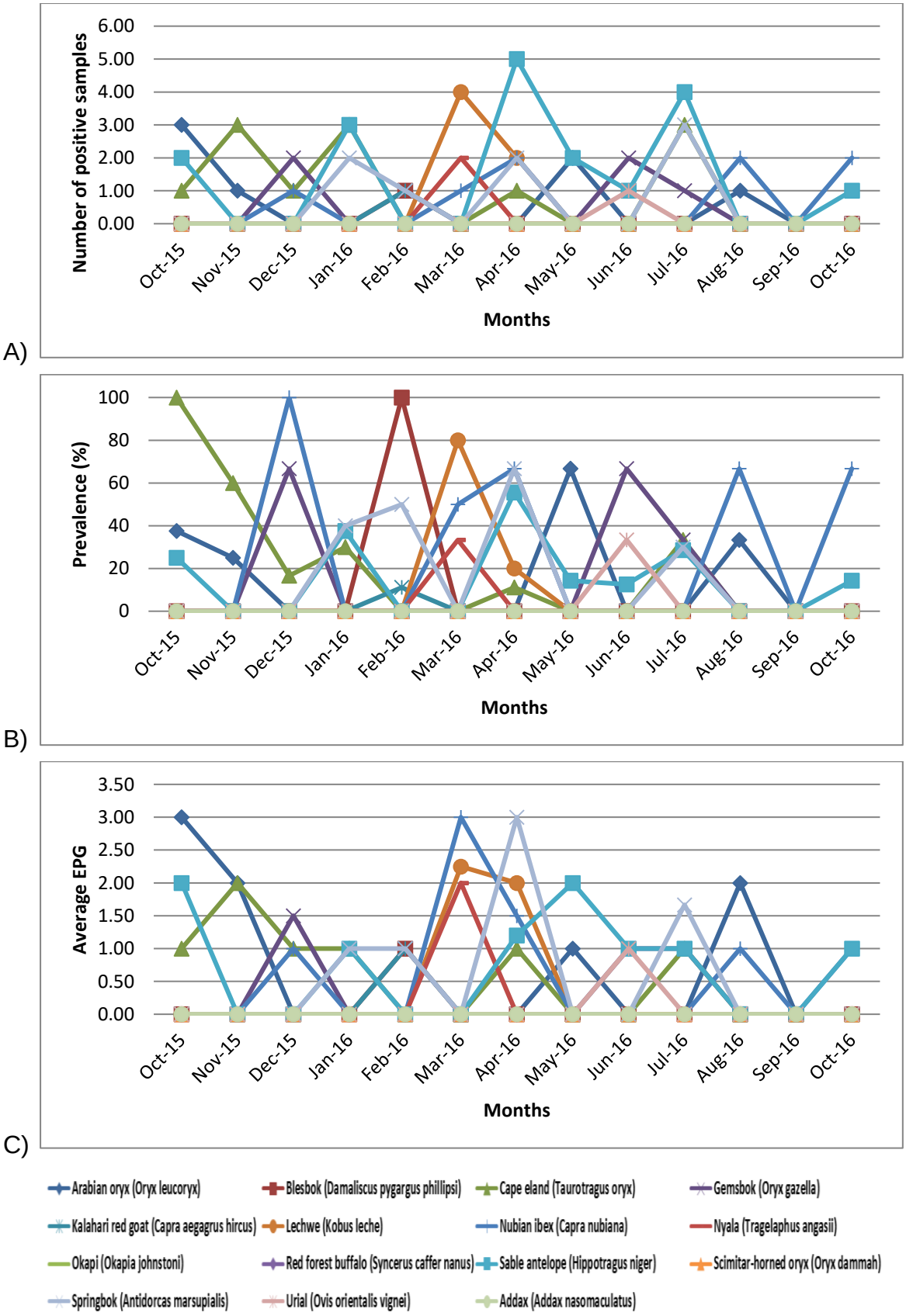


Figure 26: Distribution of coccidia from positive ungulate samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive **coccidia** samples. (B) Percentage prevalence of **coccidia** infections. (C) Average eggs per gram (EPG) of **coccidia**

4.5 Animal species level distribution of GIT parasites among selected ungulates at the NZG

The ungulates had most infections as compared to the two other animal groups. Among the ungulates, five animal species were of importance as they indicated medium to severe infections after every analysis, and these were, the cape eland, sable antelope, springbok, Arabian oryx and lechwe. This section summarises the results showing the distribution of multiple GIT parasites in in individual ungulate species throughout the study period.

4.5.1 Arabian oryx

The Arabian oryx are medium sized antelope, which are highly specialised for harsh desert environment. In the zoo, these animals were housed in very large enclosure which mimics the semi-arid biome. The prevalence of different GIT parasites fluctuated for these animals, as well as their average parasite loads (Figure 29).

Ostertagia sp. was common in the Arabian oryx, and was isolated throughout except in January, March, April and July. The highest prevalence was recorded in September at 100%, in the same month however the parasite load was very low (EPG 100). Another important parasite in these animals was *Haemonchus* sp., although not as common as *Ostertagia* sp., the parasite was observed a number of times throughout the study. The highest egg count was seen in May (EPG 1600), although only 33% of the animals were infected with *Haemonchus* sp., the parasite load was extremely high. *Trichostrongylus* sp. was also isolated from Arabian oryx in this study. The highest prevalence was in November (75.0%), and it co-occurred *Ostertagia* sp. (75.0%) and coccidia (25.0%). Overall, the prevalence of difference GIT parasites in the Arabian oryx were moderate, the average EPGs, remained low (Below 200) with the exception of October 2015 (EPG: 645) and May 2016 (EPG: 1600), and this means although more animals were infected with GIT parasites, the infections were not serious and could not have cause any harm.

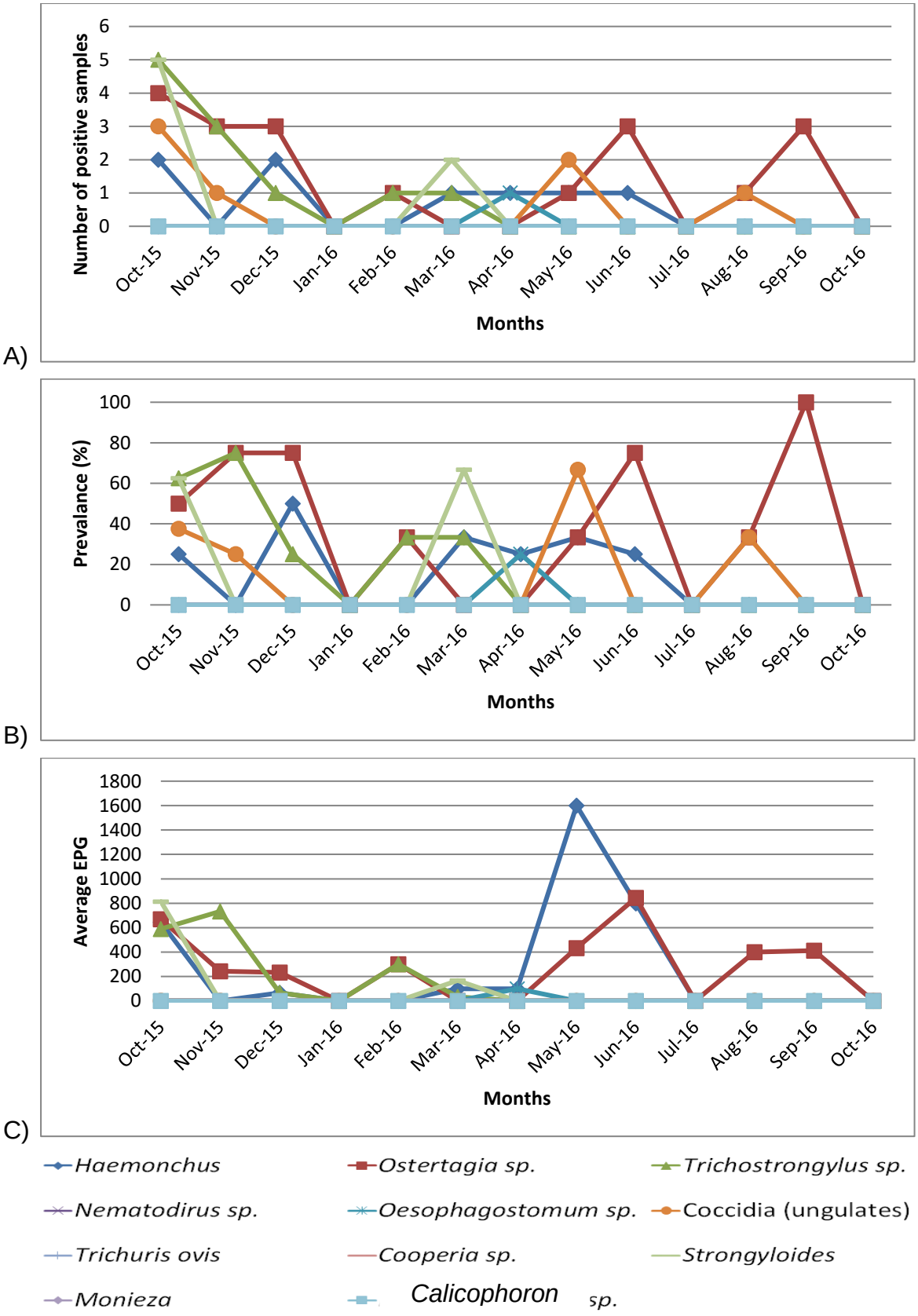


Figure 27: Distribution of GIT parasites from positive of Arabian oryx samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in Arabian oryx. (B) Percentage prevalence of GIT parasite infections in Arabian oryx. (C) Average eggs per gram (EPG) of GIT parasites in Arabian oryx.

4.5.2 Cape eland

Cape eland is a large savannah and plains antelope found in East and Southern Africa. It is said to be the second largest antelope in the world, after the giant eland (Pappas 2002). At NZG, cape eland is in a mixed exhibit enclosure, together with other antelopes such as lechwe, springbok and gemsbok.

The prevalence of GIT parasites in Cape eland was highest in October and November from coccidia and *Haemonchus* sp. infections respectively (Figure 30). A decline was observed in most GIT parasite between December, January and February, where no GIT parasite infections were recorded in the cape elands. A steady increase was noted in March to May; in most of the GIT parasites (*Trichostrongylus* sp., *Strongyloides* sp., and *Oesophagostomum* sp.) however the prevalence of *Haemonchus* sp. continued to increase through until July and then a decline is observed. Average EPG for the Cape eland varied throughout the study period. The highest parasite load was recorded in November followed by March and these were *Haemonchus* sp. infection and June, where Ostertagia sp. infection was noted.

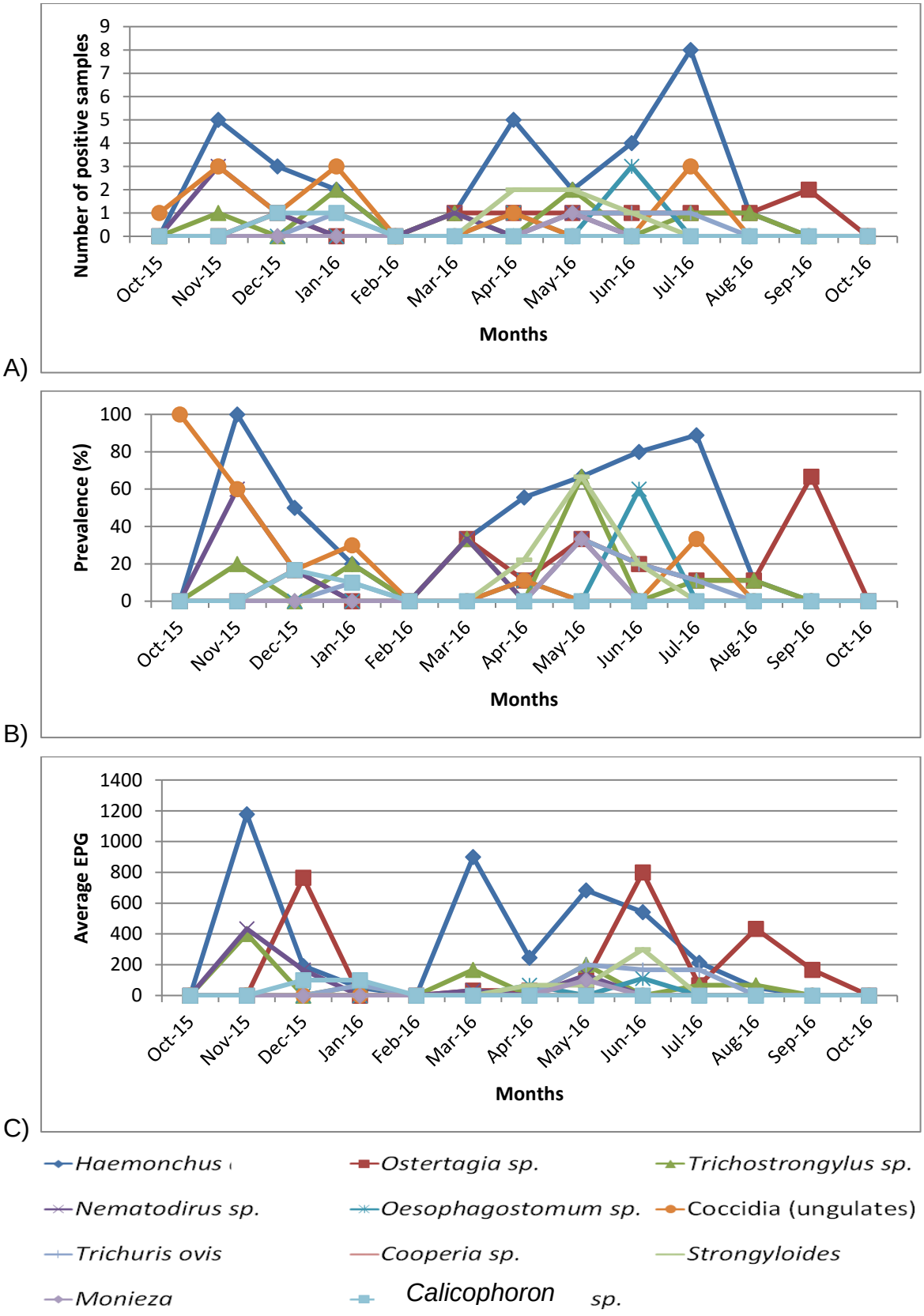


Figure 28: Distribution of GIT parasites from positive of Cape eland samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in Cape eland. (B) Percentage prevalence of GIT parasite infections in Cape eland. (C) Average eggs per gram (EPG) of GIT parasites in Cape eland.

4.5.3 Lechwe

Also known as the southern lechwe, this antelope is found in wetland of south central Africa. It is golden brown with white belly. Males are darker in colour than females, but this varies depending on subspecies. The males have long, spiral horns (Groves & Grubb 2011). At the NZG, these animals are housed in a mixed exhibit enclosure.

Overall, the prevalence of GIT parasite in lechwe was below 30%, with the exception of October 2015 and March 2016 (Figure 31). The highest prevalence was observed in March and this was coccidia infection. In October, co-infection of *Haemonchus* sp. and *Trichuris* sp. was observed in lechwe, this was the second highest prevalence in the lechwe. The average EPG for the lechwe throughout the study period was below the threshold, in February however although the EPG did not go above the threshold, it was at the borderline (*Cooperia* sp. EPG: 583), a mixed infection of *Cooperia* sp. and *Haemonchus* sp. were detected during this month. February was the only incidence of *Cooperia* sp. infections in the lechwe at the NZG.

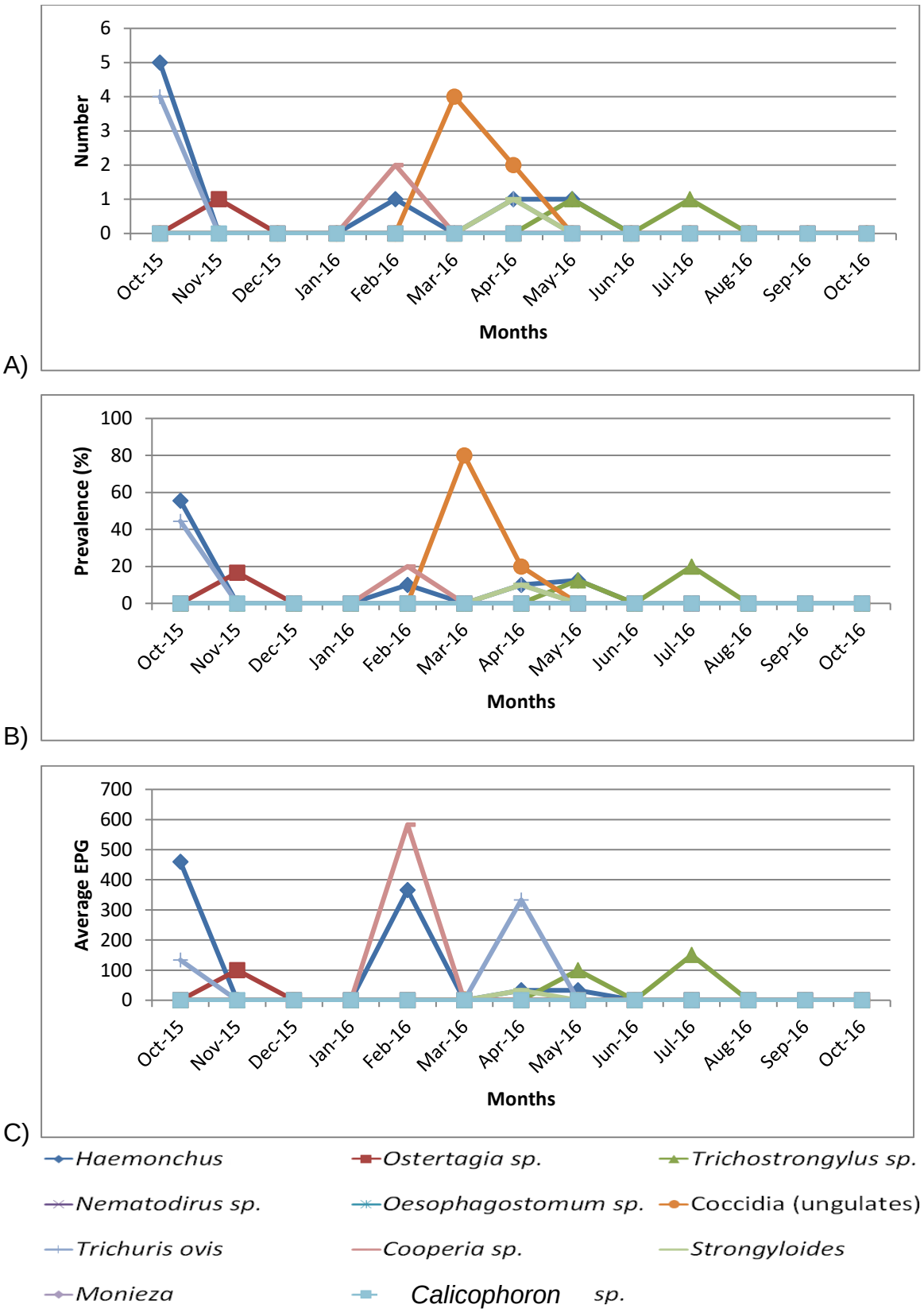


Figure 29: Distribution of GIT parasites from positive of lechwe samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in lechwe. (B) Percentage prevalence of GIT parasite infections in lechwe. (C) Average eggs per gram (EPG) of GIT parasites in lechwe.

4.5.4 Sable antelope

This antelope inhabits the wooded savannah in East Africa south of Kenya and Southern Africa. It is large, characterized by glossy black coats with white under parts and white facial markings. Both sexes have long horns, which are ridged and which are curved backwards (http://www.krugerpark.co.za/africa_sable.html). They are housed in a single exhibit enclosure at NZG, which mimics the in a grasslands biome.

The sable antelope were infected with at least one GIT parasite throughout the study period (Figure 32). Mixed infections of *Haemonchus* sp., *Trichostrongylus* sp., *Nematodirus* sp., *Ostertagia* sp. and coccidia were very common in sable antelope. In February, *Haemonchus* sp. was the only GIT parasite detected in the sable antelope, with 100% prevalence. In March, the prevalence of *Haemonchus* sp. in sable antelope decreased to 60%, an increase was observed in April. A decrease in prevalence of *Haemonchus* sp. in July was observed in sable antelope. August was the only month where *Haemonchus* sp. was not detected in sable antelope. A peak of *Nematodirus* sp. infection was observed in June. *Ostertagia* sp. infections in sable were observed in August, this was the highest prevalence for the *Ostertagia* sp. The overall average EPG of GIT parasite infections in sable antelope was low for other GIT parasites, except *Haemonchus* sp. and *Nematodirus* sp., which were above the threshold in November and August respectively.

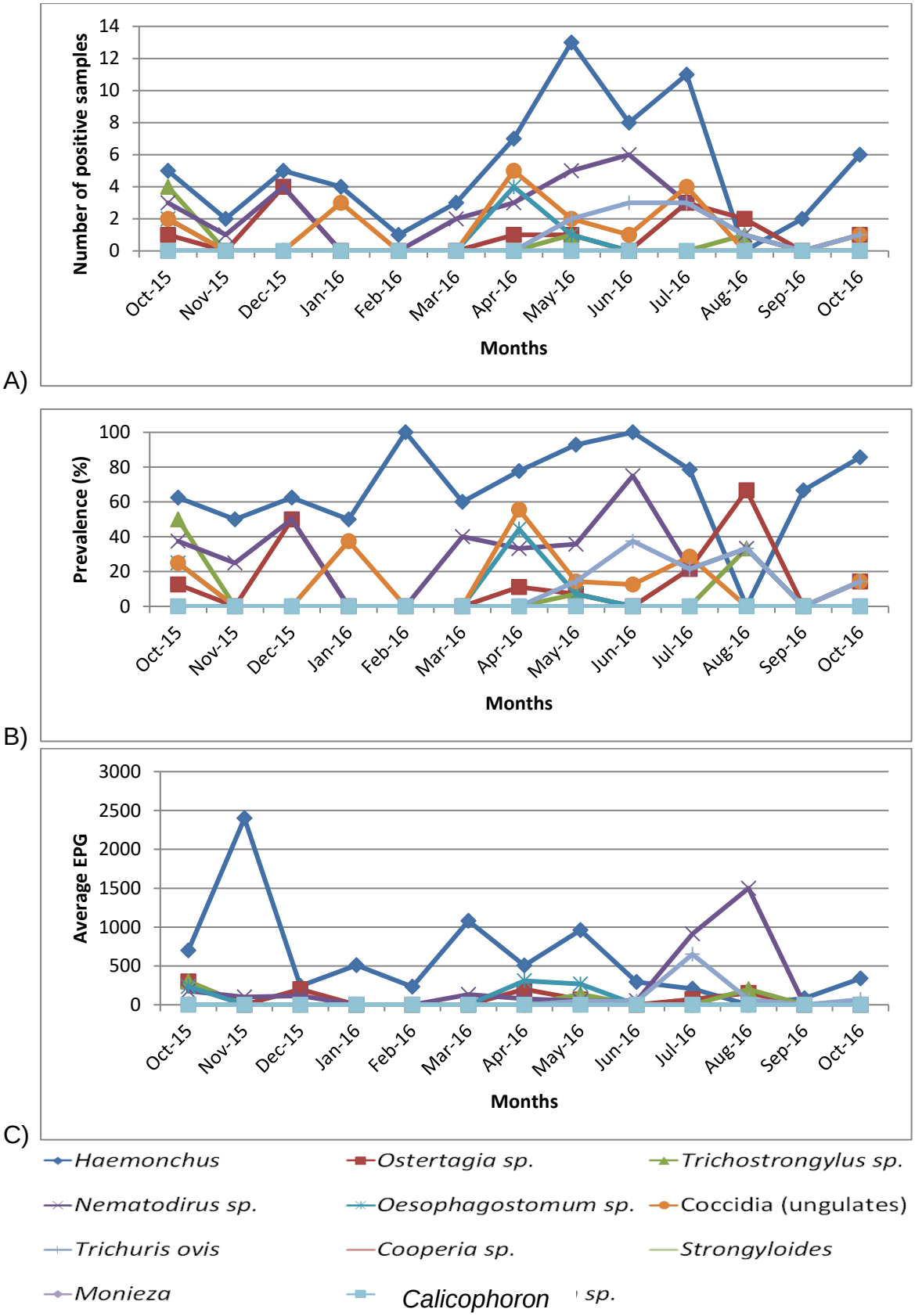


Figure 30: **Distribution of GIT parasites from positive of sable antelope samples tested at the National Zoological Gardens of South Africa.** A) Seasonal distribution of positive GIT parasites in sable antelope. (B) Percentage prevalence of GIT parasite infections in sable antelope. (C) Average eggs per gram (EPG) of GIT parasites in sable antelope.

4.5.5 Springbok

The medium-sized antelope is found mainly in southern and southwestern Africa. It is slender, long-legged antelope can grow up to 86 cm. They have striking body colour which renders them easily recognizable. Their diet comprises of both leaves and grass, as they browse in dry season and graze after the rainy season when grass sprout green (http://www.krugerpark.co.za/africa_springbok.html).

The prevalence of GIT parasite in springbok was not uniform or rather inconsistent throughout the study (Figure 33). Overall, the prevalence was above 30%. The highest peaks were noted in November, February and June, where the readings were at 100%. In February, in the springbok were detected 100% prevalence of *Cooperia* sp., *Haemonchus* sp., other infections were noted in the same month, although lower prevalence was recorded and these were coccidia and *Nematodirus* sp. infections. Average EPG in springbok was below the threshold throughout the study period, except in February 2016 (*Haemonchus* sp. EPG: 867), June 2016 (*Haemonchus* sp. EPG: 2100) and August 2016 (*Ostertagia* sp. EPG: 600).

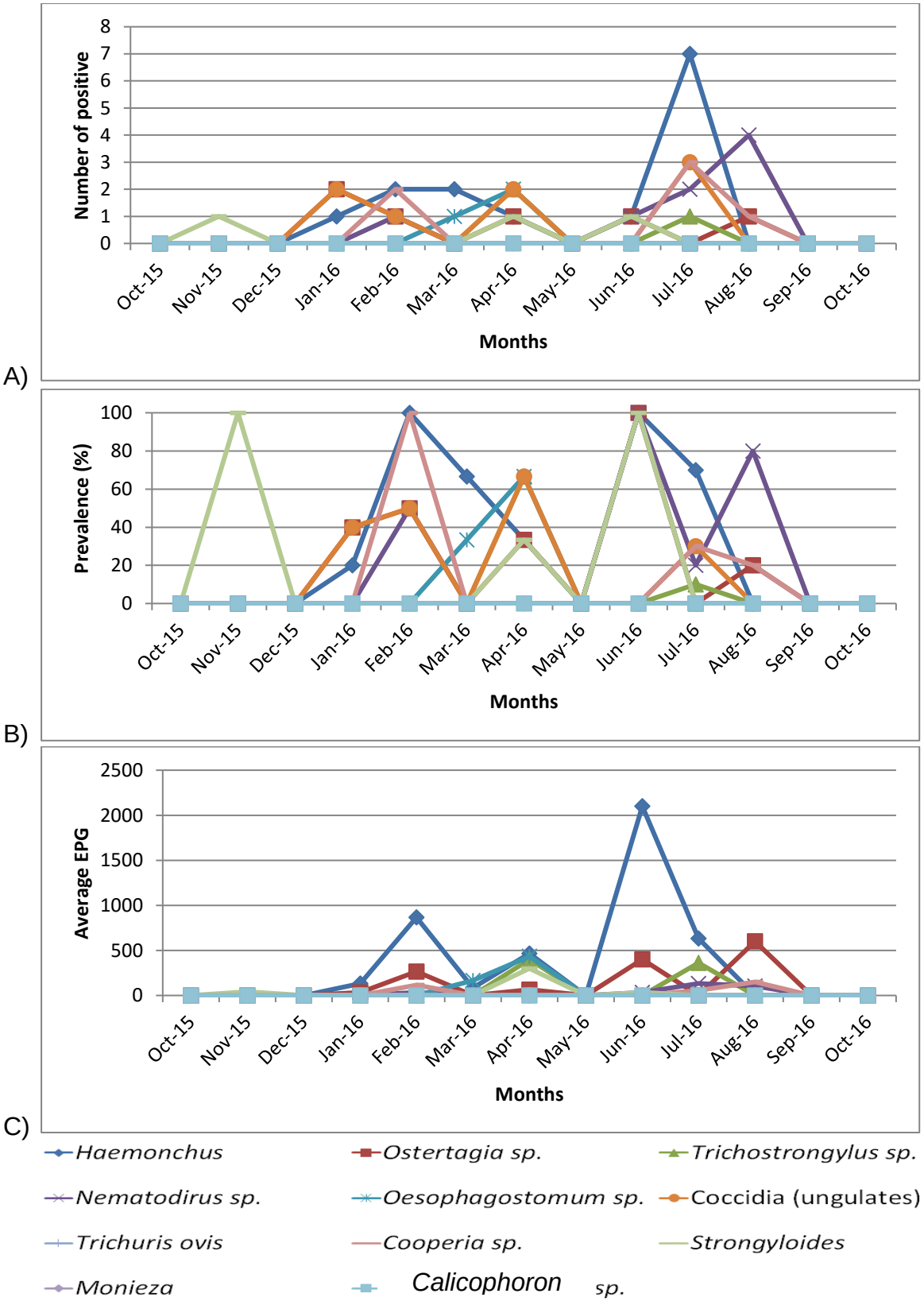


Figure 31: Distribution of GIT parasites from positive of springbok samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in springbok. (B) Percentage prevalence of GIT parasite infections in springbok. (C) Average eggs per gram (EPG) of GIT parasites in springbok.

4.6 Distribution of GIT parasites across selected captive felids at the NZG

Microscopic results revealed continuous distribution of GIT parasites in felids throughout the course of the study with the exception of November and January (Figure 34) where no GIT parasites in the felids were recovered. Prevalence of GIT parasite in felids was highest in May followed by June and September. Overall, the prevalence of GIT infections in felids was below 40%. The highest prevalence was in May 2016 and October 2016 (66.67%) followed by April 2016 (60.0%) and March 2016 (40.0%).

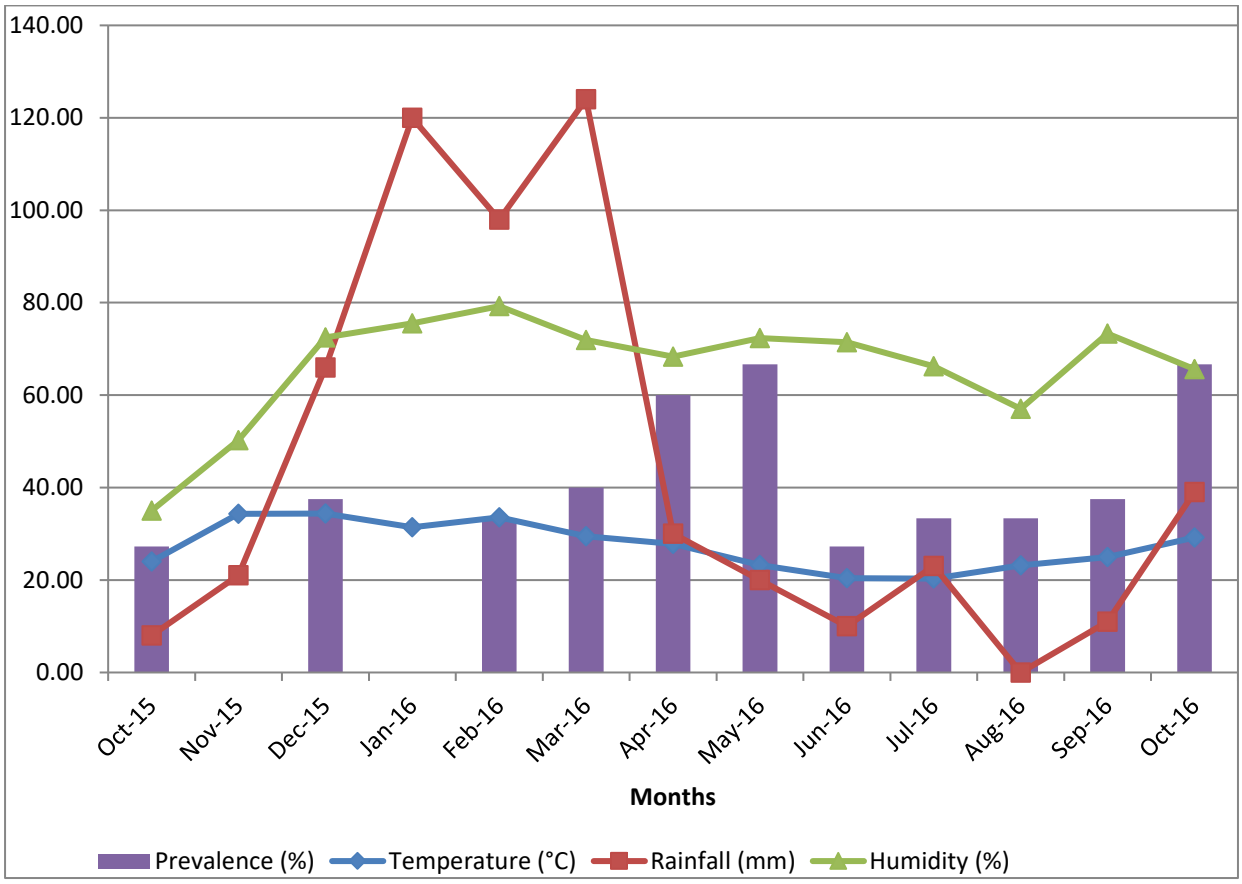


Figure 32: Prevalence of GIT parasites in felids at the NZG

The only GIT parasites recovered in felids were *Toxascaris sp.*, *Toxocara* sp. and coccidia. *Toxascaris* sp. was a common parasite in the felids; it was detected throughout the course of the study with the exception of November and January. The highest parasite load observed was in March (EPG: 2258; Figure 35), in the same month the prevalence actually dropped 57% (Figure 44), and this is troubling because

this means there were fewer samples positive for the parasite but the infection load was extremely high.

In June, the parasite load was very low (EPG: 144) as compared to other months, the prevalence on the other hand the prevalence was high, meaning although more felids were infected with *Toxascaris* sp., the infection was low or not serious. In July, August and September all samples tested were positive (100% prevalence) for *Toxascaris* sp., in those months the EPG count decreased gradually. This means, although all felids tested positive, the infection load was under control as the infection was decreasing.

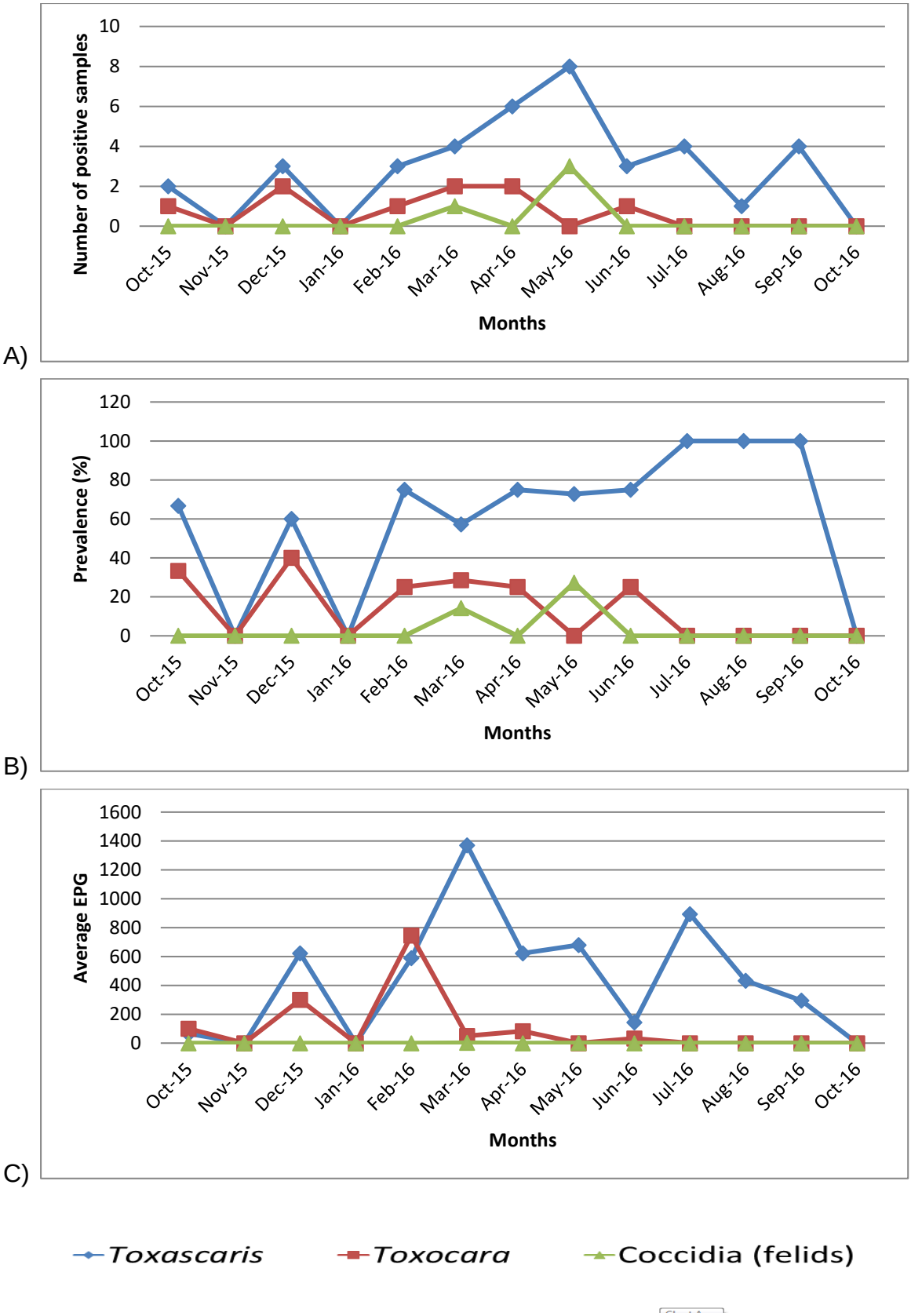


Figure 33: Distribution of GIT parasites from positive of felids samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in felids. (B) Percentage prevalence of GIT parasite infections in felids. (C) Average eggs per gram (EPG) of GIT parasites in felids.

4.5.1 *Toxascaris* species.

Toxascaris leonina is an ascarid nematode which inhabits the small intestines of both of domestic and wild canids and felids. These parasitic worms are zoonotic and therefore can infect humans (Minnaar *et al*. 2002). The parasite has worldwide distribution, but most prevalent in subtropical regions, which facilitates development of the egg in the external environment. As a general rule they are more abundant in rural regions (Sheng et al. 2012), it occurs in both canids and felids but more frequent in felids. *Toxascaris* sp. was the most common GIT parasite in felids for this study, was recovered for 10 months of the 13 months of the study.

Overall, the prevalence was above 50% throughout the study across all the felids, the infection load of *Toxascaris* sp. in felids was high, unlike the ungulates whereby these a threshold of 600 meaning ungulates can withstand an infection as high, with the felids, the opposite is observed. In the felids, an infection as low as EPG: 200 can have an impact on the animal species. *Toxascaris* sp. infection went as high as EPG: 2258 in March 2016. March, May and July 2016 were important as more than one animal species (lion and cheetah) of the felids was infected with *Toxascaris* sp. The prevalence of *Toxascaris* sp. was much higher in the African lion, especially the cubs, than in the cheetahs. Throughout the study, the parasite was detected, with the exception of November, January and October 2016 (Figure 36).

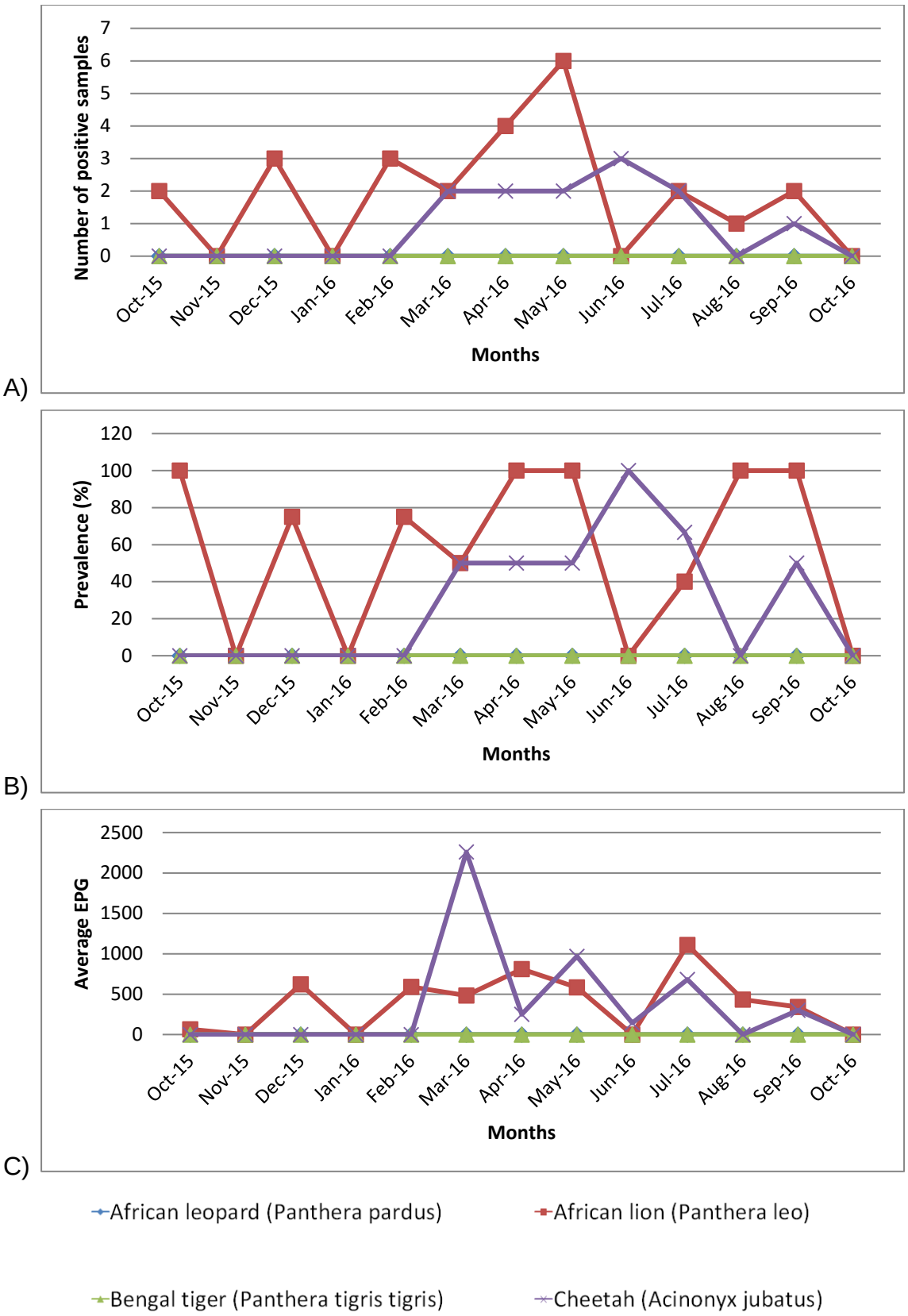


Figure 34: Distribution of *Toxascaris* sp. from positive felids samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Toxascaris* samples. (B) Percentage prevalence of *Toxascaris* sp. infections. (C) Average eggs per gram (EPG) of *Toxascaris* sp.

4.5.2 *Toxocara* species

It is believed to be the most encountered gastro-intestinal parasite in cats, Tox. *Toxocara cati* is a close relative of the *Toxocara canis* which infects the dogs. Although *T. cati* is very common round worm it does not affect livestock such as cattle, sheep, goats and horses. *Toxocara cati* may feed on body fluids depending on development stage and location of infestation. Infection can occur by host infesting viable, embryonated eggs from contaminated sources or can acquire infection from transplacentally from the infected mother when they ingest infective eggs (Despommier 2003).

Toxocara sp. was isolated in five months of the 13 months of sampling. The only incidence where the *Toxocara* sp. parasite load was higher than *Toxascaris* sp. was in October 2015, and the difference was small and again in February. Throughout, the parasite remained low-key, the highest peak was in February (EPG: 745; Figure 37). The prevalence of the parasite remained below 40% (Figure 37).

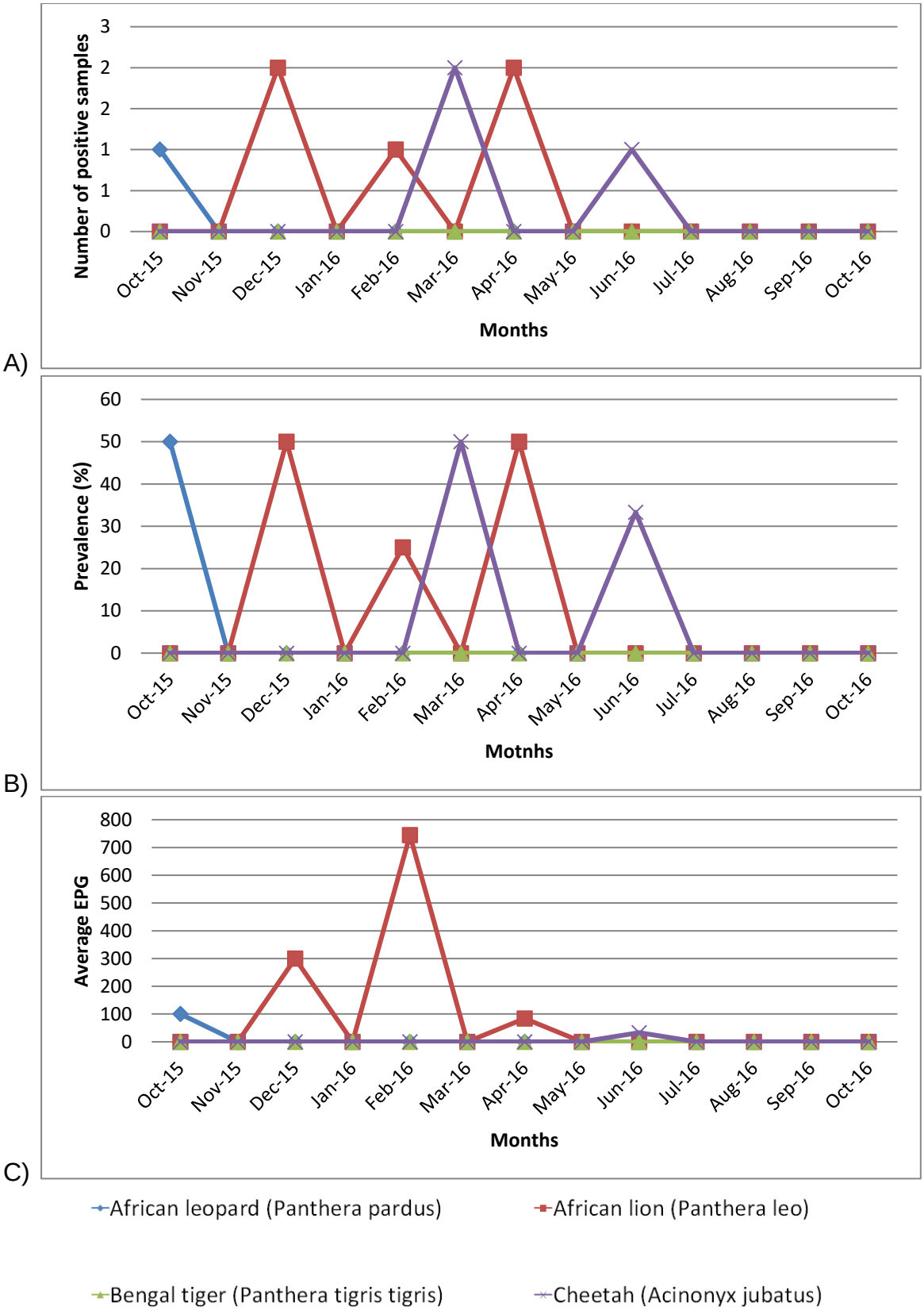


Figure 35: Distribution of *Toxocara* sp. from positive felids samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Toxocara* sp. samples. (B) Percentage prevalence of *Toxocara* sp. infections. (C) Average eggs per gram (EPG) of *Toxocara* sp.

4.5.3 Coccidia

There were two incidences of infections with coccidia in felids throughout the study, and these infections were observed in March 2016 and May 2016, infecting the cheetah and African lion respectively. Although the number of cheetahs infected with coccidia was low, the parasite load was actually higher, as compared to the African lions. More African lions were infected with coccidia, but the parasite load was much lower (Figure 38). Coccidia was an occasional parasite in the felids and it was only isolated in March (25.0%) and May (50.0%).

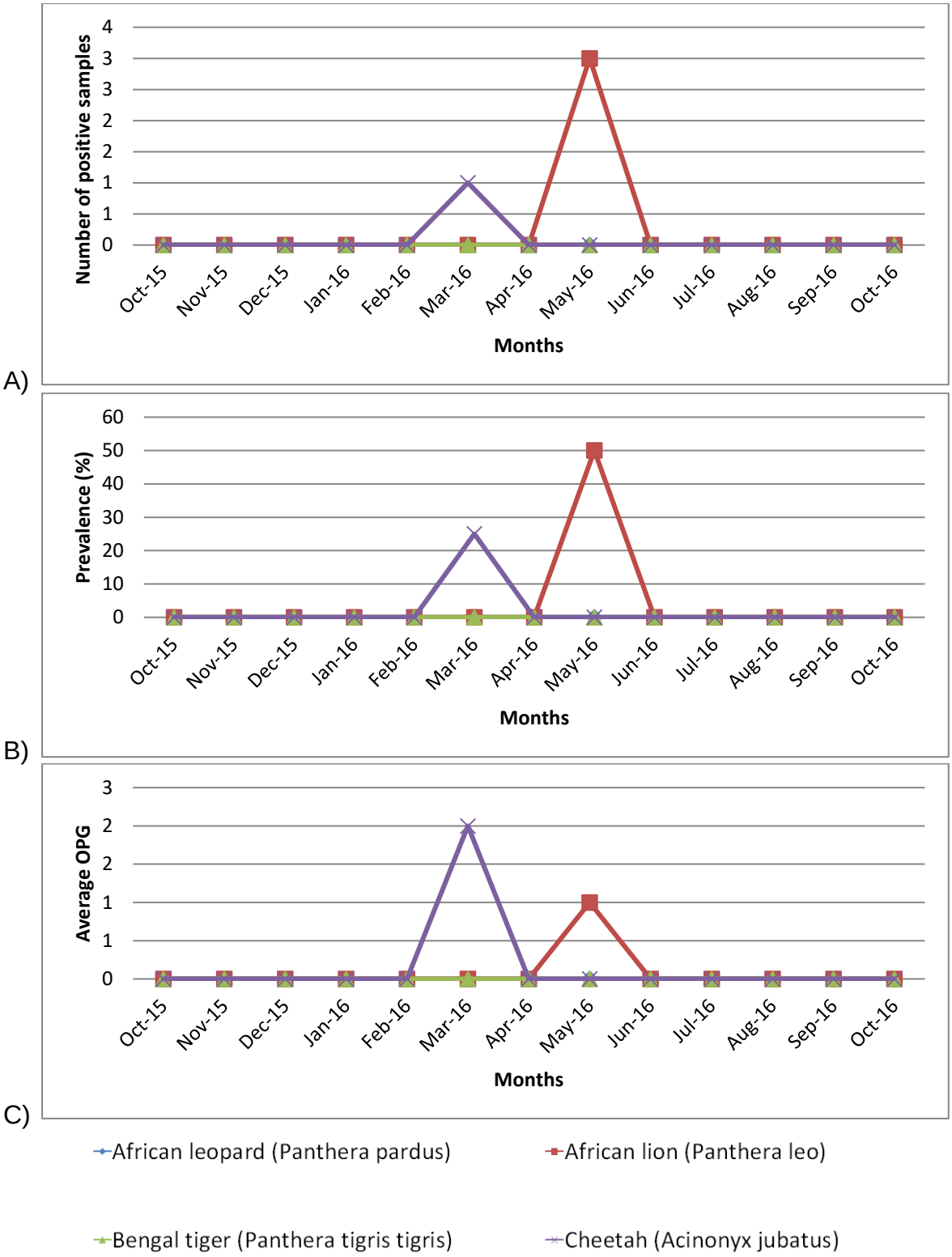


Figure 36: Distribution of coccidia from positive felids samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive coccidia samples. (B) Percentage prevalence of coccidia infections. (C) Average oocyst per gram (OPG) of coccidia.

4.6 Animal species level distribution of GIT parasites among selected felids at the NZG.

For the present study, four species were analysed from the felids and these are the African lion, African leopard, Cheetah and the Bengal tiger. Throughout the study, all these animals showed varying levels of infections. *Toxascaris* sp. was observed in African Lion and the Cheetah, while *Toxocara* sp. was isolated once in the African leopard and rarely seen in the other animal species.

The African leopard had a single infection, which was in October 2015; the animal was infected with *Toxocara* sp. Although there was 100% prevalence, the parasite load was low, and there could be considered less important. The African leopard at NZG was not susceptible to GIT parasite infections.

Gastro-intestinal infections in Bengal tigers were non-existent. The only incidence were there was suspected infection was in March and it turned out to be pollen grain in the faecal matter. No GIT parasites were ever isolated in these animals throughout the study.

4.6.1 African lion

Infections of GIT parasite where continuous in African lions with the exception of November 2015, January 2015 and June 2016. Throughout the study, there were no cases where all three GIT parasites of the felids were detected all at once. It was either a mixed infections of *Toxocara* sp. and *Toxascaris* sp. or *Toxascaris* sp. and coccidia but never all at once. Most parasite load was observed in July 2016 followed by April 2016 and December 2015 (Figure 39).

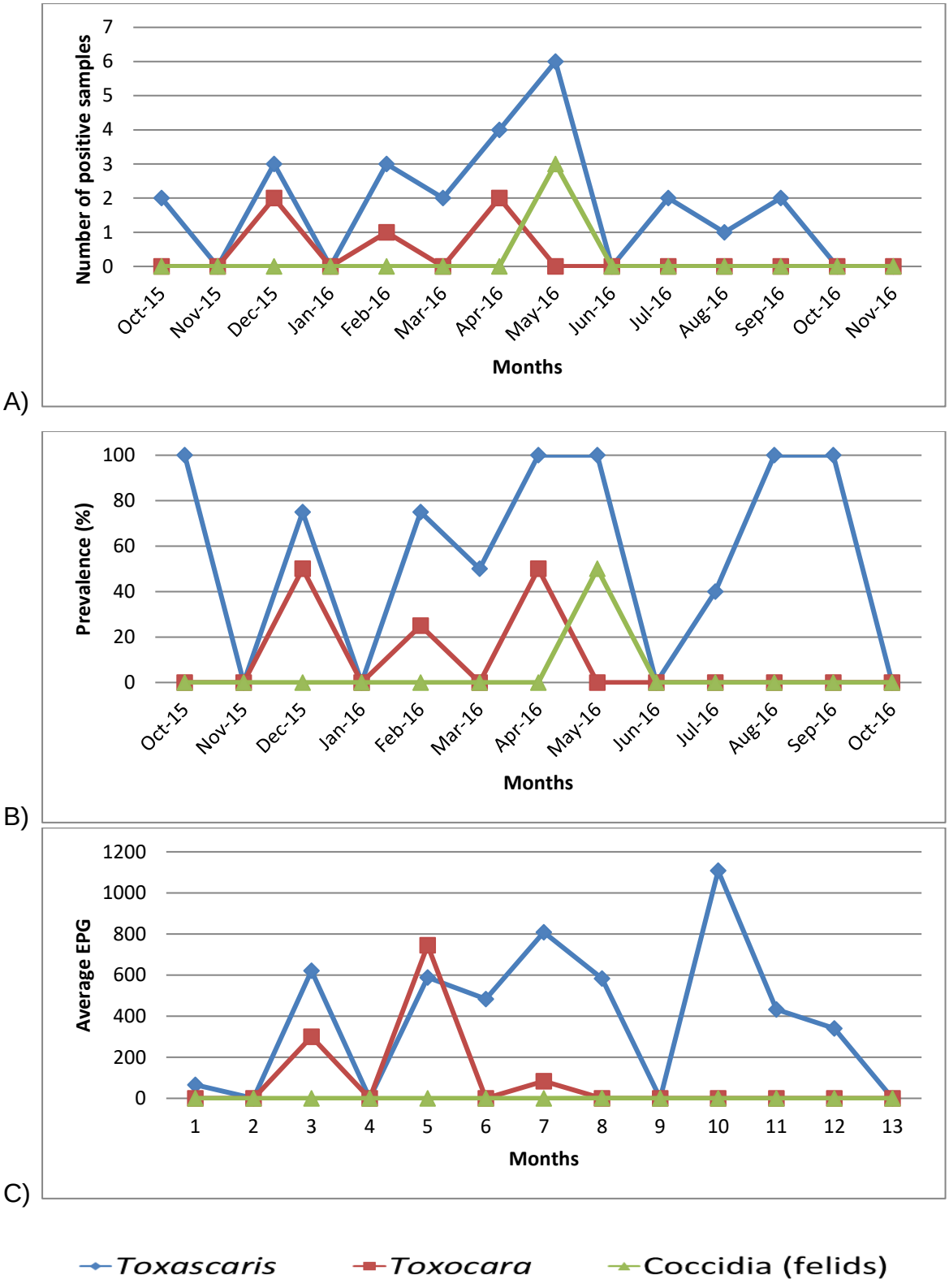


Figure 37: Distribution of GIT parasites from positive of African lion samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in African lion. (B) Percentage prevalence of GIT parasite infections in African lion. (C) Average eggs per gram (EPG) of GIT parasites in African lion.

4.6.2 Cheetah

In the cheetah, the infection of *Toxascaris* sp. was non-existent for the first five months (Figure 40). In March a prevalence of 50% was observed, this remained constant for the next two months. The infection of *Toxascaris* sp. was in March; on the same month what is to be highest record of average parasite load (EPG: 2258) of *Toxascaris* sp. infection was noted. A 100% prevalence of *Toxascaris* sp. was observed in the cheetahs in June.

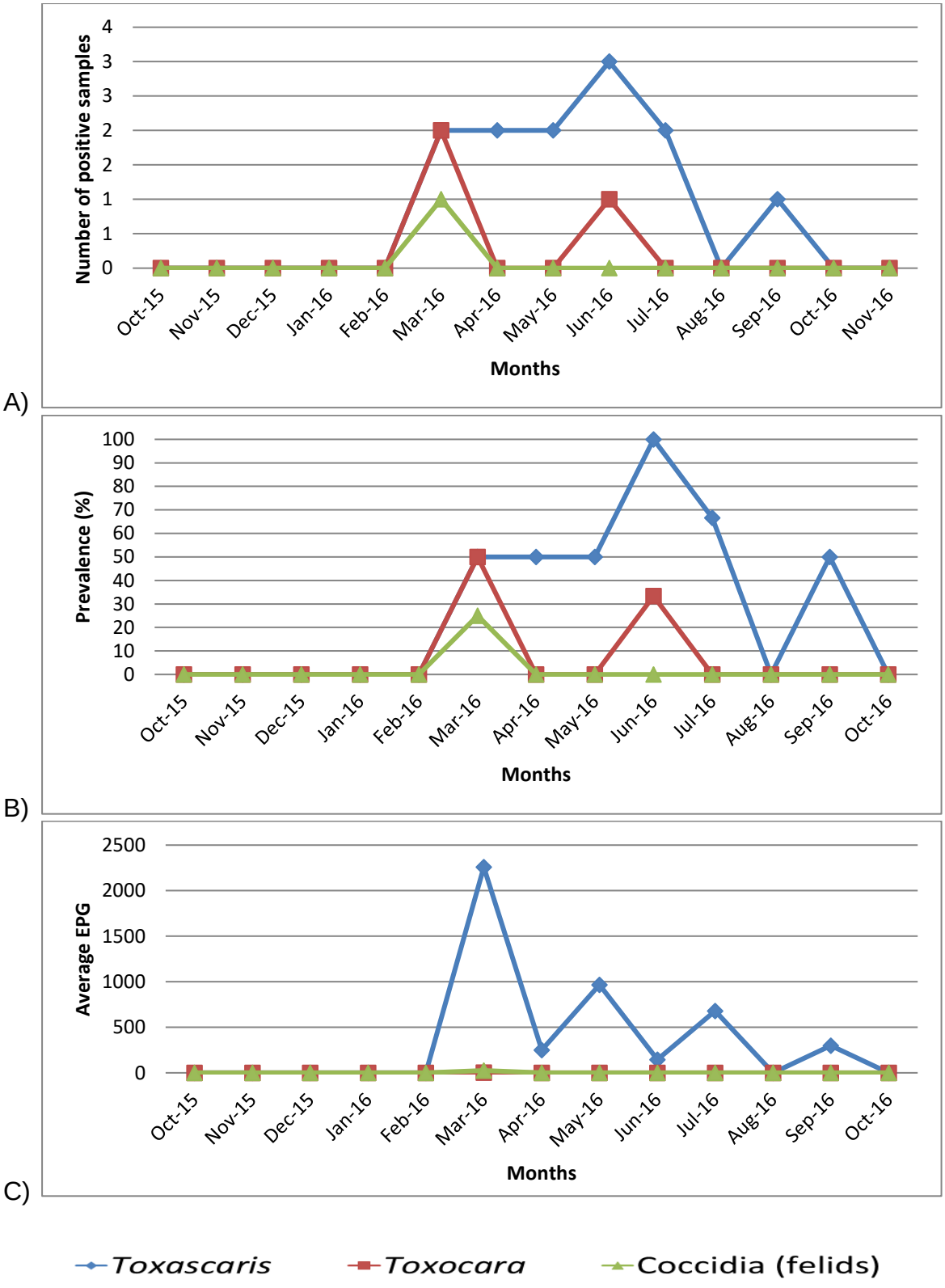


Figure 38: Distribution of GIT parasites from positive of cheetah samples tested at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in cheetah. (B) Percentage prevalence of GIT parasite infections in cheetah. (C) Average eggs per gram (EPG) of GIT parasites in cheetah.

4.7 Distribution of GIT parasites across selected captive avian species at the NZG

The distribution and prevalence of GIT parasites in avian species was not uniform throughout the course of the study (Figure 41)

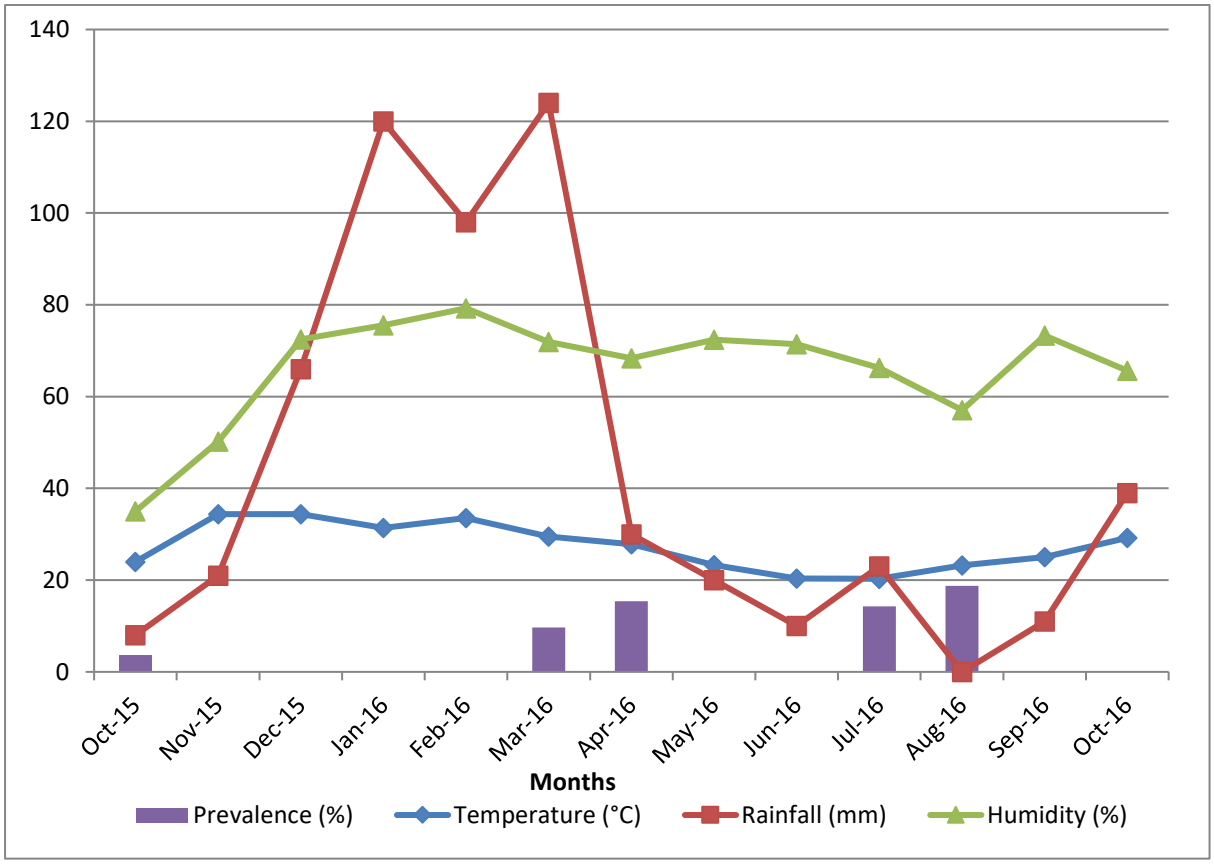


Figure 39: Monthly prevalence of GIT parasites in avian species at NZG

The highest prevalence was recorded in August (18.75%) followed by April (15.38%) and July (14.29%). There were no GIT parasites recorded in November 2015, December 2015, January, February, May, June, September and October 2016. *Capillaria* sp. was common and was detected from March to August, with the exception June and July. The highest peak was in May (DC: 85). A single case of *Heterakis* sp. (DC: 3.0) was recorded, this was in April (Figure 42). Strongyle egg type was not common in avian species, was isolated in October, March and August and the highest peak was recorded in March (DC: 9), followed by August (DC: 6) (Figure 42).

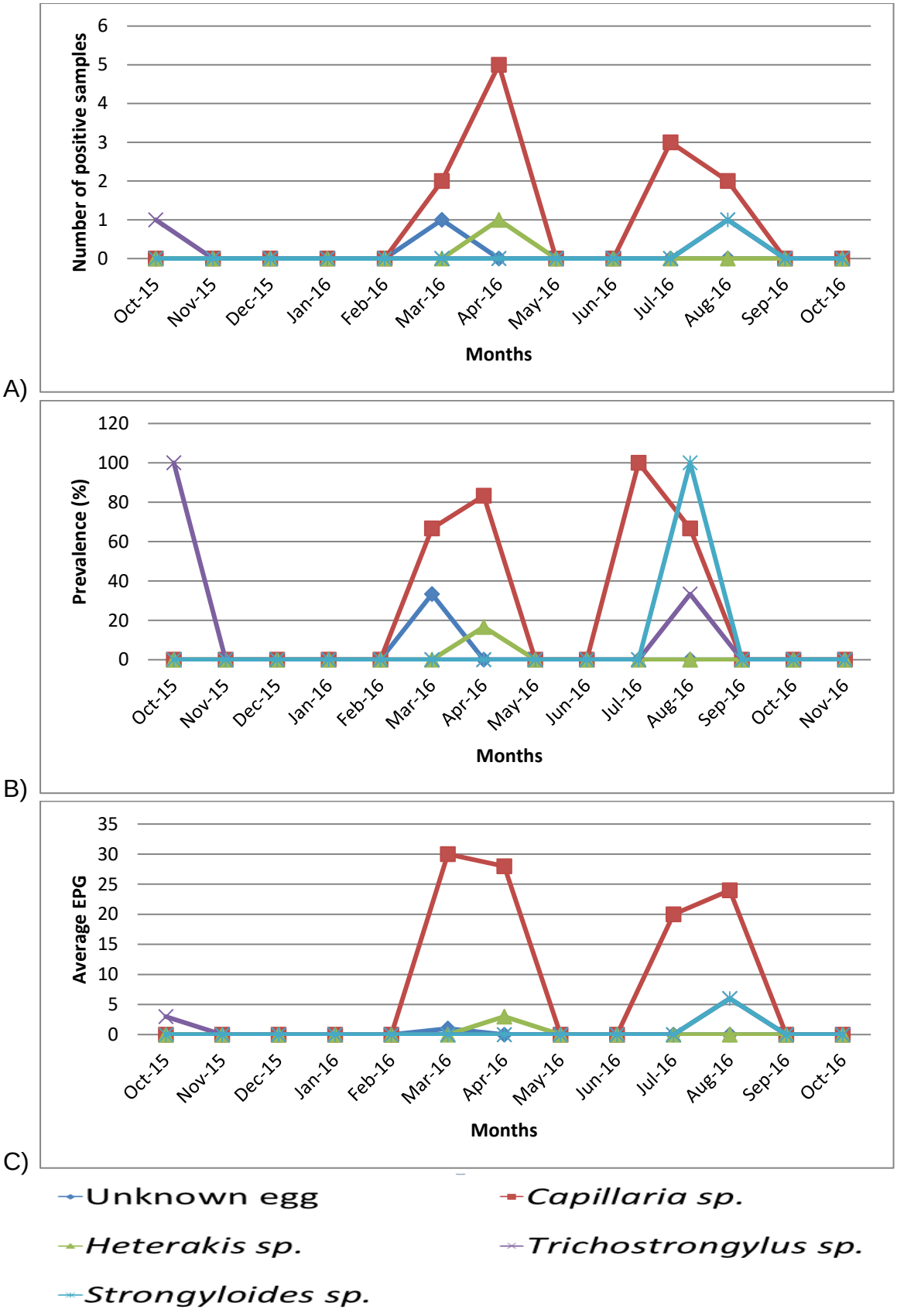


Figure 40: Distribution of GIT parasites from positive of avian species samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in avian species. (B) Percentage prevalence of GIT parasite infections in avian species. (C) Average eggs per gram (EPG) of GIT parasites in species.

For the present study, a wide variety of species were tested from the avians and these are the Illigers macaw, common ostrich, blue fronted amazon, cape vulture and domestic among a few. Throughout the study, all these animals showed varying levels of infections. The avian species were the least infected animal group as compared to the ungulates and felids.

4.7.1 *Capillaria* species and *Trichostrongylus* species

Capillaria sp. worms of birds are known as hairworms because of their extreme thinness in size, are divided into two groups: those that burrow into the epithelium of the upper digestive tract and those that burrow into the epithelium of the lower digestive tract (Park & Shin 2010). *Capillaria* sp. is rarely found and apparently does not affect the host's health (Mawson 1985).

Capillaria sp. was the most common GIT parasite in the avian species (Figure 43). The most important month for *Capillaria* sp. was April 2016 where it was five avians (Blue fronted amazon, Scalet ibis, Sun conure, Illigers macaw and Blyth hornbill). The highest egg count for April 2016, DC: 78.

Trichostongylus species

Strongyle egg of the avian species, it inhabits the caeca of avian hosts. Although, Trichostrongylus sp. were originally described in Europe from the ring necked pheasant, it has subsequently been recored in a range of hosts including the bobwhite quail, English partridge, turkey and guinea fowl and various species of ducks and geese (Watson 1989).

Trichostongylus sp. on other hand was less common (Figure 44), detected only twice, and on both occasions the parasite load was below DC: 10.

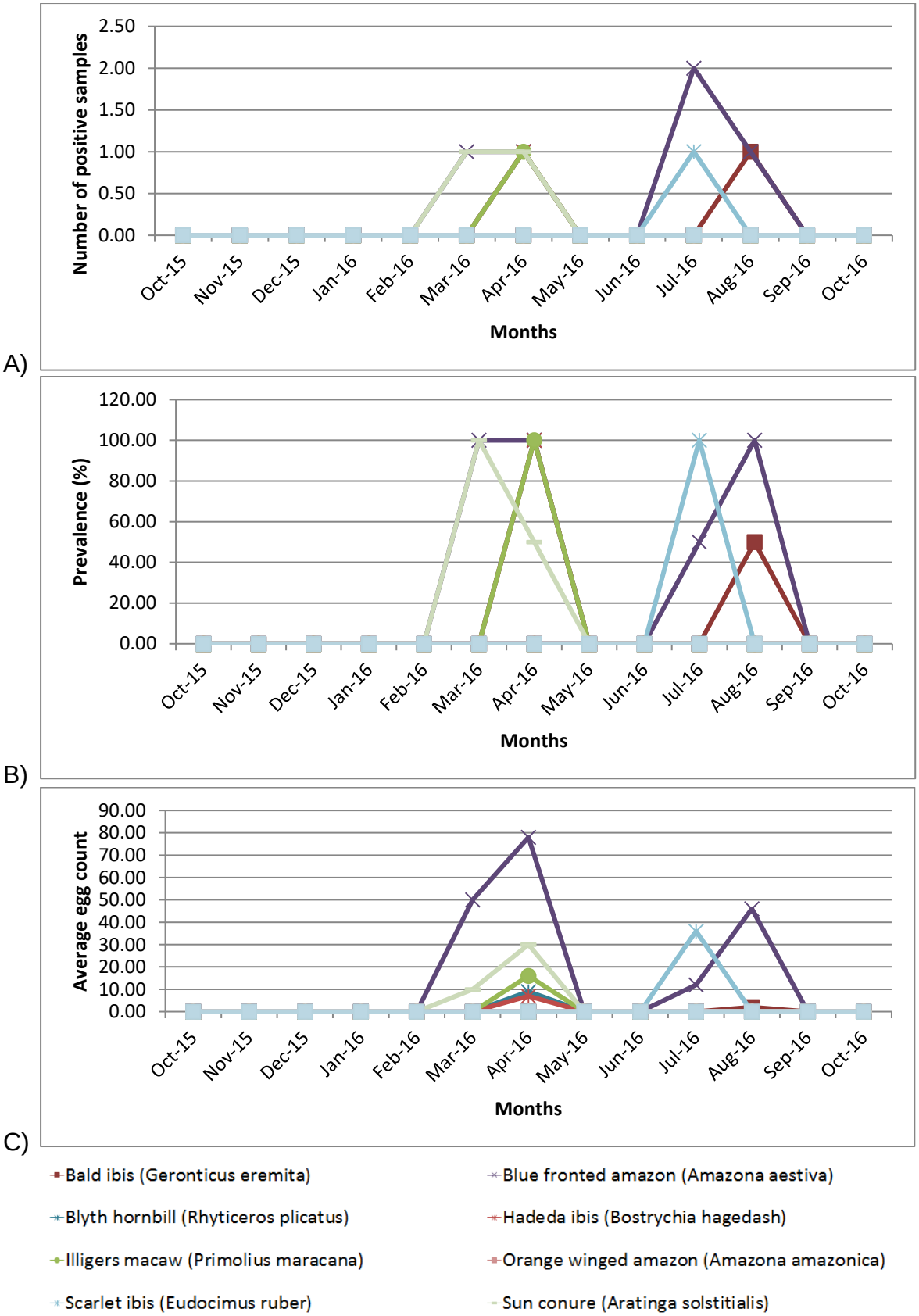


Figure 41: Distribution of *Capillaria* sp. from positive of avian species samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Capillaria* sp. in avian species. (B) Percentage prevalence of *Capillaria* sp. infections in avian species. (C) Average eggs per gram (EPG) of *Capillaria* sp. in species.

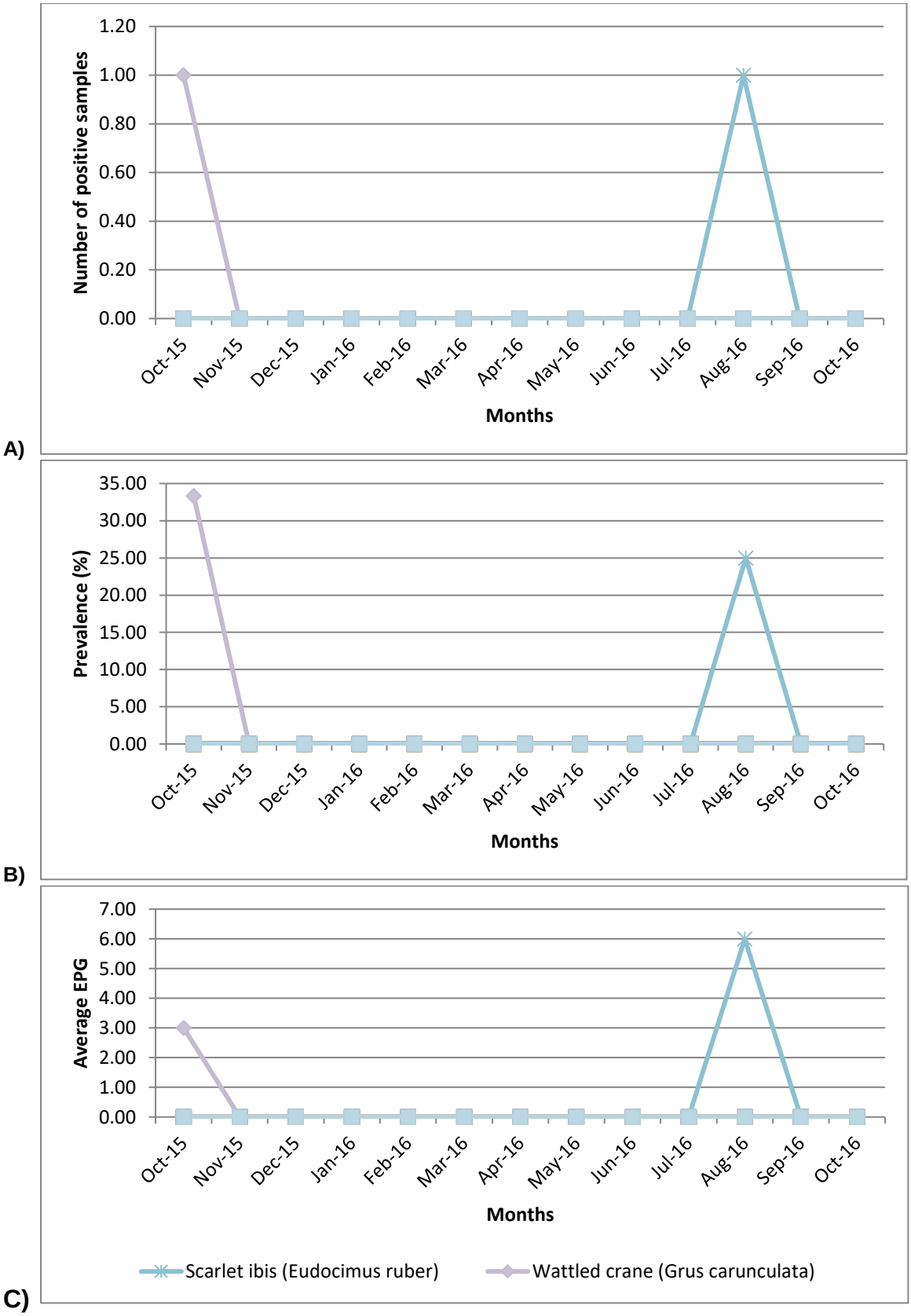


Figure 42: Distribution of *Trichostrongylus* sp. from positive of avian species samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive *Trichostrongylus* sp. in avian species. (B) Percentage prevalence of *Trichostrongylus* sp. infections in avian species. (C) Average eggs per gram (EPG) of *Trichostrongylus* sp. in species.

4.6 Animal species level distribution of GIT parasites among selected avian species at the NZG.

The avian species had low levels of infections thoughout the study. Blue fronted amazon (Figure 45) was infected with *Capillaria* sp., the first infection was in March 2016. Blue fronted amazon was the only avian species which was infected more than three times in the study. The highest infection was April 2016, with a DC: 78, followed by March 2016 DC: 50.

Scarlet ibis is the only avian species which was infected with two different GIT parasites (Figure 46), although they were on two separate occasions, *Capillaria* sp. in July 2016 and *Trichostrongylus* sp. in August 2016. In both cases, there was only one sample infected, however the infection for *Capillaria* sp. was much higher than that of *Trichostrongylus* sp.

Sun conure was infected with *Capillaria* sp. on both occasions (Figure 47), the first infection was in March 2016 followed by April 2016. The second infection was three times higher than the first infection in March 2016.

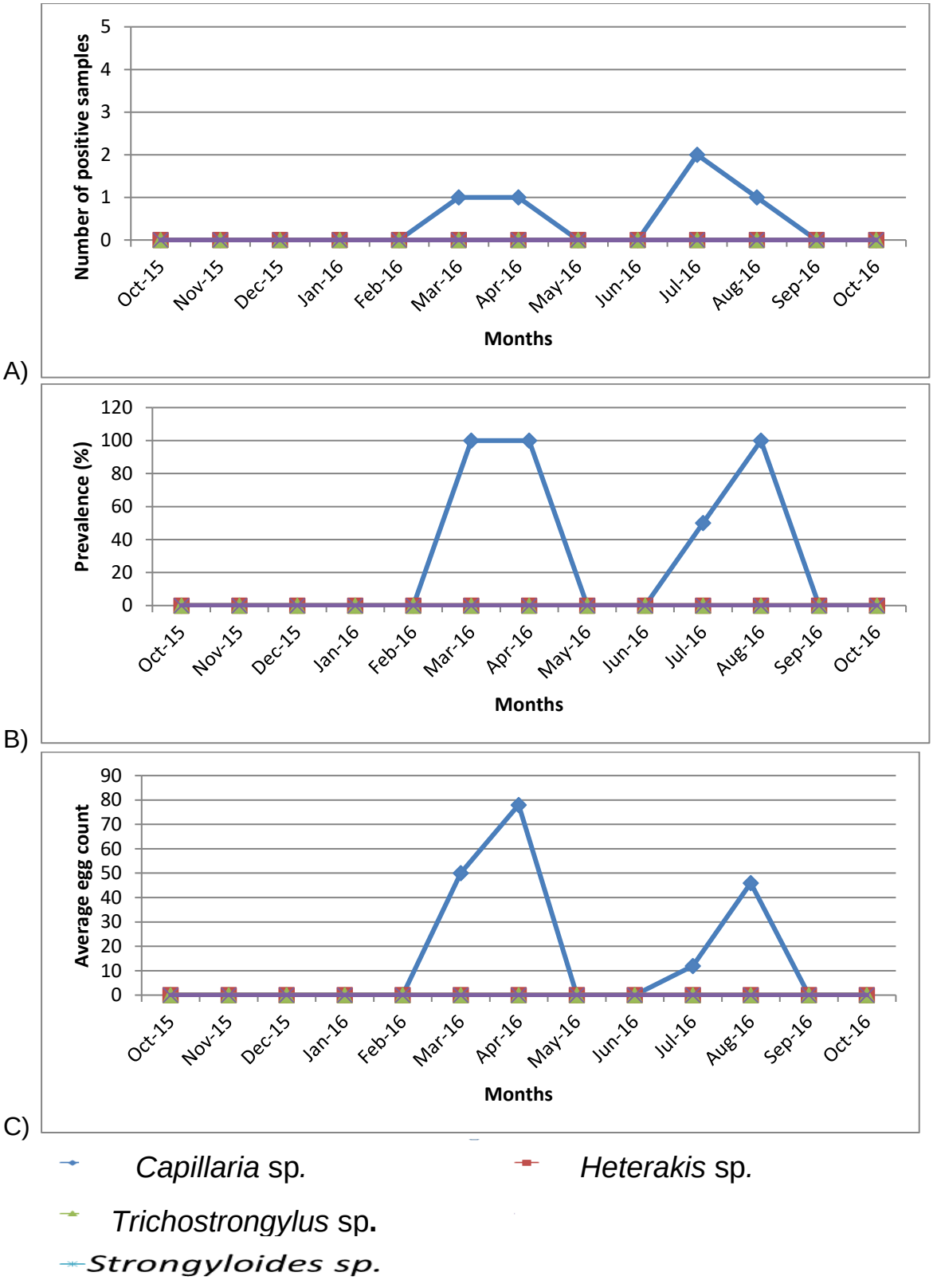


Figure 43: Distribution of GIT parasites from positive Blue fronted amazon samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in Blue fronted amazon. (B) Percentage prevalence of GIT parasite infections in Blue fronted amazon. (C) Average egg count of GIT parasites in Blue fronted amazon.

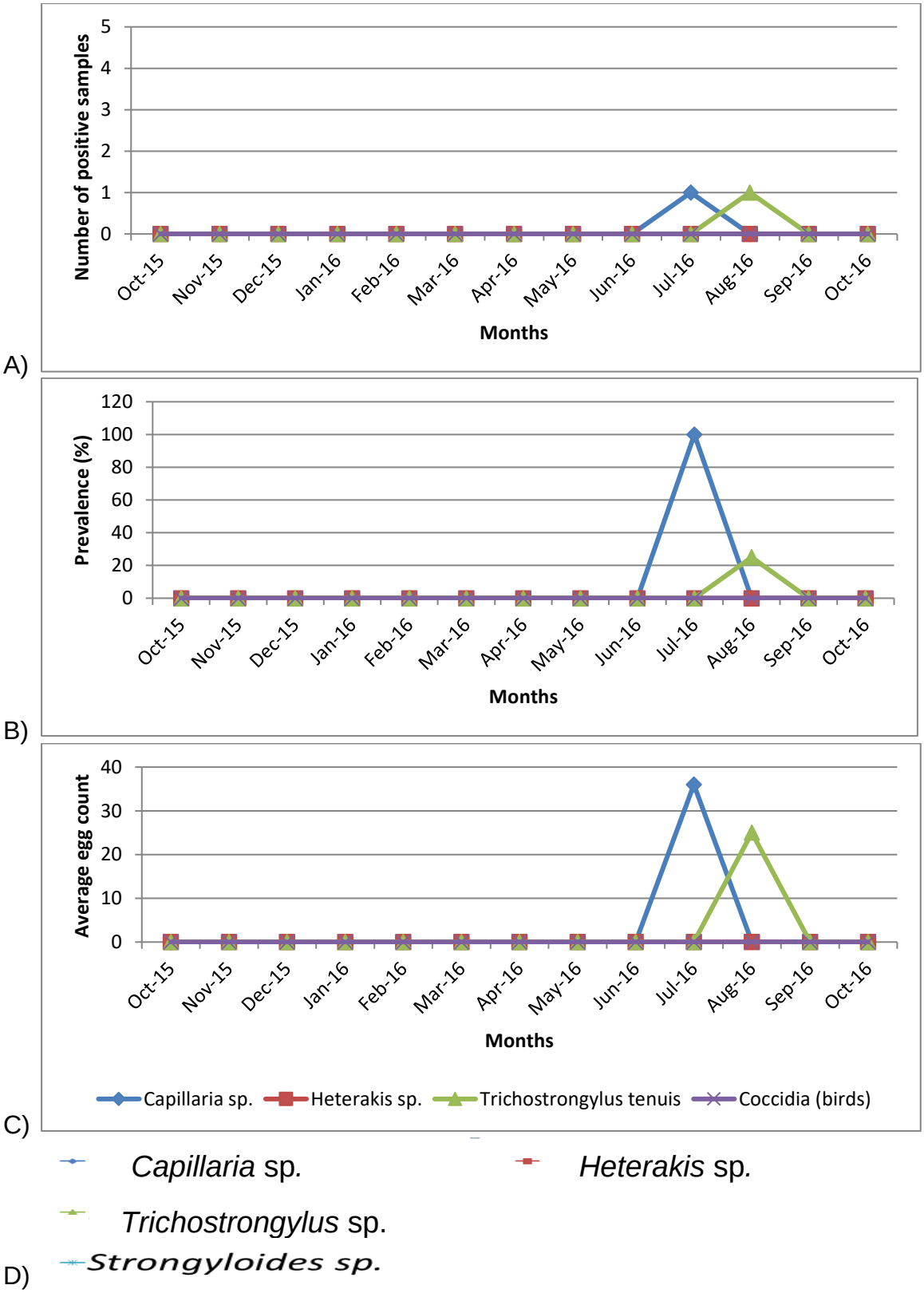


Figure 44: Distribution of GIT parasites from positive Scarlet ibis samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in Scarlet ibis. (B) Percentage prevalence of GIT parasite infections in Scarlet ibis. (C) Average egg count of GIT parasites in Scarlet ibis.

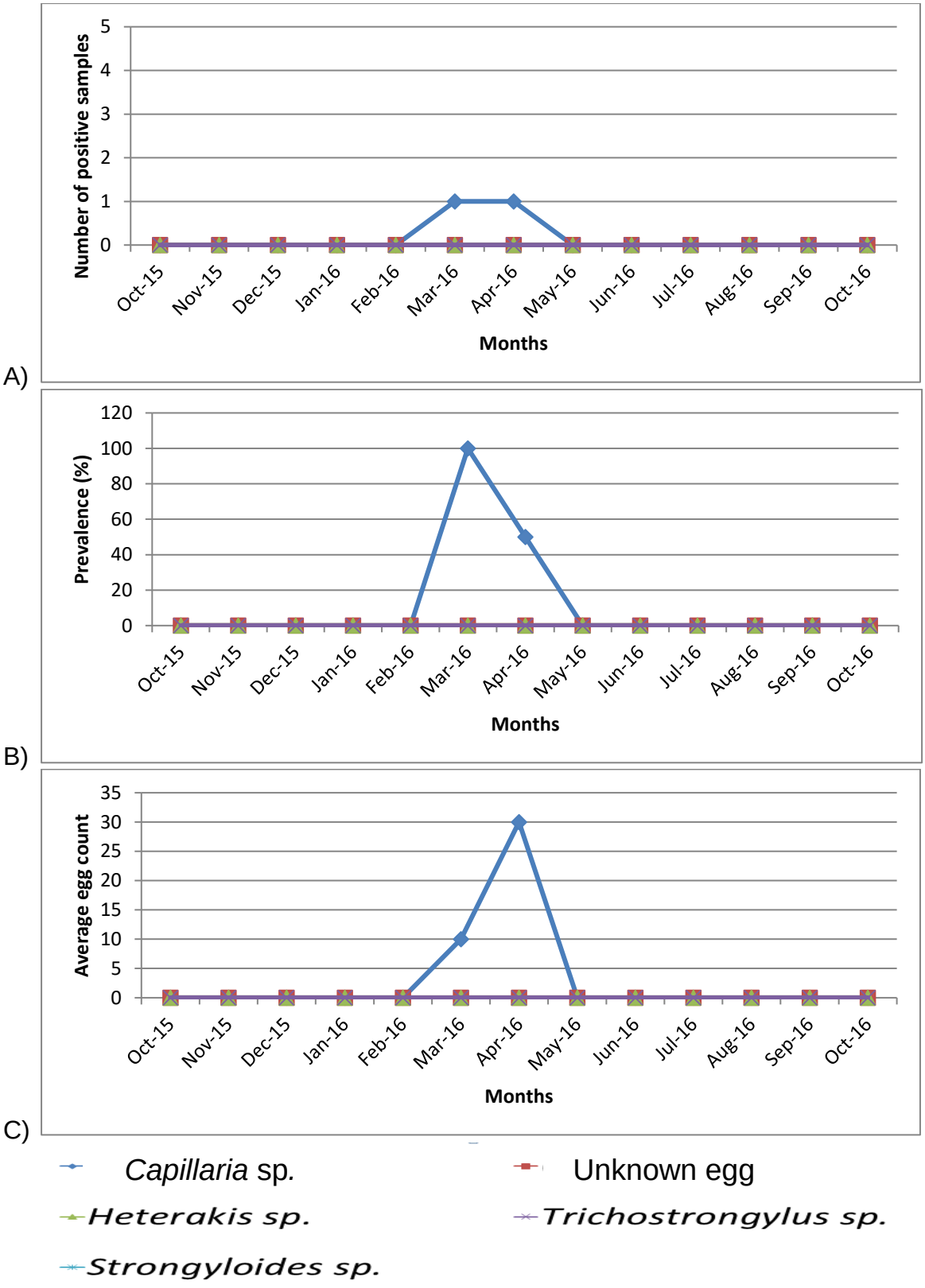


Figure 45: Distribution of GIT parasites from positive Sun conure samples at the National Zoological Gardens of South Africa. A) Seasonal distribution of positive GIT parasites in Sun conure. (B) Percentage prevalence of GIT parasite infections in Sun conure. (C) Average egg count of GIT parasites in Sun conure.

All three animal groups (ungulates, felids and avian species) showed varied levels of GIT infections, with the ungulates always having the highest prevalence with the exception of May 2016, were the felids infection was higher. The avian species were always the lowest and GIT parasite infections were occasional seen (Figure 48).

The ungulates showed higher prevalence throughout the study, with the highest record in October 2016, where all the ungulates samples tested positive for one or more GIT parasites. For the first five months, the prevalence remained constant with no great variations. There was a slight increase in the prevalence in the month of March from 57.89% to 73.33%; thereafter there was another increase in July 2016. The prevalence later decreased in August and September.

The felids did not show a pattern, the prevalence remained lower than those of the ungulates, except in May 2016, where there was a prevalence of 66.67%. From February to May there was an increase in the prevalence of GIT parasites in the felids. The prevalence dropped from 66.67% in May to 27.27% in June. There was a gradual increase in GIT parasite prevalence thereafter. The GIT parasite prevalence for the avian species was below 20% throughout the course of the study. The prevalence was highest in August 2016, followed by April 2016.

It is important to note the increase in GIT parasite prevalence in all three animal groups (ungulates, felids and avian species) from February to April. As we look at the climate data, there was a decrease in temperature and humidity but the rainfall increase in March from February but declined from 124 mm to 30 mm. A decrease in GIT parasite prevalence in all the animal groups was noted from October 2015 to November. In the first month of winter, there was an increase in prevalence in all the animal groups, the felids and the avian species continued to increase while the ungulate prevalence decreased. Overall, the ungulates had the highest prevalence while the avian species had the lowest prevalence of GIT parasites at the NZG.

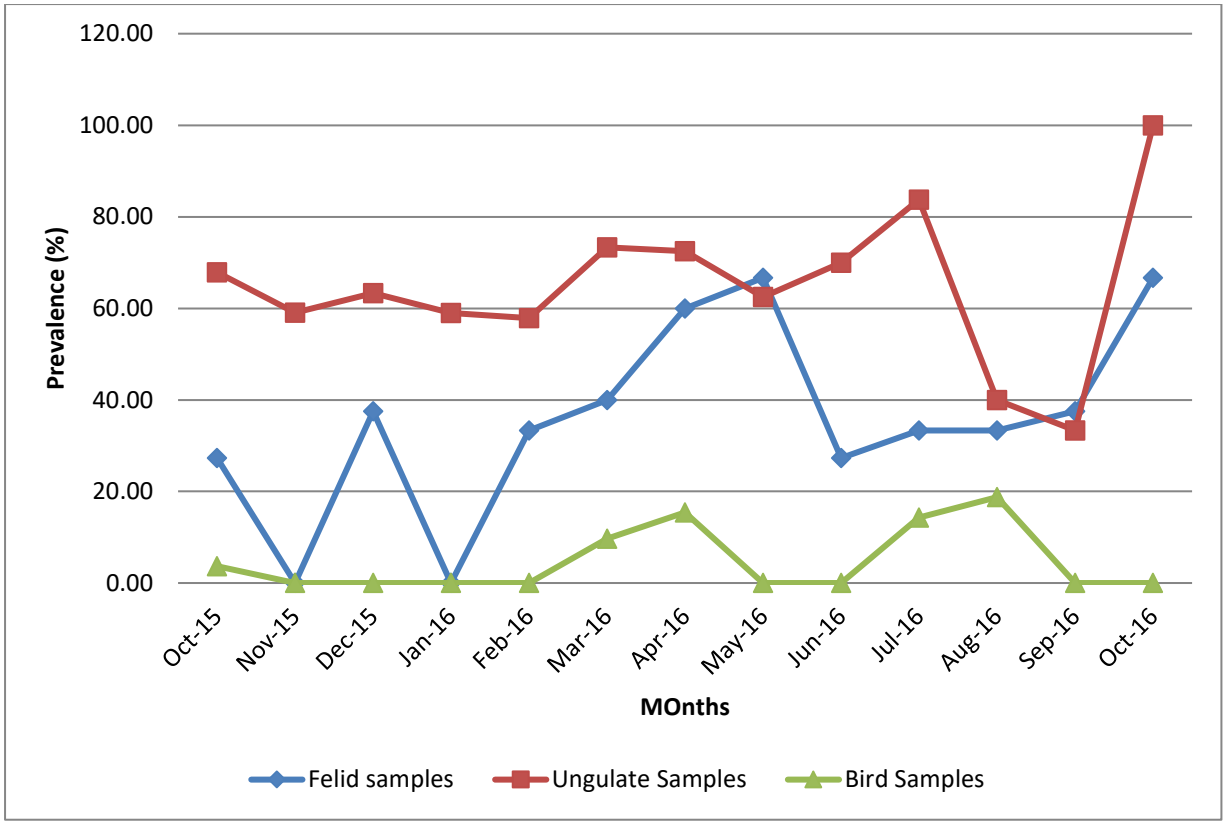


Figure 46: Overall GIT parasite prevalence in all three groups (Ungulates, Felids and Avian species [birds]) at the National Zoological Gardens of South Africa

4.5 Helminths harvested from larval cultures

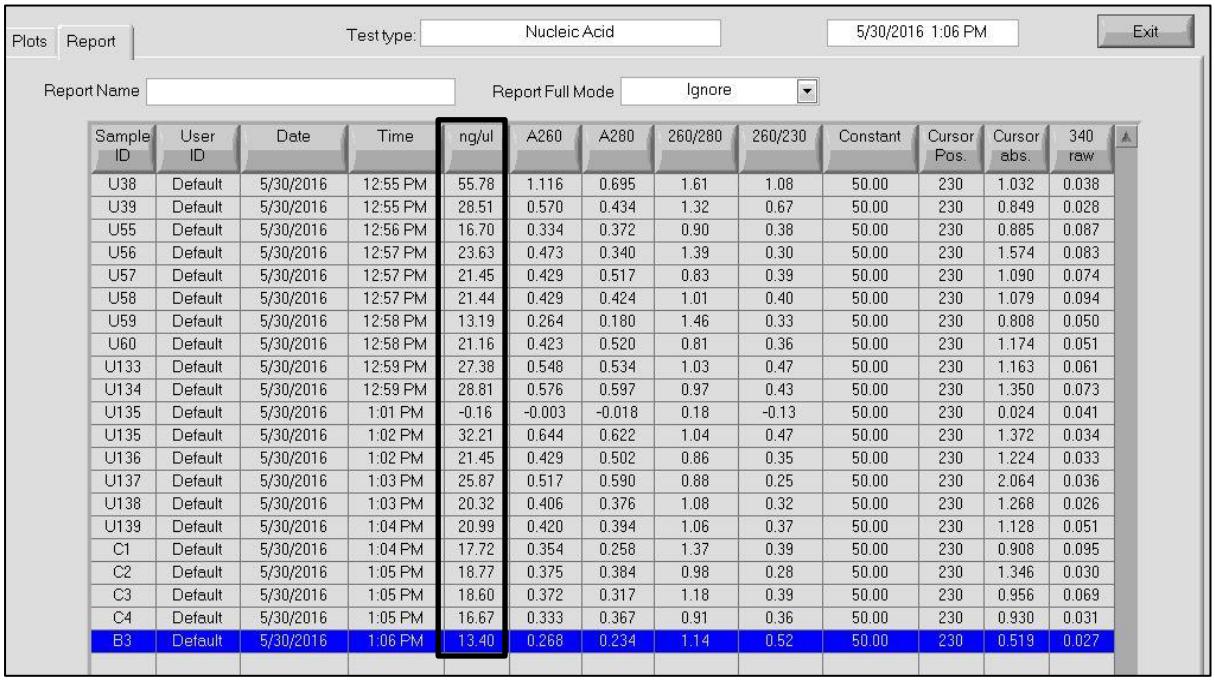
Live harvested larvae were observed under the microscope, the recovered larvae of nematodes from different ungulate species are seen in Figure 49. Fifteen of the 34 (44.11%) pooled faecal cultures prepared, were positive for GIT parasites. The number of larvae observed under the microscope correlated with the EPG recorded for that particular sample. In some cases, because of the low EPG, no larvae were seen under the microscope however PCR could pick it up. Morphological identification of the larvae harvested was not conducted, as there is currently no Identification key for nematodes in wildlife, however molecular analysis (PCR) was conducted on these samples.

Figure 47: Larvae harvested from faecal cultures various ungulates at NZG. A) Larva from Kalahari red goat, which was positive for *Trichuris* sp., *Nematodirus* sp., and *Haemonchus* sp. via faecalyser method. B) Larva from Cape eland, which was positive for *Nematodirus* sp. by faecalyser method. C) Larvae from Arabian oryx faeces which positive for *Trichuris* sp. *Nematodirus* sp., *Haemonchus* sp., *Ostertagia* sp., and *Trichostrongylus* sp. D) Larvae from Sable which was positive for *Haemonchus* sp., *Nematodirus* sp. and *Trichuris* sp.

4.5 Molecular identification and characterization of GIT parasites in selected captive wildlife at the National Zoological Gardens of South Africa

4.5.1 Quantification of extracted genomic DNA from ungulates, felids and avian species.

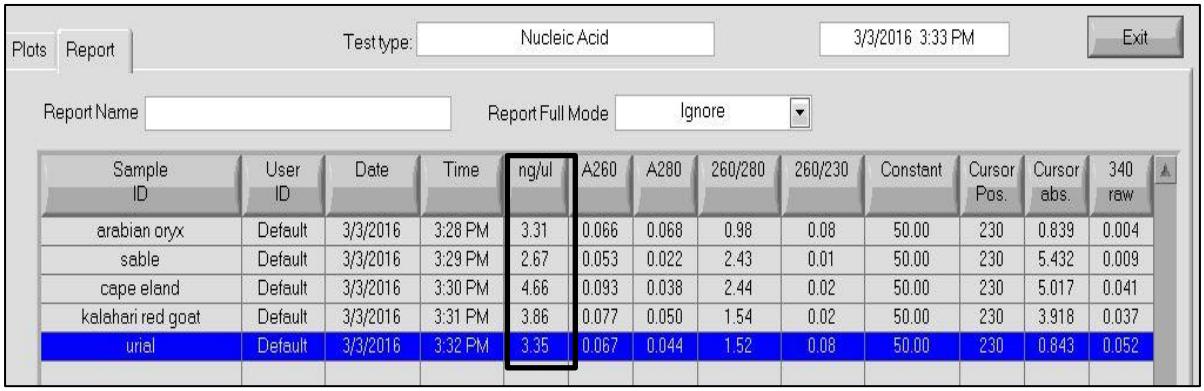
DNA concentrations from faecal samples varied for different animal groups (Figure 50). The DNA concentrations ranged between 13.40 ng/µl to 55.78 ng/µl, while the 260/280 ratio ranged between 0.83 – 1.61. The 260/280 ratio is important as it asses DNA contamination of protein solutions, since proteins absorbs light at 280 nm. Therefore 260/280 ratio has high sensitivity for nucleic acid contamination in protein.



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
U38	Default	5/30/2016	12:55 PM	55.78	1.116	0.695	1.61	1.08	50.00	230	1.032	0.038
U39	Default	5/30/2016	12:55 PM	28.51	0.570	0.434	1.32	0.67	50.00	230	0.849	0.028
U55	Default	5/30/2016	12:56 PM	16.70	0.334	0.372	0.90	0.38	50.00	230	0.885	0.087
U56	Default	5/30/2016	12:57 PM	23.63	0.473	0.340	1.39	0.30	50.00	230	1.574	0.083
U57	Default	5/30/2016	12:57 PM	21.45	0.429	0.517	0.83	0.39	50.00	230	1.090	0.074
U58	Default	5/30/2016	12:57 PM	21.44	0.429	0.424	1.01	0.40	50.00	230	1.079	0.094
U59	Default	5/30/2016	12:58 PM	13.19	0.264	0.180	1.46	0.33	50.00	230	0.808	0.050
U60	Default	5/30/2016	12:58 PM	21.16	0.423	0.520	0.81	0.36	50.00	230	1.174	0.051
U133	Default	5/30/2016	12:59 PM	27.38	0.548	0.534	1.03	0.47	50.00	230	1.163	0.061
U134	Default	5/30/2016	12:59 PM	28.81	0.576	0.597	0.97	0.43	50.00	230	1.350	0.073
U135	Default	5/30/2016	1:01 PM	-0.16	-0.003	-0.018	0.18	-0.13	50.00	230	0.024	0.041
U135	Default	5/30/2016	1:02 PM	32.21	0.644	0.622	1.04	0.47	50.00	230	1.372	0.034
U136	Default	5/30/2016	1:02 PM	21.45	0.429	0.502	0.86	0.35	50.00	230	1.224	0.033
U137	Default	5/30/2016	1:03 PM	25.87	0.517	0.590	0.88	0.25	50.00	230	2.064	0.036
U138	Default	5/30/2016	1:03 PM	20.32	0.406	0.376	1.08	0.32	50.00	230	1.268	0.026
U139	Default	5/30/2016	1:04 PM	20.99	0.420	0.394	1.06	0.37	50.00	230	1.128	0.051
C1	Default	5/30/2016	1:04 PM	17.72	0.354	0.258	1.37	0.39	50.00	230	0.908	0.095
C2	Default	5/30/2016	1:05 PM	18.77	0.375	0.384	0.98	0.28	50.00	230	1.346	0.030
C3	Default	5/30/2016	1:05 PM	18.60	0.372	0.317	1.18	0.39	50.00	230	0.956	0.069
C4	Default	5/30/2016	1:05 PM	16.67	0.333	0.367	0.91	0.36	50.00	230	0.930	0.031
B3	Default	5/30/2016	1:06 PM	13.40	0.268	0.234	1.14	0.52	50.00	230	0.519	0.027

Figure 48: DNA concentrations from faecal samples measured using a NanoDrop spectrophotometer. U – Ungulates, C – Felids, B – avian species. U38 is Sable antelope (Notch 40), U39 is Blesbok (Pooled, Female), U55 is Arabian oryx (Notch 14), U56 is Arabian oryx (Notch 20), U57 is Arabian oryx (Notch 25), U58 is Arabian oryx (Notch 11), U59 is Lechwe (Pooled, adult female), U60 is Lechwe (Pooled, adult female), U133 is Springbok (Pooled, juvenile female), U134 is Cape eland (Pooled, adult female), U135 is Cape eland (Green in, white out disk), U136 is Gemsbok (Pooled, adult female), U137 is Nubian ibex (Pooled 1), U138 is Nubian ibex (Pooled 2), U139 is Nubian ibex (Pooled 3), C1 is African lion (adult), C2 is African lion (Cub), C3 is Cheetah (Pooled 1), C4 is Cheetah (Pooled 2) and B3 is Sun conure.

DNA concentrations from harvested larval cultures were relatively lower (Figure 51), as compared to the DNA concentrations from faecal samples. The DNA concentrations ranged between 2.67 ng/µl – 4.66 ng/µl. The 260/280 ratio ranged between 0.98 – 2.44.



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
arabian oryx	Default	3/3/2016	3:28 PM	3.31	0.066	0.068	0.98	0.08	50.00	230	0.839	0.004
sable	Default	3/3/2016	3:29 PM	2.67	0.053	0.022	2.43	0.01	50.00	230	5.432	0.009
cape eland	Default	3/3/2016	3:30 PM	4.66	0.093	0.038	2.44	0.02	50.00	230	5.017	0.041
kalahari red goat	Default	3/3/2016	3:31 PM	3.86	0.077	0.050	1.54	0.02	50.00	230	3.918	0.037
urial	Default	3/3/2016	3:32 PM	3.35	0.067	0.044	1.52	0.08	50.00	230	0.843	0.052

Figure 49: DNA concentrations from larval cultures measured using a NanoDrop spectrophotometer.

4.5.2 Optimization of PCR conditions for GIT parasites in ungulates and felids

No further adjustments were required for amplification of *Haemonchus contortus* for larval cultures using Hae-F and NC2-R primer set (Figure 55 and 59). Previously published PCR conditions (Bott *et al*. 2009) were perfectly functional for the amplification of *H. contortus* DNA from larvae. Amplification of *H. contortus* DNA in faecal samples resulted in amplification of the target gene however there were non-specific bands as well.

There were several attempts to optimise the primer sets for annealing temperatures for amplification of *Trichostrongylus* sp., *Ostertagia ostertagii*, *Trichuris ovis* and *Nematodirus spathiger* using primer sets listed in Table 3.

Initially, previously published PCR conditions (Yong *et al*. 2007) were used for amplification of *Trichostrongylus* sp. larval culture samples. The expected PCR band appeared however there were non-specific bands as well. The protocol was further adjusted focusing mainly on the annealing temperature (52°C) and time (40 seconds). When the annealing temperature was increased and annealing time was decreased,

the non-specific band seemed to fade. Amplification for detection *Trichostrongylus* sp. in larval cultures (Figure 62) were observed at an annealing temperature of 55°C at the following PCR conditions; 95°C for 5 minutes followed by 35 cycles of 98°C for 20 seconds, 55°C for 15 seconds and 72°C for 30 seconds and a final extension at 72°C for 5 minutes. For faecal samples, similar adjustments were made, however in the faecal samples non-specific bands did not fade, although the expected fragment band size was observed (Figure 62).

For amplification of *N. spathiger* DNA, standard Kapa Hifi Hotstart master mix PCR conditions were used, however they resulted in non-specific DNA bands in larval cultures. Previously published PCR conditions (Oliver *et al*. 2014) were later used and they were successful for amplification of *N. spathiger* (Figure 60) in larval culture. However for amplification of *N. spathiger* in DNA extracted from faecal samples, faint single bands were obtained from the PCR assays prepared, using similar protocols and similar volumes of reagents and DNA. Figure (56) shows typical results after each reaction was conducted.

At first, previously published PCR conditions (Schnieder *et al*. 1999) tried and tested on the DNA from larval culture samples, and the *Ostertagia ostertagii* DNA was successfully amplified. However, nonspecific bands were observed on the gel. The annealing time was then decreased from 30 seconds to 15 seconds and it was only then when single specific bands were observed (Figure 61). Attempts to amplify *O. ostertagii* DNA were successful in both larval cultures and faecal samples. However, the faecal samples resulted in non-specific bands in samples with expected bands at 90bp (Figure 57).

Efforts to amplify the internal transcribed spacer 2 (ITS2) gene of the *Trichuris* sp. genomic DNA by PCR was unsuccessful on both larval culture and faecal samples. A series of reactions setup to determine optimal conditions for primer annealing gave inconclusive results, as there was amplification of specific bands. Various PCR conditions were tested as well as different reagent volumes and DNA volumes. However, the primers failed to amplify target DNA after all the efforts had been exhausted. Figure 52 shows typical results after each reaction was conducted.

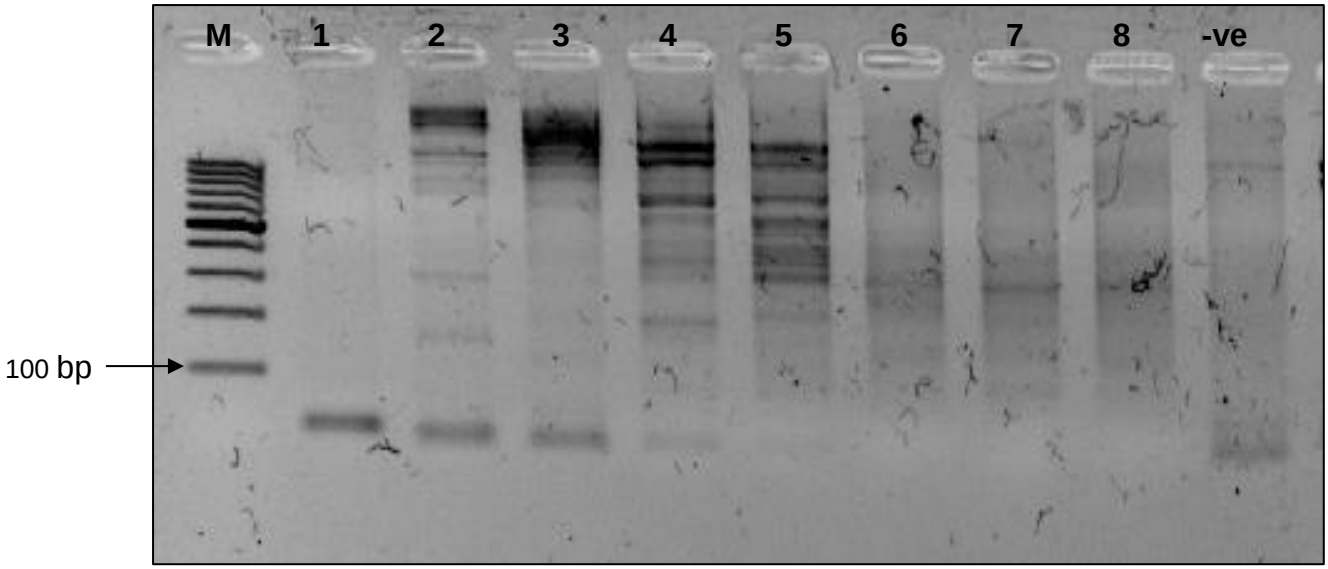


Figure 50: Gel electrophoresis of *Trichuris* sp. M as Marker of 100bp, -ve is distilled water as negative control. Expected amplicons size 0 – 100bp. Sample 1 – 8 indicate inconclusive results

For amplification of *Toxascaris leonina* and *Toxocara cati*, several attempts to amplify these GIT parasites DNA were unsuccessful in all faecal samples. To establish the optimum annealing temperature, a gradient PCR was set up, with annealing temperatures ranging from 55°C to 62°C (which was the published annealing temperature for these sets of primers), none of the attempts were successful (Figure 53 and 54)

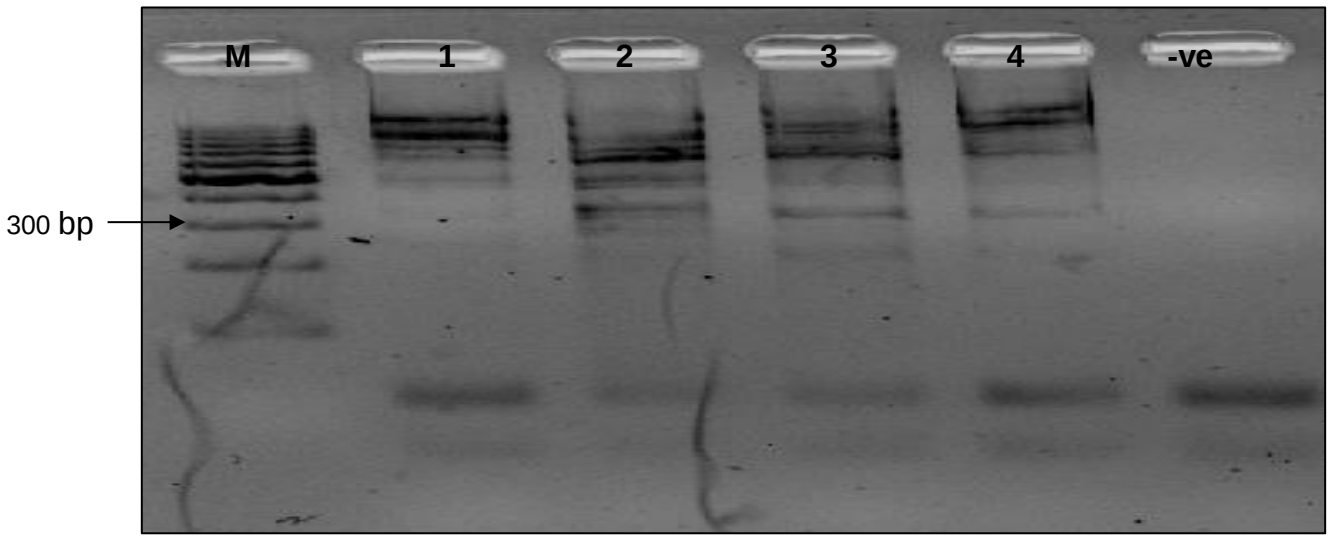


Figure 51: Gel electrophoresis of *Toxocara cati* amplicons at 370 bp. M as Marker of 100bp, -ve is distilled water as negative control. Sample 2-4 indicated inconclusive results and samples 1 indicate negative results

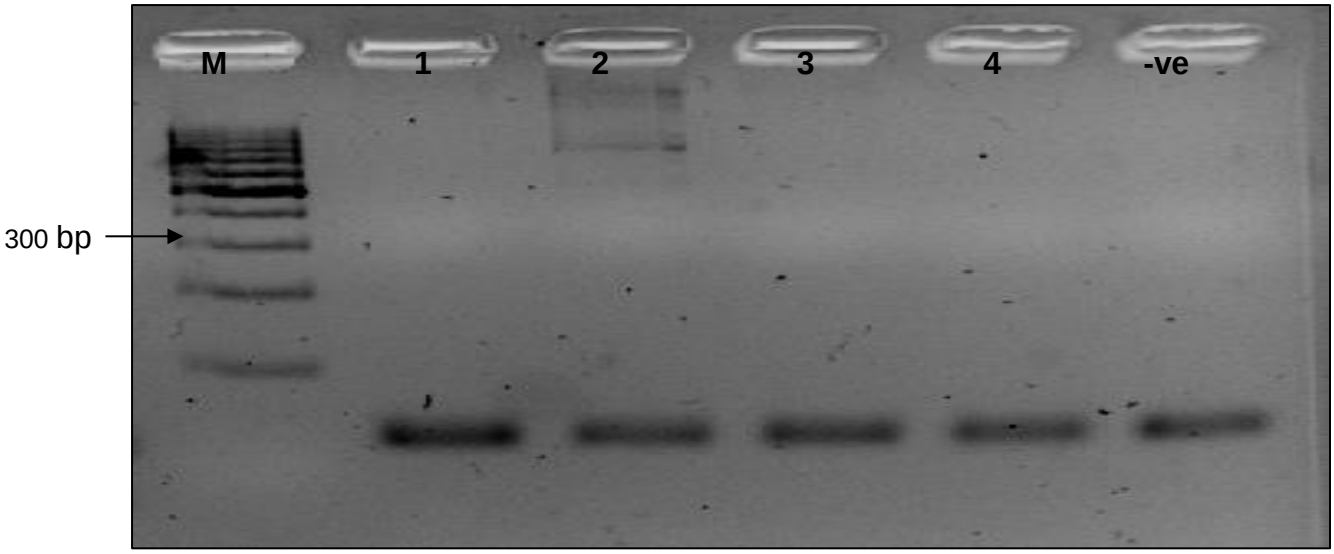


Figure 52: Gel electrophoresis of *Toxascaris leonina* amplicons at 300 bp. M as Marker of 100bp, -ve is distilled water as negative control. Samples 1 – 4, 15 indicate negative results. Sample 2 indicate secondary amplification between 800 - 900bp.

4.5.3 Molecular identification of GIT parasites in selected captive wildlife at National Zoological Gardens of South Africa

A total of 313 faecal samples were positive by microscopy; DNA was successfully extracted from 297 faecal samples, 245 from ungulates, 36 from felids and 16 from avian species. DNA samples were screened for presence of *Trichuris* sp., *Haemonchus contortus, Nematodirus spathiger, Ostertagia ostertagii* and *Trichostrongylus* sp. in ungulates. In the felids, DNA samples were screened for *Toxascaris leonina and Toxocara cati.*

DNA amplification revealed the positive band at 275bp, 860bp, 90bp and 482bp (Figure 64 – 67) respectively for 5.8S and 18S target genes. The overall infection obtained via PCR for *Haemonchus contortus, Nematodirus spathiger, Ostertagia ostertagii and Trichostrongylus* sp. was 36.23%, 10.01%, 5.38% and 45.38% respectively for the all faecal samples that were positive for GIT parasites by microscopy.

In this study, of the 38 felid samples which tested positive by microscopy DNA was successfully extracted from 36 samples and examined by PCR using the Tcat/NC2 and Tleo/NC2 primers to get a partial fragment *Toxocara cati* and *Toxascaris leonina* respectively. PCR assays prepared for detection of these GIT parasites in the felids did not yield an anticipated single amplified product of 300 bp for *T. leonina* and 370bp for *T.cati*. The primer sets did not yield any positive amplification at any of the tested annealing temperatures or at extended incubation periods for both *Toxocara cati* and *Toxascaris leonina* (Figure 62 and 63) respectively.

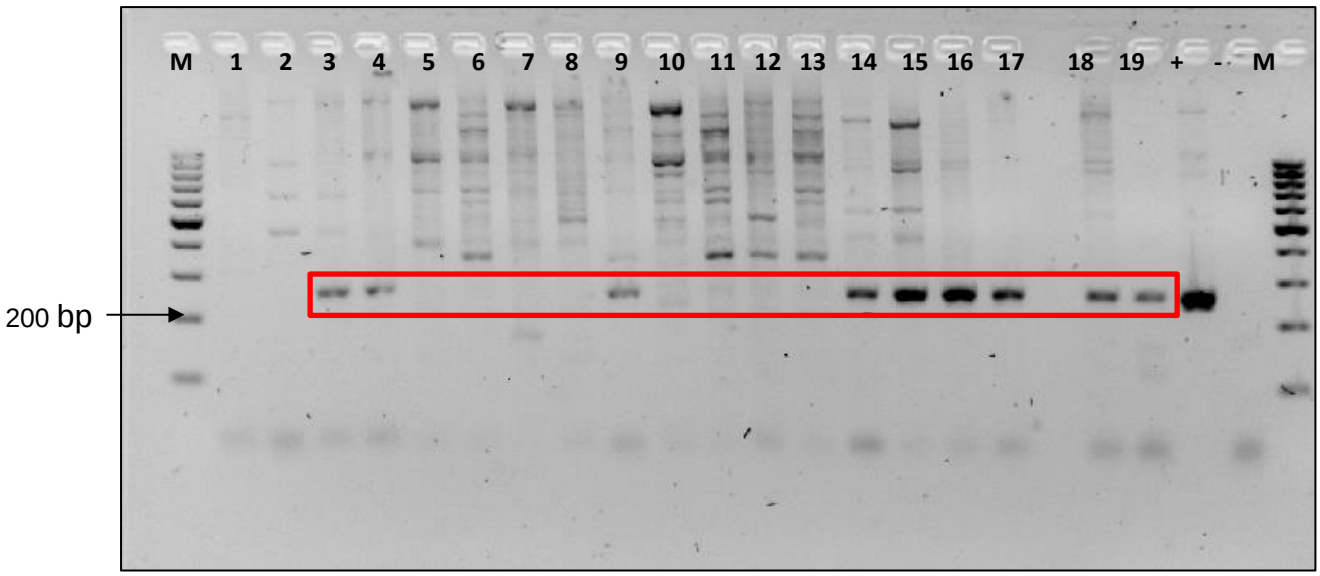


Figure 53: Gel electrophoresis of *Haemonchus contortus* amplicons at 247 bp. M as Marker of 100bp, + is *Haemonchus contortus* as positive control, - is distilled water as negative control. Samples 3, 4, 14-19 indicated positive results and samples 1, 2 5, 6, 7, 8, 10-13 indicate negative results

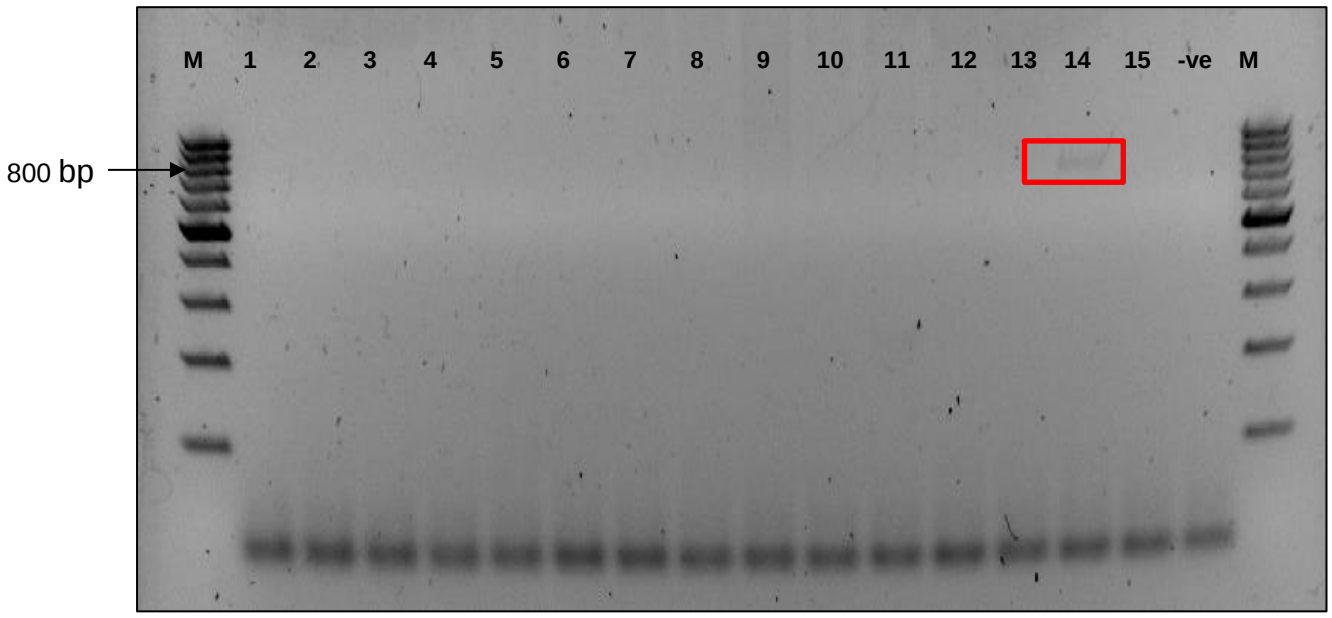


Figure 54: Gel electrophoresis of *Nematodirus spathiger* amplicons at 860 bp. M as Marker of 100bp, -ve is distilled water as negative control. Sample 14 indicated positive results and samples 1 – 13, 15 indicate negative results

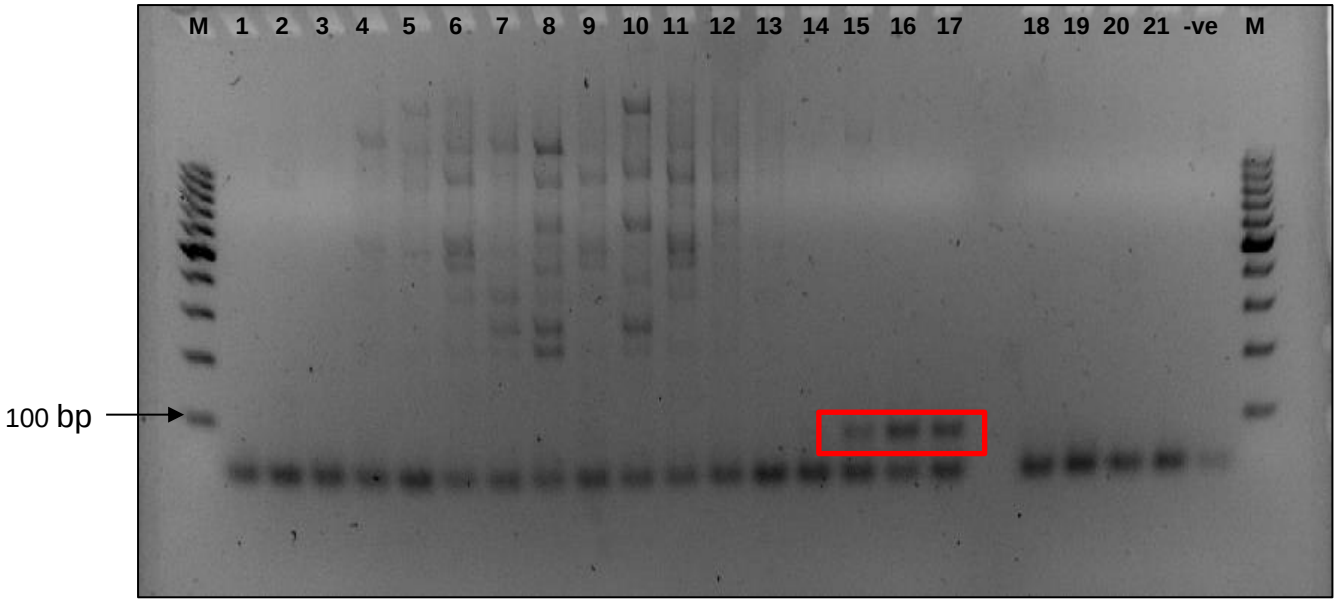


Figure 55: Gel electrophoresis of *Ostertagia ostertagii* amplicons at 90 bp. M as Marker of 100 bp, -ve is distilled water as negative control. Sample 15, 16, 17 indicate positive results and samples 1 – 14, 18, 19, 20, 21 indicate negative results

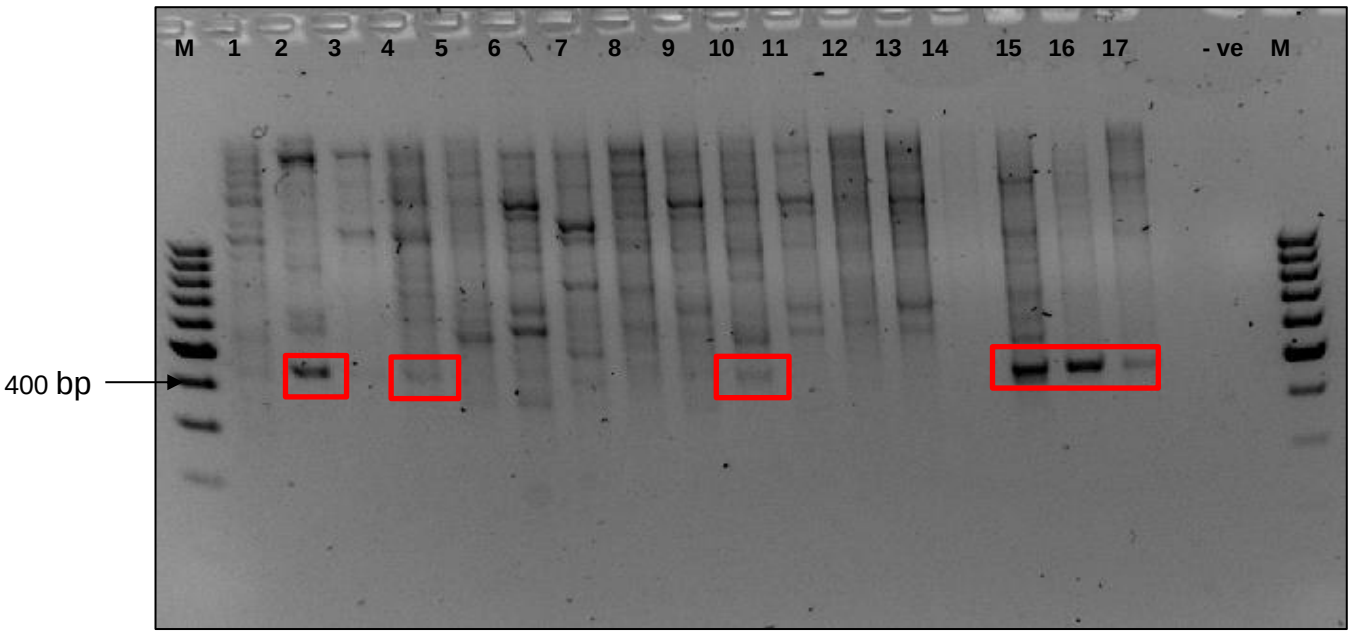


Figure 56: Gel electrophoresis of *Trichostrongylus* sp. amplicons at 482 bp. M as Marker of 100 bp, -ve is distilled water as negative control. Sample 2, 4, 10, 15, 16, 17 indicate positive results and samples 1, 3, 5 - 9, 11 - 14 indicate negative results

4.5.4 Molecular identification from larval culture samples

A total of 15 pooled larval cultures were positive by microscopy and DNA was successfully extracted from 5 larval cultures samples. Because in some cases, EPG were low, and therefore resulted in low counts of larvae harvested it was not possible to extract DNA from these samples, hence the number of samples screen for GIT parasites via PCR were lower than the initial positive culture samples.

DNA samples were screened for presence of *Haemonchus contortus, Nematodirus spathiger, Ostertagia ostertagii and Trichostrongylus* sp. and the amplification revealed the positive band at 275bp, 860bp, 72bp and 482bp (Figure 59 – 62 respectively) for 5.8S and 18S target genes. The overall infection obtained for *H. contortus N. spathiger, O. ostertagii and Trichostrongylus* sp. was 35.0%, 35.0%, 10.0% and 20.0% respectively for the larval culture samples for all positive samples. The primers were sufficiently sensitive and specific enabling the discrimination of different GIT parasites.

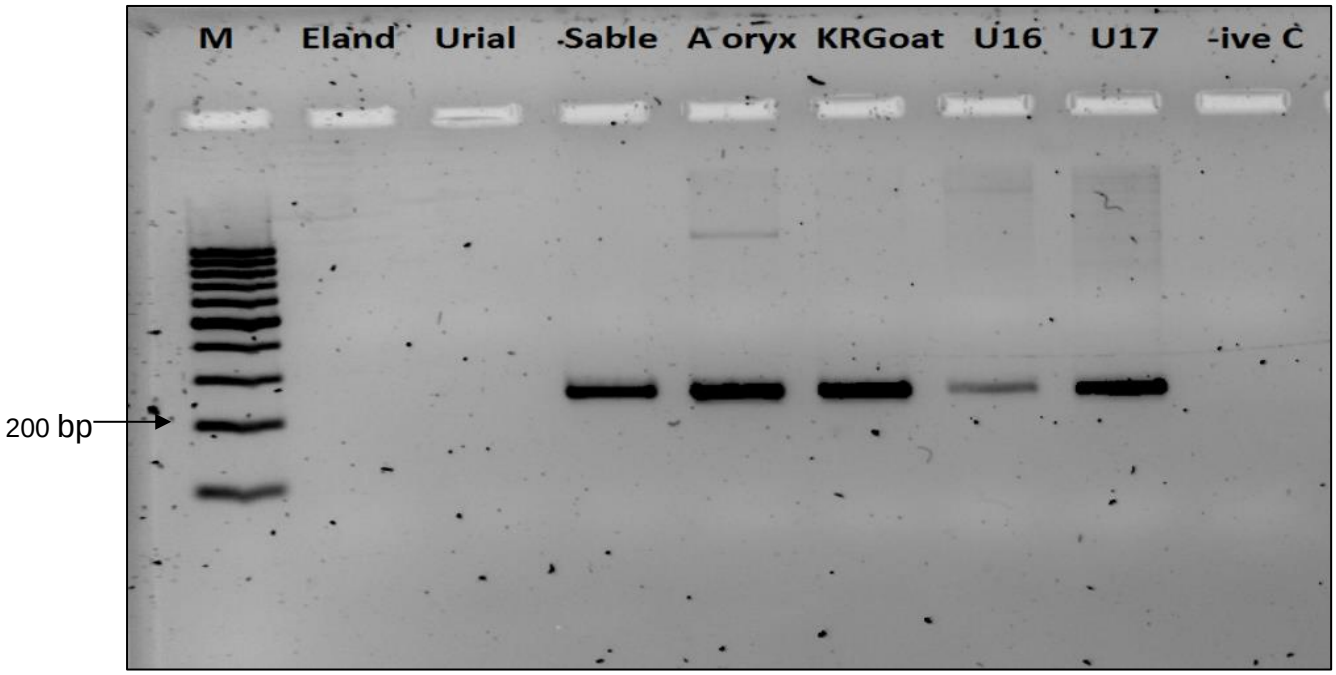


Figure 57: Gel electrophoresis of *Haemonchus contortus* amplicons at 247 bp. M as Marker of 100bp, -veC is distilled water as negative control. Samples; sable, A oryx, KRGoat, U16, U17 indicate positive results and samples eland and urial indicate negative results.

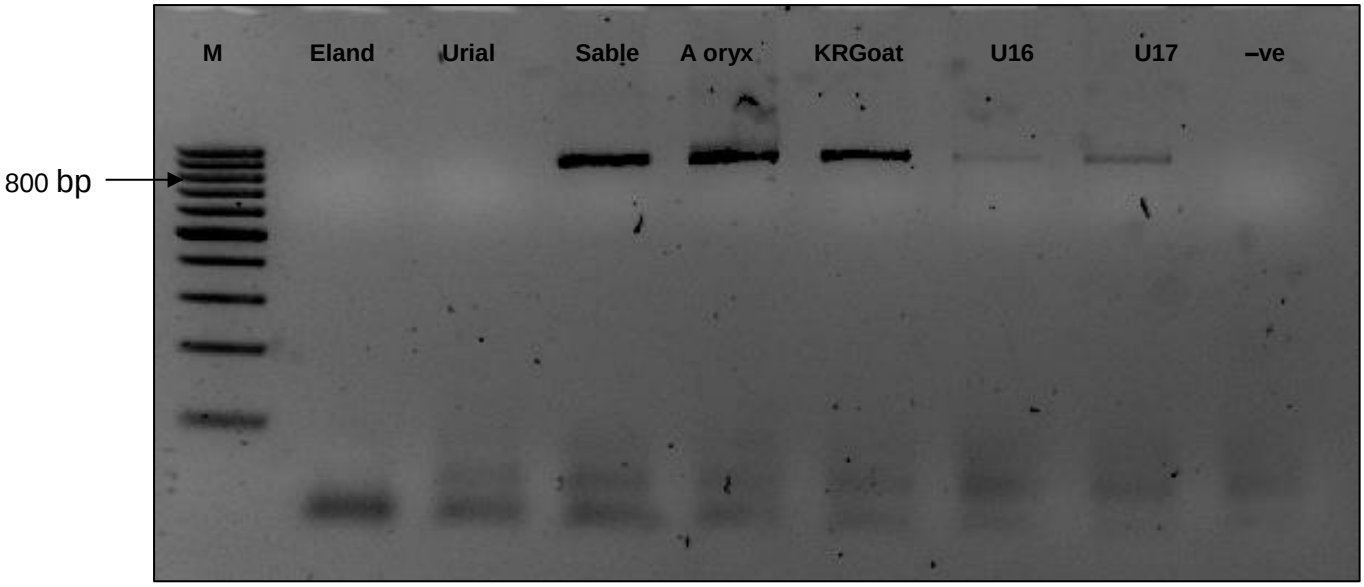


Figure 58: Gel electrophoresis of *Nematodirus spathiger* amplicons at 860b p. M as Marker of 100bp, -ve is distilled water as negative control. Samples; sable, A oryx, KRGoat, U16, U17 indicate positive results and samples eland and urial indicate negative results.

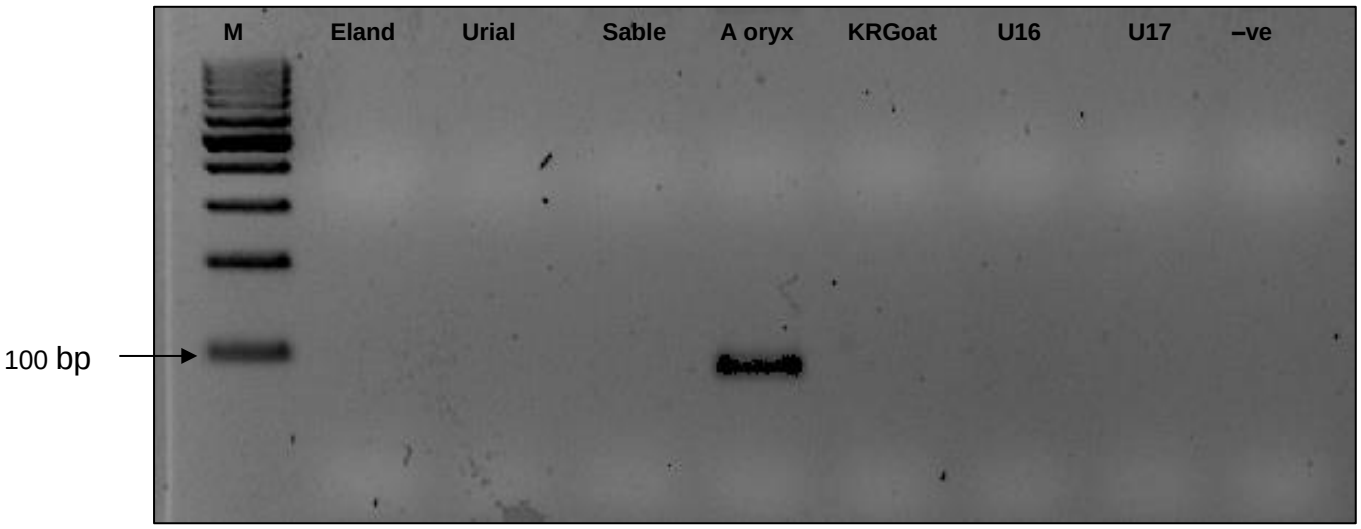


Figure 59: Gel electrophoresis of *Ostertagia ostertagii* amplicons 90 bp. M as Marker of 100bp, -ve is distilled water as negative control. Samples; A oryx, indicate positive results and samples eland, urial, sable, KRGoat, U16, U17 indicate negative results.

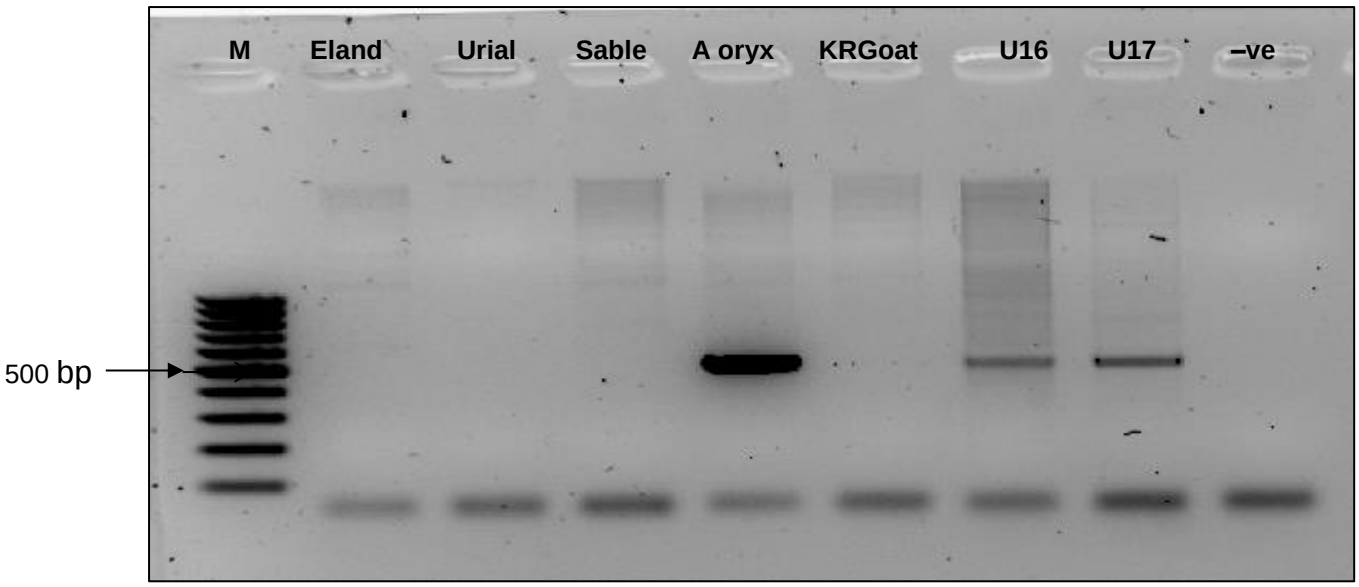


Figure 60: Gel electrophoresis of *Trichostrongylus* sp. PCR amplicons at 482 bp. M as Marker of 100bp, -ve is distilled water as negative control. Samples: A oryx, U16, U17 indicate positive results and samples eland, urial, sable, KRGoat indicate negative results.

4.5.5 Sequencing of positive PCR amplicons

Positive PCR amplicons were purified and subjected to direct Sanger sequencing using. However, sequencing was not successful for *N.* spathiger, *O. ostertagii* and *Trichostrongylus* sp. due to poor quality of DNA. Only *H. contortus* PCR amplicons were successfully sequenced. The nucleotide basic local alignment search tool (BLASTn) was used to identify the identical matching sequences from the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST). Blastn searches revealed that 2 of 6 sequence samples matched with the 18S rRNA gene sequences of *H. contortus* in the database with accession number KU891884.1 and JF680983.1 with 81% and 85% identity respectively (Figure 63 and Figure 64).

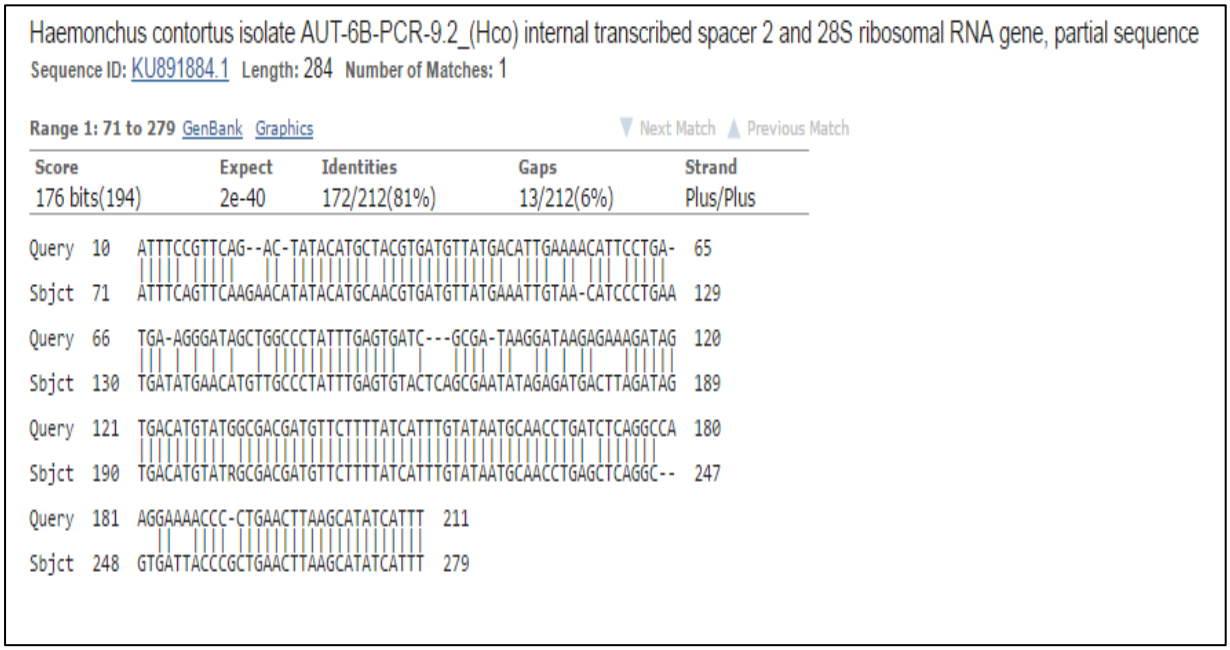


Figure 61: BLASTn alignment of *Haemonchus contortus* isolate which matched with PCR positive ungulate sample

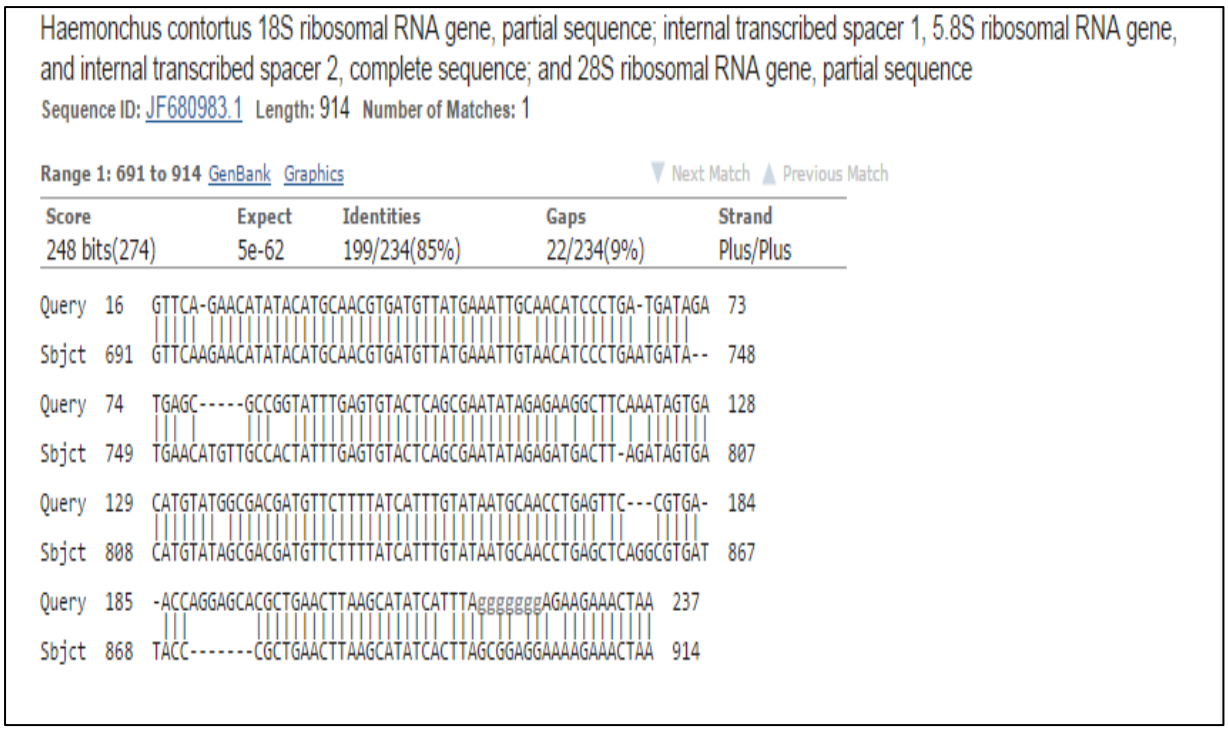


Figure 62: BLASTn alignment of *Haemonchus contortus* isolate which matched with PCR positive ungulate sample

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Prevalence and seasonal distribution of GIT parasites at NZG

Gastro-intestinal parasite infections are a universal problem for both domestic and wild animals, but are even more problematic in captive animals (Adeyini *et al.* 2015). Excessive infections of GIT parasites seldom occur in free-ranging wildlife, whereas in captivity (as in zoos), the stress to which the animals are subjected weakens their immunological system, making them more susceptible to parasite infection (Cordon *et al*. 2008). One of the major problems with captive environments is that, animals are in close proximity to one another, which provides opportunity for transmission of parasites between enclosures and from animal to animal that would normally not be in contact with one another. Another problem with GIT parasites in captive environments is the high common occurrence of re-infections (Adeyini *et al*. 2015).

Seasonal changes also play a vital role in transmission of GIT parasites. Changes in humidity and temperature can influence development, survival and transmission of parasites in the external environment, as they provide favourable environmental conditions for transmission of GIT parasites (Raharivololona & Ganzhorn 2010). The economic loss caused by GIT parasites infections in both livestock and wildlife is enormous. Economic losses are caused by GIT parasites in a variety of ways; such as lowered fertility, reduced work capacity, involuntary culling, reduced food intake, weight loss, decreased production, treatment costs, morbidity and mortality in heavily infected animals (Abouzeid *et al*. 2010). This study investigated the effects climatic conditions throughout the four seasons of the year had on the GIT parasite load in selected wildlife at NZG, by identifying and characterizing the GIT parasites using morphological and molecular techniques.

Microscopic evaluation of the level of GIT parasitic infestation by examination of the 772 faecal samples collected in this study revealed presence of GIT nematodes, trematodes, cestodes and coccidia oocysts in ungulates, felids and avian species. All parasites detected in this study have been noted in previous surveys (Lim *et al.* 2008; Opara *et al*. 2010; Rahman *et al*. 2014). The prevalence of GIT parasites was lower in NZG than in Bir Moti Bagh mini zoo, Punjab (Mir *et al*. 2016) and slightly higher than

in Nandan Van Zoo, Raipur (Thawait *et al.* 2014) . These variations may be due to different management systems, variation in climate which is necessary for development of infective larvae, different methods of diagnosis, differences in animal species and location, these factors could have potential to influence abundance and distribution of GIT parasites in the animals.

In this study, co-occurrence of helminths and protozoa in ungulates and felids was common (Table 5 and Table 6). Similar life cycles of coccidia and nematodes make correlated exposure likely, because both parasites' life cycles include faecal-oral transmission and development of infectious stages in the environment (Stewart & Penzhorn 2004). Coccidiosis is more important where animals are housed or confined in small areas. The disease is also more important in young animals as parasite infections are a source of stress and weight loss to animals when they occur in large numbers (Abouzeid *et al*. 2010). Co-infections are common in natural populations and interactions among co-infecting parasites can alter transmission and host fitness (Gorsich *et al*. 2014). Mixed infections were also common especially in the ungulates (Table 5). Mixed infections have been suggested to be a cause of morbidity and reduced production. Furthermore, mixed infection increases the risks of the host contracting other diseases or parasites (Adedipe *et al*. 2014). It is also possible for the positive association between coccidia and nematodes to be driven by hosts in poor condition being susceptible to both infections (Beldomenico & Begon 2010).

This study observed high prevalence and distribution of nematode parasites as compared to other helminths; in particular, the strongyle egg type detection rate and egg counts were more prevalent than other GIT parasites. The reason for high prevalence nematodes is that they have a direct lifecycle which do not involve any intermediate hosts and transmission is fairly easy through ingestion of contaminated food, water and soil. Nematode transmission dynamics are affected by climatic conditions. Warm, moist conditions favour nematode transmission, while a hot, dry climate is detrimental to larval survival and transmission (Turner & Getz, 2010). Helminths particularly nematodes accumulate in captive environments especially in open soil enclosure which cannot be easily disinfected. Other helminth parasites such as trematodes and some cestodes require an intermediate host for their transmission and are less likely to accumulate in captive environments (Thawait *et al*. 2014). Findings of the current study are in agreement with observations made by Thawait *et*

al. (2014) in their study of prevalence of gastro-intestinal parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhattisgarh whereby there were less encounters of the trematodes and the cestodes as compared to the nematodes in this study as shown in Table 4.

Effective control measures have been taken by the NZG to reduce the environmental contamination, such as daily faecal removal. In addition, there are three parasite control programs that are employed at the NZG: (i) Opportunistic treatment program, which involves the use of anthelminthics only when the EPG above 600 is observed in sampled ungulates during routine monitoring diagnosis; (ii) the suppressive treatment program, this approach involves the use of anthelminthics on a regular schedule and at specific time intervals; and (iii) the strategic treatment program, this approach is based on understanding the epidemiology of the parasites. The aim is to decrease the adult parasite population at critical times of the year, thus limiting the number of infective larvae in the enclosure pasture (NZG SOP – Preventative Medicine Programme 2016).

5.2. Gastro-intestinal parasites in ungulates at NZG

All the parasites recovered from the wild ungulates at the NZG are common in livestock in South Africa (Horak *et al.* 1984; Horak *et al*. 2001; Tsotetsi & Mbati 2003). All GIT parasites detected in the current study have been noted in previous survey by (Nalubamba *et al*. 2012) at a game facility located south of Lusaka, Zambia and by (Egbetade *et al*. 2014) at Federal University of Agriculture Zoological Park, Abeokuta. Among the ungulates, strongyles (*Haemonchus* sp.; *Trichostrongylus* sp. *Ostertagia* sp. *Oesophagostomum* sp. and *Cooperia* sp.) were the most prevalent throughout the course of the study. The present findings are in agreement with the report of Singh *et al*. (2006) who recorded strongyles type egg infection as the most commonly detected parasitic infection (89%) in wild herbivores in the Mahendra Choundhury Zoological Park, Chhatbir, Punjab. Gastro-intestinal parasites that have been reported in ruminants include protozoan species and helminths. Of the helminths, strongyle nematodes and *Strongyloides* sp. are reported as problematic to wild ungulates in captivity (Goossens *et al*. 2005; Ortiz *et al*. 2006) and in their natural habitat. The strongyles type eggs were more dominant in the present study, particularly *Haemonchus* sp. This is probably due to its high fecundity, which means that it is likely

to be recovered from pasture in higher numbers than larvae of other genera (Wariuru *et al*. 2001). *Haemonchus* sp. is able to tolerate a very wide range of climatic conditions (Tsotetsi & Mbati 2003). This explains why it was isolated as one of the prevalent species, and transmission is mainly restricted to rainy season and it is reflected by the increase in parasite load during rainy season in this study as shown in Figure 19 and Figure 27.

In this study, there were no GIT parasites detected in Red forest buffalo throughout the study, which contradicts a similar study by Gorsich *et al*. (2014) who observed co-occurrence of coccidia and nematodes in African buffalo, and observed higher prevalence of coccidia than that of nematodes. No occurrence of GIT parasites in Red forest buffalo could possibly be attributed to husbandry of these animals at the NZG. The enclosure for these animals is mostly covered in soil and with very little natural vegetation; this restricts the animals from grazing and may therefore reduce or prevent infection by parasites within the enclosure. Low levels of GIT parasite load (prevalence and EPG) were observed in animals such as Addax, Lechwe and Nyala. Boomker *et al*. (1991) recovered strongyles, *Taenia* sp. *Schistosoma mattheei* and *Paramphistomes* from Nyala in three localities of KwaZulu-Natal, with the exception of strongyles, none of which were observed in this study. High prevalence and EPG values were observed in Urial, Springbok, Sable, Ibex, Kalahari red goat, Gemsbok and Scimitar horned oryx (Figure 26). Strongyles were dominant in all these animals. According to the NZG Post-mortem report (Espie 2015), GIT parasites are a major problem and one of the leading causes of mortality in ungulates. In 2015, ten ungulates (5 sables, 2 urials, 1 springbok, 1 Kalahari red goat and 1 forest buffalo) died at the NZG. Post-mortems of these animals indicated severe anaemia, no fat reserves and high levels of *Haemonchus* sp., *Nematodirus* sp. and *Trichuris* sp. infection loads.

Strongyloides is a parasitic nematode that infects sheep, goats, cattle and wild ruminants with worldwide distribution. A mode of infection is through ingestion, skin penetration and through milk of lactating females (Love & Hutchinson 2003). In this study, *Strongyloides* sp. were observed in Springbok and Arabian oryx (Table 4) and the high prevalence and intensity of the parasite was observed during the first months of spring, two peaks are observed during autumn and winter (Figure 34). The larvae are not sheathed and are susceptible to adverse climatic conditions. However, warmth and moisture favour their development and survival and it also allows the accumulation

of large numbers of infective larvae in the environment. (Pienaar *et al*. 1999), this could have been the reason that the animals become heavily infected in the early spring after the first rains of the season. Localised contamination of watering and feeding areas may lead to infections, localised wet areas that may be favourable for pre-parasitic nematode developments and survival e.g. *Strongyloides* sp. that penetrate the skin of the host to initiate infection. Losses in lambs with heavy natural infections during wet period following a drought reported from Kenya (Pienaar *et al*. 1999) this could explain the presence of *Strongyloides* sp. infections during autumn months.

Trichuriasis is diseases of ruminants that are caused by *Trichuris discolor, T. globulosa* and or *T. ovis*. In the present study, highest prevalence and intensity (EPG) of this parasite occurred during winter months in sable antelopes and springboks, which is in agreement with other studies (Saha *et al*. 1996; Gul & Tak 2014). Incidences of parasitic infection during winter could be attributed to suitable climatic conditions and the availability of food e.g. (pasture during their development). The prevalence of *Trichuris* sp. varies considerably depending on local environmental conditions such as humidity, temperature, rainfall, vegetation and management practices. Climatic conditions contribute significantly to the distribution and prevalence of the disease (Padwal *et al*. 2011). A study by Umur & Yukari (2005) indicated late high wave of infection, occurring in winter which may be due to eggs deposited by juvenile and mature sheep grazing on pasture in late autumn months.

Nematodirus sp. is a roundworm that parasitizes the small intestines of the ruminants, and poses a major threat especially to young animals (lambs and calves) (Melancon 2001; Hynes 2015). The parasite was reported to be absent from Africa and southern Asia (Hoberg 2005), however it was detected in this study, and the presence was confirmed by PCR, however due to poor quality DNA, sequencing of *Nematodirus spathiger* sp. was not a success. The low infection intensity of *Nematodirus* sp. throughout the year could be attributed to its lifecycle (larval stage develops in the egg), the larval stage can be completed in 2 – 4 weeks or up to several months to develop (depending on species) and will persist on the pasture for extended periods, and does not hatch until ideal conditions (van Dijk & Morgan 2009). The sudden outbreak of *Nematodirus* sp. and *Trichuris* sp. (co-infection) were observed in young sable antelope and springbok. This is in contradiction to a study by Hutchinson (2003) who indicated that small numbers *Nematodirus* usually co-occurred with larger

numbers of *Cooperia*. *Nematodirus* sp. infections are not considered as disease causing agents of older cattle because of the immunity which calves have been shown to develop (Melancon 2001; Hynes 2015). The immune response is based on both exposure and age (Melancon 2001). *Nematodirus* sp. infection is accompanied by diarrhoea, anorexia, dehydration and dull coat; however none of these clinical signs were visible in animals of sampled enclosures in this study. High parasite load was observed during cold winter months. According to van Dijk & Morgan (2009) hatching of *Nematodirus* sp. egg is stimulated by a cold period followed by a night/day temperature of around 10 degrees.

According to Reinecke (1983), "Although *Nematodirus* sp. is rarely diagnosed in South Africa, it occurs in calves in the Western Cape Province, Eastern Cape Province and KwaZulu-Natal". He also states that its eggs are seldom detected in the faeces of calves older than 5 months. *Nematodirus* sp. would thus appear to be a parasite of young animals in the moist, temperate climates of the coastal provinces of South Africa.

Moniezia sp. is a tapeworm which requires an intermediate host to complete its lifecycle. The intermediate host (oribatid mite) are important components of the soil fauna and have worldwide distribution. Foraging mites ingest the eggs and after 1-4 months' embryos develop to form cysticercoids. Infection of the final host is by ingestion of infected mites during grazing. Seasonal distribution of *Moniezia* sp. infections can be related to activity periods of the foraging mite vectors during summer in temperate regions (Taylor *et al*. 2007). The reasons why there were such low levels of *Moniezia* sp. prevalence in this study could be due to possible scarcity of the intermediate host in the study area.

Calicophoron sp. (previously known as *Paramphistomum* sp.) is a stomach fluke found in the small intestines and rumen of cattle, sheep, goats and wild ruminants, they have a worldwide distribution, however more prevalent in humid and warm regions (Vorster & Mapham 2016). *Calicophoron* requires an intermediate host (freshwater snail) to complete its lifecycle. Snails are aquatic and live in water and are enormously prolific. The seasonal evolution of infection of *Calicophoron* might be related to climatic variations shown by Jorgensen *et al*. (1998), depending largely on rainfall. In the present study, they were observed only during rainy season from Cape eland. The

same Cape eland infected *Calicophoron* sp. during the rainy season, was later infected with *Moniezia* sp.

Some ungulates (Lechwe, Nyala, and Addax) at NZG have large enclosures with enough space; as a result, the animals appear to be defecating in areas which are far from their feeding spots, thereby reducing the chances of reinfections. It is therefore suspected that this is the possible reason of low GIT parasite prevalence and load observed in these animals in the current study.

Some ungulates are selective browsers and often switch between grazing and browsing depending on the season and habitat, but in limited spaces this is inhibited and may increase their burdens of internal and external parasites (Ezenwa 2004). Furthermore, the fact that ungulates in captivity have limited movement increases chances of re-infection cycles.

5.3 Gastro-intestinal parasites in felids at NZG

Among the felids, *Toxascaris* sp., *Toxocara* sp. and coccidia were the only parasitic infections observed in lion cubs, lions and cheetahs at the NZG in the current study. *Toxascaris* sp. was the most common GIT parasite in these felids with the highest EPG recorded (Figure 45). Occurrence of *Toxocara* and *Toxascaris* infestations in captive felids have been reported by other studies (Pawar *et al*. 2012) as an indication of poorly maintained animal enclosures (Mukarati *et al*. 2013). Another contributing factor to these infections could be the feral cats that roam the zoo which are possibly contaminating feed and thus may facilitate the transmission of these parasite infections in these animals (Otranto *et al*. 2015). Baker *et al*. (1989) indicated a prevalence of 11% of *Toxocara cati* in stray cats in the Pretoria area. Since stray cats are common at the NZG it is possible that they may be contaminating the zoo environment which indirectly results in transmission of GIT to the captive wildlife.

In the present study, there was only a single case of *Toxocara cati* infection in a leopard, and the parasite load was low (Table 6). Similar findings of low prevalence of *Toxocara* sp. in captive leopards have been reported by Thawait *et al*. (2014), however the report indicated mixed infections of *Toxocara cati* and *Diphyllobothrium* sp. in the captive leopards, and the EPG was significantly higher than in the present study. Low prevalence of GIT parasite infection in leopards in this present study could be due to

well-maintained enclosure, as well as low number of animals within the enclosure. There was only single occurrence of coccidial infection in the tigers, and the parasite load was low, this contradicts a study by Aviruppola *et al*. (2016) who reported *Toxocara* infections in captive tigers in Sri Lanka with relatively low EPGs. A study by (Thawait *et al*. 2014) reported no infections in captive tigers; this could be an indication that tigers in captive environments are less prone to GIT parasite infections.

In this study, mixed infections of *Toxocara cati* and *Toxascaris leonina* were observed in lions and cheetahs with *T. leonina* always showing higher EPGs and prevalence as compared to *T. cati*. This phenomenon was also reported by Okulewicz *et al*. (2012) in a review on *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores. The dominance of one or the other species has been reported. When the prevalence of *T. canis* is very high e.g. 61.6% in Great Britain (Smith *et al*. 2003) or 81% in Denmark (Willingham *et al*. 1996) second species occurrence is very rare or not at all. In other reports, this phenomenon was noted, where the prevalence of *T. cati* and *T. leonina* where 37.5 % and 62.5 % respectively was observed in the Iberian wild lynx in Spain (Torres *et al.* 1998) and prevalence of 35.7 % and 8.8 % of *T. leonina* and *T. cati* in cats from Brazil (Labarthe *et al.* 2004). This further explains that, in a co-infection of *Toxocara* sp. and *T. leonina*, one will dominate while the other is rare or in low numbers. Co-occurrence of *Toxocara* sp. and *T. leonina* in the definitive hosts is highly variable and depends on several factors: climate, environmental conditions, age of the hosts, and the season (Pawar *et al*. 2012). This applies to infected wildlife as well as domestic animals. In unnatural conditions, such as zoo, where periodic treatment is carried out strictly, the transmission of *T. leonina* and *Toxocara* sp. occurs through rodents. Infected rodents, captured by carnivorous animals may contribute to *T. cati* and *T. leonina* infections resulting in significant epizootic problems (Okulewicz *et al*. 2012).

The spectrum of parasites encountered in captive lions in this study agrees with results of previous similar studies elsewhere (Varadharajan & Kandasamy 2000; Lim *et al*. 2008; Dehuri *et al*. 2013). These parasites differ from the reports on internal parasites of wild free-ranging lions in terms of parasite diversity. Free-ranging African lions feed on diverse prey species including wildebeest, zebra, several members of the antelope family, and warthogs, depending on season, and parasitism may be equally diverse, with some being true parasites and others spurious. This finding is expected more

often in free-ranging African lions than those in captivity as noted by various studies (Bjork *et al*. 2000)

No cestodes or trematodes were found in the faeces of the felids sampled in this study, the report is in agreement with study by whereby only nematodes and protozoan parasites such as *Toxocara canis*, *Toxocara cati*, *Toxascaris leonina*, *Aelurastrongylus* sp. *Ancylostoma* spp. *Gnathostoma* spp., *Linguatula* spp. *Physaloptera* spp. and *Acanthocephalan* spp. were detected in captive African lions at Recreation Park in Zimbabwe (Mukarati *et al*. 2013) however differing from previously reported findings by (Thawait *et al*. 2014), who reported mixed infections of nematodes and cestodes (*Toxocara* spp. and *Diphyllobothrium* spp.) in captive lions and leopards of Nandan van Zoo, Raipur, Chhattisgarh. Absence of cestodes and trematodes in the present study could be because of lack of intermediate hosts required by these parasites to complete their lifecycles.

The *T. leonina* is known to be parasitic in dogs and cats causing health problems (Pawar *et al*. 2012). The infection of dogs with *T. canis* is common; the larvae of *Toxocara canis* are capable of infecting humans, causing ocular larva migrans (OLM) and visceral larva migrans (VLM) (Despommier 2003). Larvae of *T. leonina* and *T. cati* are known to invade the tissues of laboratory animals and also have the zoonotic potential whereby they cause diseases associated with two main clinical syndromes in humans, namely, OLM and VLM (Despommier 2003; Fisher 2003)

In this study most of the infected faecal samples were from lion cubs. Young animals are more susceptible than adults, Emery *et al*. (2000) states that young animals appear less able to resist parasite establishment and expel existing worm burdens than the older animals, this could be a plausible explanation why the lion cubs remain affected by *T. leonina,* even after they have been treated with anthelminthics. And another explanation could be due to reinfection from the environment, as the parasites are resistant to extreme unfavourable conditions (Okulewicz *et al*. 2012). Elimination of *T. leonina* and *T. cati* from the zoo environment is very difficult. Lions in the Penjab zoo in India were treated by chemotherapy (0% prevalence) exhibited reinfection after 30 days of treatment (Singh *et al*. 2006). The higher prevalence of *T. leonina* in lion cubs might be due to the direct life cycle of *T. leonina* and the development of infective stages within a week (Dehuri *et al*. 2013). Parasitic disease studies of wild felids are

not only important with respect to the animal's health but also important for their zoonotic potential. The high prevalence in younger lions could be due to the age-related immunity where younger lions in captivity are more susceptible to the parasite than adults (Sharif *et al.* 2010)

Fully developed larvae appear in eggs of *T. canis* and *T. cati* within 2 – 3 weeks depending on environmental factors while larvae of *T. leonina* reach the infective stage in eggs in 8 – 9 days at 27°C and in 3 days at 30°C. Effects of various temperatures on the development of eggs of *T. leonina*, *T. canis* and *T. cati* have been investigated and it was observed that the eggs of *T. leonina* could adapt to a greater variety of climate conditions than those of the *Toxocara* sp. (Okulewicz *et al*. 2012). This could explain the *Toxascaris* infections observed in the felids even during extreme weather conditions in this study.

5.4. Gastro-intestinal parasites in avian species at NZG

Parasitic infections cause production losses to wild birds in captivity and often results in death (Hofstatter & Guaraldo 2015). In zoological gardens, birds are under constant stress and are prone to parasitic infections (Patel *et al*. 2000). In this study, only 19 out of 276 (7.7%) bird faecal samples were infected with GIT parasites, none of these had mixed infections, and no clinical signs were observed in any of the birds in the study. Papini *et al*. (2012) statistically revealed that zoo birds were about fifteen times more likely to develop mixed nematode infections than pet birds. Previous studies however are contradictory to our results and indicated gastro-intestinal parasites prevalence ranged from 11.1–51.9% in zoo birds in Turkey (Gurler *et al.* 2010), 48.1-71.4% in zoo birds in India (Patel *et al.* 2000; Parsani *et al.* 2001), and 51.6% of zoo birds in Spain (Perez-Cordon *et al.* 2009). Ajibade *et al*. (2010) recorded no infection of birds in OAU and UI zoos in Nigeria, while Opara *et al*. (2010) and Akinboye *et al*. (2010) reported prevalence of 76.6% and 61.5% in the Nekede and U.I zoos in Nigeria respectively. The prevalence of GIT parasites in zoo birds can be explained by factors such as housing and feeding, inconsistency in treatment program, or the existence of favourable climatic conditions (Otegbade & Morenikeji 2014). These factors contribute to prevalence in this manner; housing of the bird, if the enclosure is cemented or is it sand, is the food being scattered on the enclosure ground or served on a pan as well as inconsistency in treatment which could lead to resistance to the drugs, and

favourable conditions for the development of the parasitic eggs into some infective larvae. A study of Otegbade & Morenikeji (2014) also found that *Capillaria* sp. appeared to be among the most prevalent GIT helminths, and two protozoan parasites (Coccidia and *Balantidium* sp.) in captive birds in zoological gardens in south-west, Nigeria. The overall prevalence and parasitic load in this study, for the avian species was low, this could be due to the good management system at the bird section at the NZG. High prevalence and heavy parasitic load are not natural among captive birds (Hofstatter & Guaraldo 2015). Cordón *et al*. (2009) revealed that birds in zoos show high prevalence of parasites, thus indicating that captivity may facilitate transmission between individuals and also raise the parasitic load in the hosts.

Only nematodes were isolated in the avian species faecal samples. Among these, *Capillaria* sp. was the most common in the positive samples. Its presence was confirmed in other zoos as well, whereby, *C. plagiaticia* was isolated in the gut of a dead hyacinth macaw individual (Hofstatter & Guaraldo 2015). Furthermore, strongyles type egg, *Heterakis* sp. and an unidentified nematode were present in the in a batch of GIT parasite positive samples.

Only single suspicious death of Olive thrush occurred at the NZG during this study, which may be attributed to complications caused by other health problems. Post mortem reported a suspected case of intestinal parasite infestation; floatation technique however indicated unidentified egg with low parasite load. In the present survey, all infected birds (n = 19) had single infections, and none of them showed clinical signs (Table 7). The first report of GIT parasite infection since the beginning of the study in October 2015 was in March 2016 in a sun conure (*Aratinga solstitialis*) and blue fronted amazon (*Amazona aestiva*) which were infected with *Capillaria,* the samples remained infected for the rest of the study. These were from birds that have never been sampled before. Initially, only flamingo, vultures, stork and cranes were part of the study as per ethics application. However, as the study continued it was realiased they were always giving negative results for GIT parasites and therefore, from then on, other bird species from the zoo were sampled including chickens and geese from the farmyard. The birds that were normally sampled at NZG remained negative for any GIT parasites infections throughout the study.

All the parasites found have faecal-oral route of transmission. Thus, contaminated soil, food, and water play a key role as sources of parasite infection to birds under captivity conditions (Papini *et al*. 2012). *Capillaria* include species that infect the oral cavity, pharynx, oesophagus, crop, small intestine, or caecum. Intestinal infections are usually asymptomatic, but birds with heavy parasite burden may show clinical signs of anorexia, diarrhoea, emaciation, reduced water intake, ruffled feathers, and weakness (Yabsley *et al*. 2009)

In the present study only nematodal infections were recorded whilst coccidial infections were absent. The results contrast with similar studies by Patel *et al*. (2000) and Parsani *et al*. (2003) where both nematodal and coccidial infections were observed in captive birds. Low prevalence of helminths in the avian species in the present study could be attributed to the housing; most of the avian species at NZG are housed in either cemented or rocky sand ground with daily cleaning and raking, this could be attributed to the low prevalence of infection in the avian species. The birds in some instances do not have access to their droppings which may break the cycle of transmission (Egbetade *et al*. 2014).

5.4. Molecular identification of GIT parasites in NZG

Diagnosis of intestinal parasites by microscopy is facing several important methodological issues that concern the reliability of the analysis (Stensvold & Nielsen 2012). Because so many parasite species are present in faecal specimens in low quantities only, infections are often missed in a direct smear examination (Coleman et al. 2006). More promising developments in parasite diagnostics can be found in the field of molecular parasitology. Shortly after the development of polymerase chain reaction (PCR) in 1988 (Saiki *et al*. 1988), De Bruijn (1988) predicted this technique to become a valuable way of diagnosing parasitic diseases. Recently, a range of DNA based methods for the detection of intestinal parasites has been described and postulations have been made on the tremendous impact of the implementation of automated DNA isolation and combination of multiplex real-time PCR assays for the detection of parasites, viruses, and bacteria on the differential laboratory diagnosis of diarrhoeal diseases (Monis *et al*. 2005; Espy *et al*. 2006). In the present study, PCR was used to confirm findings by microscopy and to confirm the positive PCR results, DNA amplified products were subjected to sequencing using the forward primers. ITS2

has been widely used and has become a useful and reliable genetic marker for species identification because it is highly species specific, has more conservative regions and has universal primers binding to the 5.8S and 18S ribosomal DNA genes of several nematodes and trematodes (Mangkit *et al*. 2014).

Of the ungulates samples which were positive (76.98%) by microscopy, of that 47.76% were positive via PCR, 39.23% were PCR positive for *H. contortus,* 5.38%, were PCR positive for *O. ostertagii,* 45.38% were PCR positive for *Trichostrongylus* sp. and 10.01% were PCR positive for *Nematodirus spathiger*. Overall, the lower number of positive animals detected by PCR assays for *H. contortus, O. ostertagii,* and *N. spathiger* could be because of the low parasite loads (EPG) in these samples which warrants further optimization of PCR assays for detection of GIT parasites in faecal samples. The specificity of PCR restricts the number of different pathogens that can be detected, in contrast to the broad range of different parasites that can be detected using microscopy (Stensvold & Nielsen 2012). In this present study, species-specific primers were used for *Ostertagia ostertagii*, *Haemonchus contortus*, *Nematodirus spathiger*, rather than genus level primers, which may have contributed to the observed lower levels of detection via PCR. On the other hand, primers for *Trichostrongylus* were genus specific, and could have detected other species within the genus *Trichostrongylus*. The specificity of PCR obviously restricts the number of different pathogens that can be detected, in contrast to the broad range of different parasites that can be detected using microscopy (Hove *et al*. 2007).

The *H. contortus* is predominantly GIT parasite of sheep, goats (Tiwari *et al*. 2006) and wild ruminants. Adult worms inhabit the abomasum of its host (Tiwari *et al*. 2006) where they feed on blood and cause diseases. It is a cosmopolitan species but prefers and therefore occurs in large numbers in subtropical and tropical regions of the world (Waller & Chandrawathani 2005). In the present study, the *H. contortus* PCR positive samples were successfully sequenced and obtained sequences matched *H. contortus* ITS2- 28S rRNA gene available in Genbank with accession numbers KU870653.1 and JF680983.1. This further confirms that this strongyles-type egg isolated in this study is true positive. The *Haemoncus* sp. is one of the most common GIT nematodes at NZG and results in mortalities especially in sable antelopes (NZG Post-mortem report 2015).

5.5. Environmental factors and host-parasite relationship

There were seasonal changes in the GIT parasite burdens and this corresponded with the pattern of rainfall recorded in Pretoria during the present study at the NZG. The rainy season began in November and lasted until April with two peaks in January and March, the second being the higher of the two as shown in Figure 19 During these times average temperatures were 30˚C, which favours GIT parasite growth (Chattopadhyay & Bandyopadhyay 2013), and the average humidity was 80%. Wet weather coupled with humidity and suitable temperatures are favourable for availability and abundance of nematode larvae (Oluwayiose *et al*. 2013). The higher parasite load of infections observed during wet season could be attributed to amount of rainfall that enabled the eggs passed in faeces to develop easily into infective larvae and increase the rate of infection (Kusina *et al*. 1999). Environmental conditions play a major role in development, survival and translocation of the pre-parasitic stages of the nematodes; conditions are usually favourable during rainy season. Therefore, there is a gradual build-up of adult worm population in animals so that peak worm burdens are recorded at about the peak of the rainy season. Thereafter, worm populations gradually declined during the dry season, the parasites undergo hypobiosis during dry season as a way of adaptation (Reinecke 1983), and however peak in the increase of infections was observed again in the EPG of the animals in the dry season.

Nematode infections can be carried over from one favourable season to another within the host animal (Lee *et al*. 1960), which may explain the continued presence of parasites in the ungulates (Sable antelope, Lechwe, cape eland, Arabian oryx, springbok and gemsbok) and felids (cheetah and lion cubs) even during the dry season when the environmental conditions in the study area were not favourable for development and survival of these parasites. High prevalence during dry seasons can also be due to environmental factors such as drought the country was currently experiencing. The country's average rainfall for 2015 was the lowest since 1904, according to the South African Weather Service. The drought could have led to an increased stress and lowered immunity against GIT parasites (Sahoo *et al*. 2013) in the zoo animals. Lowered immunity may increase susceptibility to parasitic infections.

In winter, animals tend to have lowered immunity due to the stress induced by the cold conditions. Winter is energetically demanding. The stress of coping with energetically

demanding conditions can also indirectly cause illness and even result in death by compromising immune function (Nelson & Demas 1996).

It was expected that during the rainy season, the GIT parasite load (EPGs) would have been at its highest for all animals than during warm and wet season – this was, however, not the case, as there was sustained increase of EPG for most animals at the zoo from April – July 2016 (cold dry season). There was no logical explanation that could be ascribed for this paradox in the mean monthly/seasonal EPG of parasites identified in this study.

5.6. CONCLUSIONS

The aim of the study was to determine the seasonal prevalence of GIT parasites in captive wildlife using both microscopic and molecular (PCR) techniques. Samples were collected from selected captive wildlife at National zoological gardens of South Africa. A total of 779 faecal samples were collected and screened using both microscopic and molecular techniques. The overall prevalence of GIT parasite infections varied throughout the seasons in different animal groups. There were no clear-cut trends in seasonal prevalence that could be discerned for some of the parasites identified in the study. This study has detected strongyle-type eggs, *Strongyloides* sp., *Trichuris* sp. *Nematodirus* sp. *Moniezia* sp., *Calicophoron* sp. in ungulates, *T. cati* and *T. leonina* in the felids and *Capillaria* sp., *Heterakis* sp. *Strongyloides* sp. and *Trichostrongylus* sp. in avian species of NZG. This study has shown that PCR can be used as diagnostic tool for species confirmation of GIT parasites. Based on the two types of samples (faeces and cultured larvae): PCR assays can be used for future screening and diagnosis of GIT parasites in captive animals as well as all other wildlife in captivity across South Africa, however PCR assays for faeces samples work better if they are cultured and therefore have the pure DNA of the parasite.

Moreover, results from this study shows that captive wildlife at NZG are harbouring parasites some which are zoonotic and therefore can potentially infect the both zoo keepers and tourists, for example *T. leonina* and *T. cati* are common roundworms of cats can cause larva migrans syndromes in individuals who accidentally ingest the infective eggs from contaminated environments.

In zoos, there is an increased risk of parasite transmission from animals to visitors or keepers as a result of direct transmission or indirect transmission via contaminated food, water, and hands. Some of the GIT parasites detected in this study are amongst those known to represent human public health concerns (*Trichuris* spp.; Huffman *et al*. 2013), *Toxoscaris* sp. and *Toxocara* sp. (Despommier 2003; Fisher 2003).

Nematode helminths are prevalent in zoo collections (Egbetabe *et al*. 2014) and NZG is not an exception. Helminths coexist with wildlife with no obvious pathology; animals screened did not show any clinical signs which may suggest low parasitic infections (Krief *et al*. 2010). Assessment of health status of most captive wildlife at NZG is based

on physical outlook. Most animals appeared healthy without any signs of helminthic infection; therefore it is important to monitor the animals as physical assessment alone can be misleading at times. Faecal parasitology offers an insight into medical status of the animals. Helminthic infection can be managed provided that proper chemo-prophylaxis and strict hygiene standards are adhered to. Parsani *et al.* (2001) stated that some captive animals do show clinical signs due to parasites even if they are regularly dewormed and some will have no clinical signs even if they are never dewormed and this depends more on the parasite host interactions than deworming practices. Although overall management of zoo including nutrition, sanitation, and deworming practices was followed, the study identifies that there is scope for improvement in the management of the zoo by re-standardizing/re-investigating or re-scheduling anthelmintic program, regular examinations for parasitic infections and early season treatments to prevent infection.

5.7. RECOMMENDATIONS

Based on the observations of this study, the following future studies are recommended in order to obtain data that will ensure correct and effective management interventions for control of GIT parasites at NZG:

- Anthelmintic resistance studies must be conducted to assess the effectiveness of the currently used drugs for control of helminth infections at NZG.
- The role of feral birds, cats and rodents in contamination of NZG environment with GIT parasite eggs must be investigated.
- The role of Oribatid mites as intermediate hosts of cestodes at NZG environment must be investigated.
- Genotyping and genetic diversity studies of GIT parasites detected in this study needs to be conducted in order to gain understanding of the population types of GIT parasites detected in the current study in comparison to other populations in livestock and free-living wildlife.

REFERENCES

AAVP. 2014. *Toxocara cati* (Schrank, 1788) Brumpt, 1927. http://www.aavp.org/wiki/nematodes/ascaridida/toxocara-cati/. (Accessed on 17 July 2015).

ABOUZEID, N, Z,. SELIM, A, M,. EL-HADY, K, M,. 2010. Prevalence of gastro-intestinal parasites infections in sheep in the Zoo garden and Sinai district and study the efficacy of anthelmintic drugs in the treatment of these parasites. *Journal of American Science*. **6**: 544 – 551.

ADAMS, V, J,. MARKUS, M, B,. ADAMS, J, F,. JORDAAN, E,. CURTIS, B,. DHANSAY, M, A,. OBIHARA, C, C,. FINCHAM, J, E,. 2005. Paradoxical helminthiasis and giardiasis in Cape Town, South Africa: epidemiology and control. *African health Sciences*. **5**: 131 – 136.

ADENIYI, I, C,. MORENIKEJI, O, A,. EMIKPE, B, O,. 2015. The prevalence of gastro-intestinal parasites of carnivores in university zoological gardens in South West Nigeria. *Journal of Veterinary Medicine and Animal Health*.**7**: 135 -139.

AHMED, A,. GROBUSCH, M, P,. KLATSER, P, R,. HARTSKEERL, R, A,. 2011. Molecular approaches in the detection and characterization of Leptospira. *Journal of Bacteriology and Parasitology*. **3**: 133

AJIBADE, W, A,. ADEYEMO, O, K,. AGBEDE, S, A,. 2010. Coprological survey and invectory of animals at Obafemi Awolowo University and University of Ibadan zoological gardens. *World Journal of Zoloogy*. **5:** 266 – 271.

AKINBOYE, D, O,. OGUNFEITIMI, A, .A,. FAWOLE, O,. AGBOLADE, O,. AYINDE, O, O,. ATULOMAH, N, O, S,. AMOSU, A, M,. LIVINGSTONE, R,. 2010. Control of parasitic infections among workers and inmates in a Nigerian zoo. *Nigeria Journal of Parasitology.* **1**:35-38.

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS 2013. *Toxascaris leonina*. http://www.aavp.org/wiki/nematodes/ascaridida/toxascaris-leonina/. Accessed 09 October 2015.

APPLETON, C, C,. KRECEK, R, C,. VESTER, A,. BRUORTON, M, R,. LAWES, M, J,. 1994. Gastro-intestinal parasites of the Samango monkey*, Cercopithecus mitis*, in Natal, South Africa. *Journal of Medical Primatology*. **23**: 52 – 55.

AVIRUPPOLA, A, J, M, K,. RAJAPAKSE, R, P, V, J,. RUPIKA, S,. 2016. Coprological survey of Gastro-intestinal parasites of mammals in Dehiwala National Zoological Gardens, Sri Lanka. *Ceylon Journal of Science*. **45**: 83 – 96.

BAKER, M, K,. LANGE, L,. VERSTER, A,. VAN DER PLAAT, S,. 1989. A survey of helminths in domestic cats in the Pretoria area of Transvaal, Republic of South Africa. Part 1: The prevalence and comparison of burdens of helminths in adult and juvenile cats. *Journal of the South African Veterinary Association*. **60**: 139 – 142.

BANDYOPADHYAY, S,. MANDAL, S,. DATTA, K, K,. DEVI, P,. DE, S,. BERA, A, K,. BHATTACHARYA, D,. 2010. Economic analysis of risk of gastro-intestinal parasitic infection in cattle in North Eastern States of India. *Tropical Animal Health and Production*. **42**: 1481 – 1486.

BARRIGA, O,. 1994. Veterinary Parasitology. Columbus: Greyden Press.

BASSERT, J, M,. THOMAS, J,. 2014. McCurnin's Clinical Textbook for Veterinary Technicians. Elsevier Health Sciences.8th Ed.

BATH, G, F. VAN WYK, J, A,. 2001. Using the FAMACHA© system on commercial sheep farms in South Africa. *5th International Sheep Veterinary Congress, Stellenbosch, South Africa, 21-25 January 2001.*

BELDOMENICO, P.M., BEGON, M., 2010. Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecology and Evolution*. **25**: 21 – 27. http://dx.doi.org/ 10.1016/j.tree. A2009.06.015.

BELGRADER, P,. BENETT, W,. HADLEY, D,. RICHARDS, J,. STRATTON, P,. MARIELLA JR, R,. MILANOVICH, F,. 1999. PCR Detection of bacteria in seven minutes. *Science*. **284**: 449 – 450.

BERENTSEN, A, R,. BECKER, M, S,. STOCKDALE-WALDEN, H,. MATANDIKO, W,. MCROBB, R,. DUNBAR, M, R,. 2012. Survey of Gastro-intestinal parasite infection in African lion (*Panthera leo*), African wild dog (*Lycaonpictus*) and spotted hyaena (*Crocutacrocuta*) in the Luangwa Valley, Zambia. *African Zoology*. **47**: 363 – 368.

BJORK, K, E,. AVERBECK, G, A,. STROMBERG, B, E,. 2000. Parasites and Parasite stages of free-ranging wild lions (*Pathera leo*) of Northern Tanzania. *Journal of Zoo and Wildlife Medicine*. **31**: 56 – 61.

BOOMKER, J,. 2014. Helminth infections of ruminants. http://www.afrivip.org/sites/default/files/01_helminth_infections_complete-1.pdf. Accessed 10 June 2016

BOOMKER, J,. HORAK, I, G,. FLAMAND, J, R, B,. 1991. Parasites of South African Wildlife. XII. Helminths of nyala, *Tragelaphus angasi*, in Natal. *Ondersterpoort Journal of Veterinary Research.* **58**: 275 – 280.

BOOMKER, J,. HORAK, I, G,. MACIVOR, K, M,. DE F. 1989. Helminth parasites of grysbok, common duikers, and Angora and Boer goats in the Valley Bushveld in the Eastern Cape Province. *Ondersterpoort Journal of Veterinary Research*. **56**: 165 – 172.

BORAY, P, A,. STRONG, M, B,. ALLISON, J, R,. VON OREILLI, M,. SARASIN, G,. GFELLER, W,. 2008. Nitroscanate: a new broad spectrum anthelmintic against nematodes of cats and dogs. *Australian Veterinary Journal*. **55**: 45 – 53.

BORKOVCOVA, M,. KOPRIVA, J,. 2005. Parasitic helminths of Reptiles (Reptilia), South Moravia, Czech Republic. *Journal of Parasitology Research*. **95**: 77 – 78.

BOTT, N.J., CAMPBELL, B.E., BEVERIDGE, I., CHILTON, N.B., REES, D., HUNT, P.W., GASSER, R.B., 2009. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep, *International Journal for Parasitology*. **39**: 1277 – 1287.

BOWMAN, D, D,. HENDRIX, C, M,. LINDSAY, D, S,. BARR, S, C,. 2002. Feline Clinical Parasitology. Iowa State University Press. Iowa, United States of America.

BUSH, B, M,. 1975 Veterinary Laboratory Manual. Collection and Examination of Faeces. William Heinemann medical books limited. United Kingdom. London. 1st Ed. Pg 318-319

CARDINI, G,. GRASSOTTI, G,. PAPINI, R,. VALLE, V, C,. 1997. Anthelmintic activity and tolerance of a new micronized mebenzole pharmaceutical formulation (Lendue) for treating intestinal helminthoses in dogs and cats. *Veterinaria Cremona*. **11**: 125 – 131.

CENTERS FOR DISEASE CONTROL AND PREVENTION 2013.DPDx – Laboratory Identification of Parasitic Diseases of Public Health Concern,

Trichostrongylosis.http://www.cdc.gov/dpdx/trichostrongylosis/index.html. Accessed 09 October 2015.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 2013. Parasites – Trichuriasis (also known as Whipworm Infection). http://www.cdc.gov/parasites/whipworm/epi.html. Accessed 10 June 2016.

CHAKRABORTY, A,. GOGOI, A, R,. CHOUDHARY, B,. 1994. Prevalence of parasitic infection in captive wild herbivores in a zoo in Assam, India. *The Indian Journal of Animal Sciences*. **9**: 149 – 152.

CHATTOPADHYAY, A, K,. BANDYOPADHYAY, S,. 2013. Seasonal variations of EPG levels in gastro-intestinal parasitic infection in a southeast Asian controlled locale: a statistical analysis. *SpringerPlus*. **2**: 205.

CHIEJINA, S, N,. BEHNKE, J, M,. FAKAE, B, B. 2015. Haemonchotolerance in West African Dwarf goats: contribution to sustainable, anthelmintics-free helminth control in traditionally managed Nigerian dwarf goats. *Parasite*. **22**: 7

COLDITZ, I, G,. 2008a. Challenges to the development of new tests for diagnosis of infection and prediction of resistance of sheep to gastro-intestinal nematodes. *Tropical Biomedicine*. **25**: 41 – 49.

COLDITZ, I.G. 2008b. Six costs of immunity to gastro-intestinal nematode infections. *Parasite Immunology*. **30**: 63 – 70.

COLLEY, D. G. BUSTINDUY, A. L. SECOR, W. E. KING, C. H. 2014. Human schistosomiasis. *Lancet*. **383**: 2253 – 2264.

CONTI, J, A,. HOWERTH, E, W,. 1987. Ostertagiosis in a whitetailed deer due to *Ostertagia ostertagi*. *Journal of Wildlife Diseases*. **23**:159 – 162.

CORDON, G, P,. PRADOS, A, H,. ROMERO, D,. MORENO, M, S,. PONTES, A,. OSUNA, A,. ROSALES, M, J,. 2008. Intestinal parasitism in the animals of the zoological garden “Pen˜a Escrita” (Almuñecar, Spain). *Veterinary Parasitology*. **156**: 302 – 309.

DEHURI, M, M, R,. PANDA, B, N,. MOHNTY, SAHOO, N,. 2013. Prevalence and evaluation of anthelmintics against nematodes in Lions (*Panthera leo*) in Nandakanan zoo. *Journal of Wildlife Research*. **1**: 5 – 7.

DEMELER, J,. RAMÜNKE, S,. WOLKEN, S,. LANIELLO, D,. RINALDI, L,. GAHUTU, J, B,. CRINGOLI, G,. VON SAMSON-HIMMELSTJERNA, G,. KRÜCKEN, J,. 2013. Discrimination of gastro-intestinal Nematode Eggs from Crude Fecal Egg

Preparations by Inhibitor-Resistant Conventional and Real-Time PCR. PLoS ONE. 8(4): e61285. doi:10.1371/journal.pone.0061285

DENEGRI, G,. BERNADINA, W,. PEREZ-SERRANO, J,. RODRIGUEZ-CAABEIRO, F,. 1998. Anoplocephalid cestodes of veterinary and medical significance: a review. *Folia Parasitologica.* **45**: 1 – 8.

DESPOMMIER, D,. 2003. Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Review*. **16**: 265 – 272.

DONG, H,. ZHAO, Q,. HAN, H,. JIANG, L,. ZHU, S,. LI, T,. KONG, C,. HUANG, B,. 2002. Prevalence of coccidial infection in dairy cattle in Shanghai, China. *The Journal of Parasitology*. **98**: 963 – 966.

DOUGHTY, B, L,. 1996. Schistosomes and Other Trematodes. *Medical microbiology*. 4th Ed. The University of Texas Medical Branch at Galveston

DREYER, K,. FOURIE, L, J,. KOK, D, J,. 1999. Gastro-intestinal parasites of cattle in the communal grazing system of Botshabelo in Free State. *Ondersterpoort Journal of Veterinary Research*. **66**: 145 – 149.

EGBETADE, A,. AKINKUOTU, O,. JAYEOLA, O,. NINIOLA, A,. EMMANUEL, N,. OLUGBOGI, E,. ONADEKO, S,. 2014. Gastro-intestinal helminths of resident wildlife at the Federal University of Agriculture Zoological Park, Abeokuta. *Sokoto Journal of Veterinary Sciences*.**12**: 26 – 31.

EMIKPE, B, O,. ADENIRAN, G, A,. ALAKA, O, O,. OHORE, O, G,. ANTIA, R, E,. AJAYI, O, L,. OMOBOWALE, O, T,. 2007. Valvular endocarditis in a captive monkey in Idaban, Nigeria: a case report. *Nigerian Veterinary Journal*. **28**: 49 – 52.

ESPY, M, J,. UHL, J, R,. SLOAN, L, M,. BUCKWALTER, S, P,. JONES, M, F,. VETTER, J, D, C,. YAO, N, L,. WENGENACK, J, E,. ROSENBLATT, J, E,. COCKERILL, F, R,. SMITH, T, F,. 2006. Real-Time PCR in Clinical Microbiology: Applications for routine laboratory testing. *Clinical Microbiology Reviews*. **19**: 165 – 256.

EZENWA, V.O. 2004. Parasite infection rates of impala (*Aepyceros melampus*) in fenced game reserves in relation to reserve characteristics. *Biological Conservation*. **118**: 397–401.

FAGIOLINI, M., LIA, R.P., LARICCHIUTA, P., CAVICCHIO, P.,MANNELLA, R., CAFARCHIA, C., OTRANTO, D., FINOTELLO, R. PERRUCCI, S. 2010.

Gastro-intestinal parasites in mammals of two Italian zoological gardens. *Journal of Zoo and Wildlife Medicine.* **41**: 662–670.

FISHER, M,. 2003. *Toxocara cati*: an underestimated zoonotic agent. *Trends in Parasitology*. **19**: 167 – 170.

FOX, J, E,. 1985. Coccidiosis in cattle. *Modern Veterinary Practice.* **66**: 113 – 116.

Gastrointestinal parasites of birds in zoological gardens in south-west Nigeria (PDF Download Available). Available from: https://www.researchgate.net/publication/262644483_Gastrointestinal_parasites_of_birds_in_zoological_gardens_in_south-west_Nigeria [accessed Jun 18, 2017].

GELDHOF, P,. KNOX, D,. 2008. The intestinal contortin structure in *Haemonchus contortus*: an immobilised anticoagulant? *International Journal for Parasitology*. **38**: 1579 – 1588.

GHASEMIKHAH, R,. MIRHENDI, H,. KIA, E, B,. MOWLAVI, G, H,. SARMADIAN, H,. MESHGI, B,. GOLESTAN, B,. MOBEDI, I,. 2011. Morphological and Morphometrical Description of *Trichostrongylus* species Isolated from Domestic Ruminants in Khuzestan Province, Southwest Iran. *Iranian Journal of Parasitology*.**6**: 82 – 88.

GHOLAM, S,. BABAMAHMOODI, F,. ABEDIAN, R,. SHARIF, M,. SHAHBAZI, A,. PAGHEH, A,. FAKHAR, M,. 2015. *Trichostrongylus colubriformis*: Possible most common cause of human infection in Mazandaran Province, North of Iran. *Iranian Journal of Parasitology*. **10**: 110 – 115.

GILFILLAN, C,. 2010. Conical Fluke (*Calicophoron microbothrium*) Control. Afrivet. (http://www.afrivet.co.za/downloads/articles/Conical%20Fluke%20(Calicophoron%20microbothrium)%20Control.pdf). Accesed 15 July 2016.

GOOSSENS, E,. DORNY, P,. BOOMKER, J,. VERCAMMEN, F,. VECRUYSSE, J,. 2005. A 12-month survey of the gastrointestinal helminths of antelopes, gazelles and giraffids kept at two zoos in Belgium. *Veterinary Parasitology*. **127**: 303 – 312.

GORSICH, E, E,. EZENWA, V, O,. JOLLES, A, E,. 2014. Nematode-coccidia parasite co-infections in African buffalo: Epidemiology and associations with host conditions and pregnancy. *International Journal for Parasitology: Parasites and Wildlife*. **3**: 124 – 134

GUL, N,. TAK, H,. 2014. Prevalence of *Trichuris* spp. in small ruminants slaughtered in Srinagar District (J&K). *Journal of Parasitic Diseases*. **40**: 741 – 744.

GURLER, A, T,. BEYHAN, Y, E,. ACICI, M,. BOLUKBAS, C, S,. UMUR, S,. 2010. Helminths of mammals and birds at the Samsun Zoological Garden, Turkey. *Journal of Zoo and Wildlife Medicine*. **2**: 218 – 223.

HALE, M., 2006. Managing internal parasites in sheep and goats. *Attra*. 1 – 8

HELKE, K, L,. COOPER, T, K,. MANKOWSKI, J, L,. POYNTON, S, L,. 2006. Disseminated visceral coccidiosis indo-gangetic flap-shelled turtles, *Lissemys punctate andersonii. Journal of Wildlife Diseases*. **42**: 788 – 796.

HERD, R, P,. MCNAUGHT, D, J,. 1975. Arrested development and the histotropic phase of Ascaridia galli in the chicken. *International Journal for Parasitology*. **5**: 401 – 406.

HOBERG, E, P,. KOCAN, A, A,. RICKARD, L, G,. 2001. Gastro-intestinal Strongyles in Wild Ruminants. *Faculty Publications from the Harold W. Manter Laboratory of Parasitology*. Paper 623 .http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1633&context=parasitologyfacpubs. Accessed 09 October 2015.

HOFSTATTER, P, G,. GUARALDO, A, M, A,. 2015. Parasitological survey on birds at some selected Brazilian zoos. *Brazilian Journal Veterinary Parasitology*. **24**: 87 – 91.

HOORMAN, J, J,. 2011. The role of souil protozoa and nematodes. Fact sheet: Africulture and natural resources. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.231.647&rep=rep1&type=pdf. (Accessed on 16 July 2017)

HORAK, I, G,. DE F. MACIVOR, K, M,. GREEFF, C, J,. 2001. Parasites of domestic and wild animals in South Africa. XXXIX. Helminth and arthropod parasites of Angora goats in the southern Karoo. *Ondersterpoort Journal of Veterinary Research.* **68**: 27 – 35

HUFFMAN, M,. NAHALLAGE, C,. HASEGAWA, H,. EKANAYAKE, S,. DE SILVA, L,. ATHAUDA, I,. 2013. Preliminary survey of the distribution of four potentially zoonotic parasite species among primates in Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*. **41**:319 – 326.

HYNES, F,. 2015. *Nematodirus* in Young Lambs. https://www.teagasc.ie/media/website/about/farm-advisory/Nematodirus-Article_April-21-2015.pdf. Accessed 16 October 2016

JACOBS, D, E,. ZHU, X,. GASSER, R, B,. CHILTON, N, B,. 1997. PCR-based methods for identification of potentially zoonotic ascaridoid parasites of the dog, fox and cat. *ActaTropica.* **68**: 191–200

JORGENSEN, L, T,. LEATHWICK, D, M,. CHARLESTON, W, A,G,. GODFREY, P, L,. VLASSOFF, A,. SUTHERLAND, I, A,. 1998. Variation between hosts in the developmental success of the free-living stages of trichostrongyle infections of sheep. *International Journal of Parasitology*. **28**: 1347 – 1352.

JUNQUERA, P,. 2015. *Trichostrongylus* species, parasitic roundworms of CATTLE, SHEEP, GOATS, PIGS and HORSES. Biology, prevention and control; *Trichostrongylus colubriformis, Trichostrongylus axei*. *Parasitipedia.net*. (http://parasitipedia.net/index.php?option=com_content&view=article&id=2628&Itemid=2908). Accessed 06 May 2016.

KAJEROVA, V,. BARUS, V,. LITERAK, I,. 2004. Nematodes from the genus Ascaridia parasitizing psittaciform birds: A review and determination key. *International Journal for Veterinary and Biomedical Science*.**49**: 217 – 223.

KATOCH, R,. YADAV, A,. GODARA, R,. KHAJURIA, J, K,. BORKATAKI, S,. SODHI, S, S,. 2012. Prevalence and impact of gastro-intestinal helminths on body weight gain in backyard chickens in subtropical and humid zone of Jammu, India. *Journal of Parasitic Diseases*. **36**: 49 – 52.

KAUFMANN, J,. 1996. Parasitic infection of Domestic Animals: A Diagnostic Manual. Birkhäuser. Berlin. Germany.

KRIEF, S,. VERMUELEN, B,. LAFOSSE, S,. KASENENE, J, M,. NIEGUITSILA, A,. L'HOSTIS, M,. BAIN, O,. GUILLOT, J,. 2010. Nodular worm infection in wild chimpanzees in western Uganda: A risk for human health. *PLoS Neglected Tropical Diseases.* **16**: e630.

KUCHAI, J, A,. AHMAD, F,. CHISHTI, M, Z,. DAR, J, A,. TAK, H,. 2013. On morphology and morphometry of *Trichuris ovis* Abildgaard, 1795 Recovered from ruminants of Ladakh, India. *Journal of Buffalo Science*. **2**: 49 – 52.

KUMAR, A,. SINGLA, L,D,. SINGLA, N,. AULAKH, G, S,. SINGH, J,. 2005. Managemment of piperazine resistance toxocariosis with ivermectin in lions (*Panthera leo*). *Journal of Parasitic diseases*. **29**: 156-160.

KUMAR, R, R,. VATSYA, S,. YADAV, C, L,. SINGH, V, S,. 2006. Economic Impact of Helminth infestation control on Milk Production in Cattle. *Journal of Veterinary Parasitology*. **20**: 133 – 136.

KUSINA, N, T,. KAHIYA, C,. MUKARATIRWA, S,. CHIKURA, S,. SIBANDA, S,. 1999. Seasonal patterns in parasitic infection in Zimbabwean Communal areas. *Zimbabwe Veterinary Journal*. **30**: 41 – 54.

LABARTHE, N, SERRAO, M, L,. FERREIRA, A, M,. ALMEIDA, N, K,. GUERRERO, J, A,. 2004. Survey of gastrointestinal helminths in cats of the metropolitan region of Rio de Janeiro, Brazil. *Veterinary Parasitology*. **123**: 133 – 139.

LARSEN, J,. 2007. *Ostertagia* in cattle. http://agriculture.vic.gov.au/agriculture/pests-diseases-and-weeds/animal-diseases/beef-and-dairy-cows/ostertagia-in-cattle. Accessed 29 September 2015

LEE, R, P,. ARMOUR, J, ROSS, J, G,. 1960. The seasonal variations in strongyle infections in Nigeria Zebu cattle. *Brittish Veterinary Journal*. **116**: 1 – 13.

LEVINE N.D. 1985. *Veterinary Protozoology.* Iowa State University Press, Ames,Iowa.

LI, M,. ZHAO, B,. LI, B,. WANG, Q,. NIU, L,. DENG, J,. GU, X,. PENG, X,. WANG, T,. YANG, G,. 2015. Prevalence of gastro-intestinal parasites in captive non-human primates of twenty-four zoological gardens in China. *Journal of Medical Primatology*. **44**: 168 – 173.

LIČINA, T,. 2014. Gastro-intestinal parasites in moose (Alcesalces) – which ones and what consequences? http://brage.bibsys.no/xmlui/bitstream/id/243455/Tina%20Licina.pdf. Accessed 05 September 2015.

LIM, Y, A, L,. NGUI, R,. SHUKRI, J,. ROHELA, M,. MAT NAIM, H, R,. 2008. Intestinal parasites in various animals at a zoo in Malaysia. *Veterinary Parasitology*. **157**: 154 – 159.

LOVE, S, C, J,. HUTCHINSON, G, W,. 2003. Pathology and diagnosis of internal parasites in ruminants. *In Gross Pathology of Ruminants*. **16**: 309 – 338.

MABE, L, T,. 2012. Development of molecular diagnostic methods (LAMP and PCR) for detection of *Haemonchus contortus, Fasciola* spp. and *Trichostrongylus* spp. infections in livestock. http://etd.uovs.ac.za/ETD-db/theses/available/etd-02282014-114441/unrestricted/MabeLT.pdf. Accessed 09 July 2015.

MACHEN, R,. CRADDOCK, F,. GRAIG, T,. FUCHS, T,. 2002. A *Haemonchus contortus* Management Plan for Sheep and Goats in Texas. *AgriLife Extension*.

http://animalscience-extension.tamu.edu/publications/13268717-haemonchus.pdf. Accessed 23 April 2016.

MACPHERSON, C, N,. 2013. The epidemiology and public health importance of toxocariasis: A zoonosis of global importance. *International Journal of Parasitology*. **43**: 999 – 1008.

MAFF. 1980. Manual of veterinary parasitological techniques. Technical Bulletin 18. London, Ministry of AgricultureFisheries and Food.

MAGNAVAL, J, F,. GLICKMAN, L, T,. DORCHIES, P,. MORASSIN, B,. 2001. Highlights of human toxocariasis. *Korean Journal of Parasitology*.**39**: 1–11.

MAHMOUD, M, A,. 2015. Prevalence of some pathogens in a population of zoo animals. *Alexandria Journal of Veterinary Sciences*. **45**: 139 – 145.

MAICHOMO, M, W,. KAGIRA, J, M,. WALKER, T,. 2004. The point prevalence of gastro-intestinal parasites in calves, sheep and goats in Magadi division, south-western Kenya. *Onderstepoort Journal of Veterinary Research*. **71**: 257 – 261.

MANGKIT, B,. THAENKHAM, U,. ADISAKWATTANA, P,. WATTHANAKULPANICH, D,. JANTASURIYARAT, C,. KOMALAMISRA, C,. 2014. Molecular characterization of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from small ruminants in Thailand based on the second internal transcribed spacer of Ribosomal DNA. *Kasetsart Journal*. **48**: 740 – 758.

MATJILA, P, T,. PENZHORN, B, L,. 2002. Occurrence and diversity of bovine coccidia at three localities in South Africa. *Veterinary Parasitology*. **104**: 93 – 102.

MAWSON, P, M,. 1985. Nematodes (*Ascaridia* species) from some captive and feral parrots. *South Australian Ornithologist*. **29**: 190 – 191.

MEDEROS, A,. FERNANDEZ, S,. PEREGRINE, A,. MENZIES, P,. VANLEEUWEN, J,. LEBOEUF, A,. KELTON, D. MARTIN, R,. 2007. Study of the population dynamics of gastrointestinal nematodes in organic sheep farms in Canada with an attempt to introduce sustainable parasite control measures. http://www.organicagcentre.ca/Docs/Parasite_Report_2007.pdf. Accessed 16 July 2017

MEHLHORN, H. 2015. Encyclopedia of Parasitology: *Ascaridia Species of Birds*. Springer Berlin Heidelberg. Berlin. Germany

MELANCON, J, J,. 2001. Nematodirus Infections in cattle. Merial Veterinary Bulletin Bulletin.

http://us.merial.com/pdf/page_pdf/nematodirus_infections_in_cattle.pdf. Accessed 15 July 2016.

MINNAAR, W, N,. KRECEK, R, C,. FOURIE, L, J,. 2002. Helminths in dogs from a peri-urban resource-limited community in Free State Province, South Africa. *Veterinary Parasitology*. **107**: 343 – 349.

MIR, A, Q,. DUA, K,. SINGLA, L, D,. SHARMA, S,. SINGH, M, P,. 2016. Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh mini zoo (Deer Park), Pataila, Punjab. *Veterinary World*. **9**: 540 – 543.

MONIS, P, T,. GIGLIO, S,. SAINT, C, P,. 2005. Comparison of SYTO9 and SYBR Green I for real-time polymerase chain reaction and investigation of the eVect of dye concentration on amplication and DNA melting curve analysis. *Analytical Biochemistry*. **340**: 24 – 34.

MUKARATI, N, L,. VASSILEV, G, D,. TAGWIREYI, W, M,. TAVENGWA, M,. 2013. Occurrence, prevalence and intensity of internal parasite infections of African lions (*Panthera leo*) in enclosures at a recreation park in Zimbabwe. *Journal of Zoo and wildlife medicine.* **44**: 686 – 693.

MUKARATIRWA, S,. SINGH, V, P,. 2010. Prevalence of Gastro-intestinal parasites of stray dogs impounded by the Society for the Prevention of Cruelty to Animals (SPCA), Durban and Coast, South Africa. *Journal of the South African Veterinary Association*. **81**: 123 – 125.

MUNGUBE, E, O,. WAMAE, L, W,. OMONDI, G, A,. MWANGI, G,. 2015. Prevalence of multiple resistant *Haemonchus* and *Ostertagia* species in goats and cattle in Machakos, Eastern Kenya. *Livestock Research for Rural Development*. **27**(12)

MYERS, G, H,. TAYLOR, R, F,. 1989. Ostertagiasis in cattle. *Journal of Veterinary Diagnostic Investigation*. **1**: 195 – 200.

NALUBAMBA. K, S,. MUDENDA, N, B,. MALAMO, M, R,. 2012. A seasonal survey of Gastro-intestinal parasites in captive wild impala antelope on a game facility south of Lusaka, Zambia. *Journal of Helminthology*. **86**: 418 – 425.

NCBI: http://www.ncbi.nlm.nih.gov/BLAST

NELSON, R, J,. DEMAS, G, E,. 1996. Seasonal changes in immune function. *The Quarterly Review of Biology.* **71**: 511 – 548.

NEWTON, C, R,. GRAHAM, A,. 1997. PCR. 2nd Edition. BIOS Scientific Publishers. United Kingdom, England.

NIELSEN, M, K,. KAPLAN, R, M,. THAMSBORG, S, M,. MONRAD, J,. OLSEN, S, N,. 2007. Climatic influences on development and survival of free-living stages of equine strongyles: Implications for worm control strategies and managing anthelmintic resistance. *The Veterinary Journal*. **174**: 23 – 32.

NTONIFOR, H, N,. SHEI, S, J,. NDALEH, N, W,. MBUNKUR, G, N,. 2013. Epidemiological studies of Gastro-intestinal parasitic infections in ruminants in Jakiri, Bui Division, North West Region of Cameroon. *Journal of Veterinary Medicine and Animal Health*. **5**: 344 – 352.

OETJEN B.D. 1993. Management of coccidiosis in dairy calves and replacement heifers. *Compendium on Continuing Education for the Practicing Veterinarian.* **15:** 891 – 895.

OKULEWICZ, A,. PEREC-MATYSIAK, A,. BUNKOWSKA, K,. HILDEBRAND, J,. 2012. *Toxocaracanis*, *Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores. *Helminthologia*. **49**: 3 – 10.

OLIVER, A-M,B,. LEATHWICK, D, M,. POMROY, W, E,. 2014. A survey of the prevalence of *Nematodirus spathiger* and *N. filicollis* on farms in the North and South Islands of New Zealand. *New Zealand Veterinary Journal*. **62** (5): 286 – 289.

OLUWAYIOSE, O,A,. JEJE, O,. MORENIKEJI, O,A,. EMIKPE, B,O,. 2014. Prevalence of gastrointestinal parasites of goats fed to captive animals at the University of Ibadan Zoological gardens*. Nigerian Veterinary Journal*. **34**: 836–840.

OPARA, M, N,. OSUJI, C, T,. OPERA, J, A,. 2010. Gastro-intestinal parasitism in captive animals at the Zoological Garden Nekede, Owerri, Southeast Nigeria. *Report and Opinion*. **2**(5): 21 – 28.

OTEGBADE, A, C,. MORENIKEJI, O, A,.2014. Gastro-intestinal parasites of birds in zoological gardens in South-West Nigeria. *Tropical Biomedicine Journal*. **31**: 54 – 62.

OTRANTO, D,. CANTACESSI, C,. DANTAS-TORRES, F,. BRIANTI, E,. PFEFFER, M,. GENCHI, C,. GUBERTI, V,. CAPELLI, G,. DEPLAZES, P,. 2015. The role of wild canids and felids in speading parasites to dogs and cats in Europe. Part II: Helminths and arthropds. *Veterinary Parasitology*. **213**: 24 – 37.

PADWAL, N,. NUMBE, A,. JADHAV, S,. BORDE, S, N,. 2011. Seasonal variation of intestinal *Trichuris* sp. in sheep and goats from Maharashtra State. *International Multidisciplinary Research Journal*. **1**: 17 – 18.

PANAYOTOVA-PENCHEVA, M, S,. 2013. Parasites in captive animals: A Review of Studies in Some European Zoos. *Der Zoologische Garten*. **82**: 60 – 71.

PAPAZAHARIADOU, M,. FOUNTA, A,. PAPADOPOULOS, E,. CHLIOUNAKIS, S,. ANTONIADOU-SOTIRIADOU, K,. THEODORIDES, Y,.2007. Gastro-intestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece.*Veterinary Parasitology*. **148**: 170 – 173.

PAPINI, R,. GIRIVETTO, M,. MARANGI, M,. MANCIANTI, F,. GIANGASPERO, A,. 2012. Endoparasite infections in Pet and Zoo birds in Italy. *The Scientific World Journal.* **2012**: doi:10.1100/2012/253127

PAPPAS, L, A,. 2002. *Taurotragus oryx*. *Mammalian species*. **689**:1 – 5.

PARK, S,. SHIN, S,. 2010. Concurrent *Capillaria* and *Heterakis* Infections in Zoo Rock Partridges, *Alectorisgraeca*. *Korean Journal of Parasitology*. **48**: 253 – 257.

PARSANI, H, R,. MORMIN, R, R,. MARADIA, M, G,. VEER, S,. 2001. A survey of gastrointestinal parasites of captive animals at Rajkot Municipal Corporation Zoo, Rajkot, Gujarat. *Zoo's Print Journal*. **16**: 604 – 606.

PATEL, P, V,. PATEL, A, I,. SAHU, R, K,. VYAS, R,. 2000. Prevalence of gastrointestinal parasites in captive birds of Gujarat zoos. *Zoo's Print Journal*. **15**: 295 – 296.

PAWAR, R, M,. LAKSHMIKANTAN, U,. HASAN, S,. POORNACHANDAR, A,. SHIVAJI, S,. 2012. Detection and molecular characterization of ascarid nematode infection (*Toxascaris leonina* and *Toxocara cati*) in captive Asiatic lions (*Pantheraleopersica*). *Acta Parasitologica.* **57**: 67–73.

PECSON, B, M,. BARRIOS, J, A,. JOHNSON, D, R,. NELSON, K, L,. 2006. A real-time PCR method for quantifying viable *Ascaris* eggs using the first Internal Transcribed Spacer region of Ribosomal DNA. *Applied and Environmental Microbiology.***72**: 7864 – 7872.

PEREZ-CORDON, G,. HITOS PRADOS, A,. ROMERO, D,. 2009. Intestinal and haematic parasitism in the birds of the Almu˜Necar (Granada, Spain) Oornithological Garden. *Veterinary Parasitology* **3-4**: 361 – 366.

PERMIN, A,. HANSEN, J, W,. 1998. Epidemiology, diagnosis and control of poultry parasites. Food and Agriculture Organization of the United Nations. Rome. Italy

PETRIGH, R,S,. FUGASSA, M, H,. 2014. Molecular identification of *Nematodirus spathiger* (Nematoda: Molineidae) in *Lama guanicoe* from Patagonia, Argentina. *Helminthologia*. **51**: 79 – 82.

PFUKENYI, D, M,. MUKARATIRWA, S,. 2013. A review of the epidemiology and control of gastro-intestinal nematode infections in cattle in Zimbabwe. *Onderstepoort Journal of Veterinary Research.* **80**(1): 612

PIENAAR, J, G,. BASSON, P, A,. DU PLESSIS, J, L,. MARIA COLLINS, H,. NAUDE, T, W,. BOYAZOGLU, P, A,. BOOMKER, J,. REYERS, F,. PIENAAR, W, L,. 1999. Experimental studies on *Strongyloides papillosus* in goats. *Onderstepoort Journal of Veterinary Research*. **66**: 191 – 235.

PRESTON, S, J, M,. SANDEMAN, M,. GONZALEZ, J,. PIEDRAFITA, D,. 2014. Current status for gastro-intestinal nematode diagnosis in small ruminants: Where are we and where are we going? *Journal of Immunology Research*. doi.org/10.1155/2014/210350

QAMAR, M, F,. MAQBOOL, A,. KHAN, S, M,. AHMAD, N,. MUNEER, M, A,. 2009. Epidemiology of Haemonchosis in sheep and goats under differential managemental conditions. *Veterinary World*. **2**: 413 – 417.

RAHARIVOLOLONA, B, M,. GANZHORN, J, U,. 2010. Seasonal variations in gastrointestinal parasites excreted by the gray mouse lemur Microcebus murinus in Madagascar. *Endangered Species Research*. **be**: 113 – 122.

RAHMAN, W, A,. HAMID, S, A,. 2007. Morphological characterization of *Haemonchus contortus* in goats (*Capra hircus*) and sheep (*Ovis aries*) in Penang, Malaysia. *Tropica Biomedicine*. **24**: 23 – 27.

RALPH, A,. O'SULLIVAN, M, V, N,. SANGSTER, N, C,. WALKER, J, C,. 2006. Abdominal pain and eosinophilia in suburban goat keepers – trichostrongylosis. *Medical Journal of Australia.***184**: 467 – 469.

REINECKE, R., 1983. Veterinary Helminthology, Butterworths, Durban, pp 392

REPERANT, L, A,. HEGGLIN, D,. FISCHER, C,. KOHLER, L,. WEBER, M,. DEPLAZES, P,. 2007. Influence of urbanization on the epidemiology of intestinal helminths of the red fox (*Vulpesvulpes*) in Geneva, Switzerland. *Parasitology Research*.**101**: 605 – 611.

ROBLES, M, dR. CUTILLAS, C,. PANEI, C, J,. CALLEJON, R,. 2014. Morphological and molecular characterization of a new *Trichuris* species (Nematoda-Trichuridae), and Phylogenetic Relationships of *Trichuris* species of Cricetid

rodents from Argentina. *PLoS ONE*. **9**: e112069. doi:10.1371/journal.pone.0112069

ROEBER, F,. JEX, A, R,. GASSER, R, B,. 2013. Impact of Gastro-intestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. *Parasites & Vectors*. **6**: 153

RUBINSKY-ELEFANT, G,. HIRATA, C, E,. YAMAMOTO, J, H,. FERREIRA, M, U,. 2010. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Annals of tropical medicine and parasitology. **104**: 3 – 23.

SAHA, S, B,. PRAMANIK, S,. MUKHERJEE, G, S,. 1996. Prevalence of gastrointestinal nematodes of goats in West Bengal. *Indian Journal of Animal Science*. **11**: 51 – 52.

SAHOO, A,. KUMAR, D,. NAQVI, S, M, K,. 2013. Climate resilient small ruminant production. National initiative on climate resilient agriculture, central sheep and wool research institute. Izatnagar. India. Pg 1- 106.

SANCHEZ, V, V, V,.PATIñO, A, S,. SEGUNDO, V, J, P,. SANDOVAL, J, A, C, S,. ESQUIVEL, C, V, C,. SANCHEZ, T, A, C,. 2009. Prevalence of gastro-intestinal parasites among captive primates in Panama. *Journal of Animal and Veterinary Advances*. **8**: 2644 – 2649.

SCHEUERLE, M, C,. MAHLING, M,. PFISTER, K,. 2009. Anthelminthic resistance of *Haemoncus contortus* in small ruminants in Switzerland and Southern Germany. *Wiener Klinische Wochenschrift*. **3**: 46 – 49.

SCHNIEDER, T,. HEISE, M,. EPE, C,. 1999. Genus-specific PCR for the differentiation of eggs or larvae from gastro-intestinal nematodes of ruminants. *Parasitology Research*. **85**: 895–898.

SENDOW, J,. 2003. *Haemoncus contortus*. *Animal Diversity Web*. (http://animaldiversity.org/accounts/Haemonchus_contortus/) Accessed: 23 April 2016.

SHARIF, M,. DARYANI, A,. NASROLAHEI, M,. ZIAPOUR, S, P,. 2010. A survey of gastrointestinal helminthes in stray cats in northern Iran. Comparative Clinical Pathology. **19**: 257 – 261.

SHENG, Z,. CHANG, Q,. TIAN, S,. LOU, Y,. ZHENG, X,. ZHAO, Q,. WANG, C,. 2012. Characterization of *Toxascaris leonina* and *Toxocara canis* from cougar

(*Panthera leo*) and common wolf (*Canis lupus*) by nuclear ribosomal DNA sequences of internal transcribed spacers. *African Journal of Microbiology Research.* **6**: 3545 – 3549.

SINGH, P,. GUPTA, M, P,. SINGLA, L, D,. SHARMA, S,. SANDHU, B, S,. SHARMA, D, R,. 2006. Parasitic infections in wild herbivores in the Mahendra Choundhury Zoological Park, ChhatBir, Punjab. *Zoo's Print Journal*. **21**: 2459 – 2461.

SMITH, G, C,. GANGADHARAN, B,. TAYLOR, Z., LAURENSON, M, K,. BRADSHAW, H,. HIDE, G,. HUGHES, J, M,. DINKEL, A,. ROMIGE, T,. CRAIG, P, S,. 2003. Prevalence of zoonotic important para-sites in the red fox (*Vulpes vulpes*) in Great Britain. Veterinary Parasitology. 118: 133 – 142.

SMITH, S, A,. 1993. Diagnosis and treatment of helminths in birds of prey: Diagnosis and treatment of helminths in birds of prey. *Raptor Biomedicine*.

SOULBY, E, J,. 1982. Helminths, arthropods and protozoa of domesticated animals. Lea & Febiger: Philandelphia. 7th Ed. Pg 340 – 341.

SOUZA, R, P,. SOUZA, J, N,. MENEZES, J, F,. ALCÂNTARA, L, M,. SOARES, N, M,. TEIXEIRA, M, C, A,. 2013. Human infection by *Trichostrongylus* spp. in residents of urban areas of Salvador city, Bahia, Brazil. *Biomédica*. **33**: 446 – 458.

STEWART, C.G., PENZHORN, B.L., 2004. COETZER, J, A, W,. TUSTIN, R, C,. In: Infectious diseases of livestock, 2nd Ed.Oxford University Press, Cape Town, South Africa.

STRUBE, C,. HEUER, L,. JANECEK, E,. 2013. *Toxocara* spp. infections in paratenic hosts. *Veterinary Parasitology.* **193**: 375 – 89.

SWAI, E, S,. MSHANGA, D,. FYUMAGWA, R,. MPANDUJI, D,. CHUMA, I,. KUYA, S,. EBATE, E,. KATALE, Z,. KEYYU, J,. 2013. Prevalence and spectrum of helminths in free-ranging African buffaloes (*Syncerus caffer*) in wildlife protected areas, Tanzania. *Journal of Coastal Life Medicine*.**1**: 145 – 150.

SWAI, E, S,. MTUI, P, F,. MBISE, A, N,. KAAYA, E,. SANKA, P,. LOOMU, P, M,. 2006. Prevalence of gastro intestinal parasite infections in Maasai cattle in Ngorongoro District, Tanzania. *Livestock Research for Rural Development*.**18** (8).http://www.lrrd.org/lrrd18/8/swai18107.htm. Accessed 01 October 2015.

TAMURA, K,. STECHER, G,. PETERSON, D,. FILIPSKI, A,. KUMAR, S,. 2013. Mega6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. **30**(12): 2725 – 2729.

TAYLOR, M, A,. COOP, R, L,. WALL, R, L,. 2007. Veterinary parasitology. *Blackwell Publishing*, pp.600

THAWAIT, V, K,. MAITI, S, K,. DIXIT, A, A,. 2014. Prevalence of gastro-intestinal parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhattisgarh. *Veterinary World.***7**: 448 – 451.

THE MEDICAL LETTER. 1998. Drugs for parasitic infections. *The Medical letter on drugs and therapeutics*. **40**: 28.

THIENPONT, D,. ROCHETTE, F,. VANPARIJS, O, F, J,. 1979. Diagnosing helminthiasis through coprological examination. Janssen Research Foundation. Beerse, Belgium.

THOMAS H.S. 1994. Coccidiosis in calves. *The Cattleman.* **81**: 21 – 32.

TISDELL, C, A,. HARRISON, S, R,. RAMSAY, G, C,. 1999. The economic impacts of endemic diseases and disease control programmes. *Revue scientifique et technique* .**18**: 380 – 398.

TIWARI, J,. KUMAR, S,. KOLTE, A, P,. SWARNKAR, C, P,. SINGH, D,. PATHAK, K, M, L,. 2006. Detection of benzimidazole resistance in *Haemonchus contortus* using RFLP-PCR technique. *Veterinary Parasitology*. **138**: 301 – 307.

TORRES, J,. GARCIÁ-PEREA, R,. GISBERT, J,. FELIU, C,. 1998. Helminth fauna of the Iberian lynx*, Lynx pardinus*. *Journal of Helminthology*. **72**: 221 – 226.

TSOTETSI, A, M. MBATI, P, A,. 2003. Parasitic helminths of veterinary importance in cattle, sheep and goats on communal farms in the north-eastern Free State, South Africa. *Journal of the South African Veterinary Association*. **74**: 45 – 48.

TURNER, W, C,. GETZ, W, M,. 2010. Seasonal and Demographic factors influencing Gastro-intestinal parasitism in ungulates of Etosha National Park. *Journal of Wildlife Diseases*.**46**: 1108 – 1119.

UMUR, S,. YUKARI, B, A,. 2005. An abattoir survey of gastrointestinal nematodes in sheep in the Burdur region. *Turkish Journal of Veterinary and Animal Sciences*. **29**: 1195 – 1201.

VAN DIJK, J,. MORGAN, E, R,. 2009. Hatching behaviour of *Nematodirus filicollis* in a flock co-infected with *Nemadirus battus*. *Parasitology*. **136**: 805 – 811.

VAN WYK, J, A,. MAYHEW, E,. 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal of Veterinary Research*. **80**, http://dx.doi.org/10.4102/ojvr.v80i1.539.

VARADHARAJAN, A,. KANDASAMY, A,. 2000. A survey of Gastro-intestinal parasites of wild animals in captivity in the V.O.C Park and mini zoo, Coimbatore. *Zoo's Print Journal*. **5**: 257 – 258.

VORSTER, J, H,. MAPHAM, P, H,. 2016. Conical flukes in ruminants. http://www.cpdsolutions.co.za/publications/article_uploads/conical_flukes_cpd_article.pdf. Accessed 29 November 2016.

WALLER, P, J,. CHANDRAWATHANI, P,. 2005. *Haemonchus contortus*: parasite problem No.1 from tropics – Polar Circle. Problems and prospects for control based on epidemiology. *Asian Pacific Journal of Tropical Biomedicine*. **22**: 131 – 137.

WARUIRU, R, M,. NGOTHO, J, W,. MUKIRI, J, G,. 1997. Multiple anthelmintic resistance in *Haemoncus contortus* on a sheep farm in Kenya. *Veterinary Research Communications*. **21**: 483 – 491.

WIDEMAN, G, N,. 2004. Fatal *Trichuris* spp. infection in a Holstein heifer persistently infected with bovine viral diarrhoea virus. *The Canadian Veterinary Journal*. **45**: 511 – 512.

WILSON, G, H,. GREENACRE, C, B,. HOWERTH, E, W,. AMBROSE, D, L,. FONTENOT, D,. 1999. Ascaridosis in a Group of Psittacine Birds. *Journal of Avian Medicine and Surgery*. **13**: 32 – 39.

WINDON, R, G,. 1996. Genetic control of resistance to helminths in sheep. *Veterinary Immunology and Immunopathology*. **54**: 245 – 254.

WU, J,. YANG, J,. WEI, S,. LI, D,. CHEN, C,. ZHENG, T,. LI, Q,. PENG, J,. 2015. Isolation, identification, and characterization of the nematophagous fungus Monacrosporium salium from China. *Journal of Basic Microbiology*. **54**: 1 – 10.

YABSLEY, M, J,. ATKINSON, C, T,. THOMAS, N, J,. HUNTER, D, B,. 2009. Capillarid Nematodes: Parasitic Diseases of Wild birds. John Wiley & Sons. Oxford, UK. Pp.463 – 497

YAMAMOTO, Y,. 2002. PCR in Diagnosis of Infection: Detection of Bacteria in Cerebrospinal Fluids. *Clinical and Diagnostic Laboratory Immunology*. **9**: 508 – 514.

YONG, T,S,. LEE, J,H,. SIM, S,. LEE, J,. MIN, D,Y,. CHAI, J,Y,. EOM, K,S,. SOHN,W,M,. LEE, S,H., RIM, H,J,. 2007. Differential diagnosis of *Trichostrongylus* and hookworm eggs via PCR using ITS-1 sequence. *Korean Journal of Parasitology*. **45**: 69 – 74.

ZAJAC, A,. 2006. Gastro-intestinal nematodes of small ruminants: Lifecycle, anthelminthics, and diagnosis. *Veterinary Clinics of North America Food Animal Practice*. **22**: 529-541

ZASITYTE, R,. GRIKIENCIENE, T,. 2002. Some data in endoparasites of common mole in Lithnani. *Acta Zoological Lituanica*. **4**: 403.

ANNEXURES

NZG RESC Approval Letter

Table 1 - 3 represents samples collected from different animal groups; Ungulates, Felids and Avian species at the National Zoological Gardens of South Africa indicating which samples were positive and negative when using microscopy and molecular technique.

Table 1: Ungulates microscopy vs PCR

	Haemonchus		***Trichostrongylus***		***Ostertagia***		***Nematodirus***		***Trichuris***	
Sample	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR
U1	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U2	pos	neg	pos	pos	pos	neg	neg	neg	neg	*x*
U3	pos	pos	neg	neg	neg	neg	pos	neg	neg	*x*
U5	neg	pos	pos	neg	neg	neg	pos	pos	neg	*x*
U6	neg	neg	neg	neg	neg	neg	neg	pos	neg	*x*
U7	pos	neg	neg	neg	neg	neg	neg	pos	pos	*x*
U8	neg	neg	neg	neg	neg	neg	neg	pos	neg	*x*
U9	pos	neg	neg	neg	neg	neg	neg	pos	neg	*x*
U10	pos	pos	neg	neg	neg	neg	neg	pos	pos	*x*
U11	neg	neg	neg	neg	neg	neg	neg	pos	neg	*x*
U12	pos	neg	neg	neg	neg	neg	neg	pos	pos	*x*
U13	neg	neg	neg	neg	neg	neg	neg	pos	neg	*x*

U14	pos	neg	neg	neg	neg	neg	neg	pos	pos	*x*
U15	neg	pos	neg	pos	pos	neg	neg	neg	neg	*x*
U16	pos	pos	pos	pos	pos	pos	neg	pos	neg	*x*
U17	neg	pos	pos	pos	pos	pos	neg	neg	neg	*x*
U18	neg	pos	pos	pos	pos	pos	neg	neg	neg	*x*
U19	neg	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U20	neg	pos	pos	neg	pos	neg	neg	neg	neg	*x*
U21	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U22	pos	pos	pos	neg	neg	neg	neg	neg	neg	*x*
U23	pos	neg	pos	pos	pos	neg	pos	neg	neg	*x*
U24	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U25	neg	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U26	pos	pos	neg	pos	neg	neg	neg	pos	neg	*x*
U27	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U28	pos	pos	neg	pos	neg	neg	neg	pos	neg	*x*
U29	pos	neg	pos	neg	neg	neg	pos	pos	neg	*x*
U30	pos	neg	neg	neg	neg	neg	pos	neg	neg	*x*

U31	pos	pos	neg	neg	neg	neg	pos	neg	neg	*x*
U32	neg	neg	pos	pos	pos	neg	neg	neg	neg	*x*
U33	pos	pos	pos	neg	pos	neg	neg	neg	neg	*x*
U34	neg	pos	pos	pos	pos	neg	neg	neg	neg	*x*
U35	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U36	pos	pos	neg	pos	neg	neg	pos	neg	neg	*x*
U37	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U38	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U39	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U40	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U41	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U42	pos	neg	pos	neg	neg	neg	neg	neg	neg	*x*
U43	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U44	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U45	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U46	pos	pos	pos	pos	pos	neg	neg	pos	neg	*x*
U47	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*

U48	neg	neg	neg	neg	neg	neg	pos	neg	neg	*x*
U49	neg	neg	neg	pos	neg	neg	pos	neg	neg	*x*
U50	pos	neg	neg	neg	pos	neg	neg	neg	neg	*x*
U51	pos	neg	neg	pos	pos	neg	pos	neg	neg	*x*
U52	pos	neg	neg	pos	pos	neg	pos	neg	neg	*x*
U53	neg	neg	neg	pos	neg	neg	pos	neg	neg	*x*
U54	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U55	pos	pos	pos	pos	pos	neg	neg	neg	neg	*x*
U56	pos	neg	neg	neg	pos	neg	neg	neg	neg	*x*
U57	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U58	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U59	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U60	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U61	pos	pos	neg	neg	neg	neg	neg	pos	neg	*x*
U62	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U63	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U64	pos	pos	neg	neg	neg	neg	neg	pos	neg	*x*

U65	pos	neg	pos	pos	neg	neg	neg	pos	neg	*x*
U66	neg	neg	neg	pos	neg	neg	neg	pos	pos	*x*
U67	neg	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U68	neg	pos	neg	pos	neg	pos	neg	neg	neg	*x*
U69	neg	pos	neg	pos	neg	neg	neg	pos	neg	*x*
U70	neg	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U71	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U72	pos	pos	neg	pos	neg	pos	neg	neg	neg	*x*
U73	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U74	neg	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U75	pos	pos	neg	neg	neg	neg	neg	pos	neg	*x*
U76	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U77	neg	neg	neg	neg	pos	neg	neg	pos	neg	*x*
U78	neg	pos	neg	pos	pos	neg	neg	pos	neg	*x*
U79	neg	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U80	pos	pos	pos	pos	neg	neg	neg	pos	neg	*x*
U81	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*

U82	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U83	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U84	pos	pos	pos	neg	neg	neg	neg	neg	neg	*x*
U85	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U86	neg	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U87	pos	pos	neg	neg	neg	neg	neg	pos	pos	*x*
U88	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U89	pos	pos	neg	neg	neg	neg	neg	neg	pos	*x*
U90	neg	neg	neg	neg	neg	neg	neg	neg	pos	*x*
U91	neg	neg	neg	pos	neg	neg	neg	neg	pos	*x*
U92	neg	neg	pos	neg	pos	neg	neg	neg	neg	*x*
U93	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U94	pos	pos	pos	pos	neg	neg	pos	neg	neg	*x*
U95	neg	pos	pos	pos	neg	neg	neg	neg	neg	*x*
U96	pos	neg	pos	pos	neg	neg	pos	neg	neg	*x*
U97	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U98	pos	neg	neg	pos	neg	neg	pos	neg	neg	*x*

U99	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U100	neg	neg	neg	neg	neg	neg	pos	neg	neg	*x*
U101	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U102	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U103	pos	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U104	neg	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U105	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U106	neg	pos	pos	pos	pos	neg	neg	neg	neg	*x*
U107	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U108	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U109	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U110	pos	pos	neg	pos	neg	neg	neg	pos	neg	*x*
U111	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U112	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U113	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U114	neg	pos	neg	neg	neg	neg	neg	pos	neg	*x*
U115	pos	pos	pos	pos	pos	neg	neg	neg	pos	*x*

U116	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U117	pos	pos	neg	neg	neg	pos	neg	neg	neg	*x*
U118	neg	neg	neg	neg	neg	pos	neg	neg	neg	*x*
U119	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U120	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U121	pos	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U122	pos	pos	neg	pos	neg	neg	pos	neg	neg	*x*
U123	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U124	neg	pos	neg	pos	pos	neg	pos	neg	neg	*x*
U125	pos	pos	neg	pos	neg	neg	neg	neg	neg	*x*
U126	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U127	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U128	pos	pos	pos	neg	neg	neg	neg	neg	neg	*x*
U129	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U130	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U131	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U132	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*

U133	neg	neg	pos	neg	pos	neg	neg	neg	neg	*x*
U134	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U135	neg	pos	neg	pos	pos	neg	neg	neg	neg	*x*
U136	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U137	neg	neg	pos	pos	pos	neg	neg	neg	neg	*x*
U138	neg	pos	neg	pos	pos	neg	neg	neg	neg	*x*
U139	neg	pos	neg	pos	pos	neg	neg	neg	neg	*x*
U140	pos	neg	pos	pos	neg	neg	pos	pos	neg	*x*
U141	pos	neg	neg	pos	neg	neg	neg	pos	pos	*x*
U142	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U143	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U144	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U145	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U146	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U147	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U148	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U149	neg	neg	neg	neg	neg	neg	neg	pos	neg	*x*

U150	pos	neg	pos	neg	pos	neg	pos	neg	neg	*x*
U151	pos	neg	neg	neg	neg	neg	neg	pos	neg	*x*
U152	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U153	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U154	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U155	pos	pos	pos	pos	pos	neg	pos	pos	pos	*x*
U156	pos	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U157	neg	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U158	pos	neg	neg	neg	pos	neg	neg	neg	neg	*x*
U159	neg	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U160	pos	neg	neg	pos	pos	neg	pos	neg	pos	*x*
U161	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U162	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U163	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U164	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U165	pos	neg	pos	pos	pos	neg	neg	pos	neg	*x*
U166	pos	neg	pos	pos	neg	neg	neg	pos	neg	*x*

U167	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U168	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U169	pos	neg	pos	pos	pos	neg	neg	neg	neg	*x*
U170	pos	neg	pos	pos	pos	neg	neg	pos	neg	*x*
U171	pos	neg	neg	neg	pos	neg	pos	neg	neg	*x*
U172	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U173	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U174	pos	neg	neg	neg	neg	neg	pos	neg	pos	*x*
U175	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U176	pos	neg	neg	neg	neg	neg	pos	pos	pos	*x*
U177	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U178	pos	neg	neg	neg	neg	neg	pos	pos	pos	*x*
U179	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U180	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U181	pos	neg	neg	pos	neg	neg	pos	pos	neg	*x*
U182	pos	neg	pos	pos	pos	neg	pos	neg	pos	*x*
U183	pos	neg	neg	pos	pos	neg	neg	neg	neg	*x*

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U186	neg	neg	neg	pos	pos	neg	neg	neg	pos	*x*
U187	neg	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U188	neg	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U189	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U190	pos	neg	neg	neg	neg	neg	pos	neg	neg	*x*
U191	pos	pos	neg	pos	neg	neg	neg	pos	neg	*x*
U192	neg	pos	neg	neg	neg	neg	neg	pos	neg	*x*
U193	pos	pos	neg	neg	neg	neg	neg	pos	neg	*x*
U194	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U195	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U196	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U197	pos	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U198	pos	neg	neg	pos	pos	neg	pos	neg	pos	*x*
U199	pos	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U200	pos	neg	neg	neg	neg	neg	neg	neg	neg	*x*

U201	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U202	pos	neg	pos	neg	pos	neg	pos	neg	pos	*x*
U203	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U204	pos	neg	neg	neg	neg	neg	pos	neg	neg	*x*
U205	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U206	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U207	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U208	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U209	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U210	neg	neg	pos	neg	neg	neg	neg	neg	neg	*x*
U211	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U212	neg	neg	neg	pos	pos	neg	neg	pos	neg	*x*
U213	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U214	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U215	pos	neg	neg	pos	neg	neg	neg	neg	pos	*x*
U216	pos	neg	neg	neg	pos	neg	pos	neg	pos	*x*
U217	pos	neg	neg	pos	neg	neg	neg	pos	neg	*x*

U218	neg	neg	neg	pos	neg	neg	neg	pos	neg	*x*
U219	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U220	pos	neg	pos	pos	pos	neg	pos	neg	pos	*x*
U221	neg	neg	neg	pos	pos	neg	neg	neg	neg	*x*
U222	neg	neg	pos	pos	pos	neg	pos	neg	pos	*x*
U223	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U224	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U225	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U226	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U227	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U228	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U229	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U230	neg	neg	neg	pos	neg	neg	pos	neg	neg	*x*
U231	neg	neg	pos	pos	neg	neg	pos	neg	neg	*x*
U232	neg	neg	neg	pos	neg	neg	pos	neg	neg	*x*
U233	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U234	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*

U235	neg	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U236	neg	neg	neg	pos	neg	neg	neg	neg	pos	*x*
U237	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U238	pos	neg	neg	pos	neg	neg	neg	neg	neg	*x*
U239	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U240	pos	pos	pos	pos	neg	neg	neg	neg	neg	*x*
U241	neg	pos	pos	pos	neg	neg	neg	neg	neg	*x*
U242	neg	neg	pos	pos	neg	neg	neg	neg	neg	*x*
U243	neg	pos	neg	neg	neg	neg	neg	neg	neg	*x*
U244	neg	neg	neg	neg	neg	neg	neg	neg	neg	*x*
U245	neg	pos	neg	neg	pos	neg	neg	neg	neg	*x*
Total pos	150	74	55	162	54	7	45	69	25	0

neg: Negative
pos: Positive
x: PCR did not work or did not conduct PCR

Table 2: Felids microscopy vs PCR

	Toxascaris		***Toxocara***		**Coccidia**	
Sample	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR
C1	pos	x	neg	x	neg	x
C2	pos	x	neg	x	neg	x
C3	neg	x	neg	x	neg	x
C4	neg	x	neg	x	neg	x
C5	neg	x	pos	x	neg	x
C6	pos	x	pos	x	neg	x
C7	pos	x	pos	x	neg	x
C8	pos	x	neg	x	neg	x
C9	pos	x	neg	x	neg	x
C10	pos	x	neg	x	neg	x
C11	pos	x	neg	x	neg	x
C12	pos	x	neg	x	neg	x
C13	pos	x	neg	x	neg	x
C14	pos	x	neg	x	neg	x
C15	pos	x	neg	x	neg	x
C16	pos	x	neg	x	neg	x
C17	pos	x	neg	x	neg	x
C18	pos	x	neg	x	neg	x
C19	pos	x	pos	x	pos	x
C20	pos	x	pos	x	pos	x
C21	pos	x	neg	x	neg	x
C22	pos	x	neg	x	neg	x
C23	pos	x	neg	x	neg	x
C24	pos	x	neg	x	neg	x
C25	pos	x	neg	x	pos	x
C26	pos	x	neg	x	pos	x
C27	pos	x	neg	x	pos	x
C28	pos	x	pos	x	neg	x

C29	pos	x	neg	x	neg	x
C30	pos	x	neg	x	neg	x
C31	pos	x	neg	x	neg	x
C32	pos	x	neg	x	neg	x
C33	pos	x	neg	x	neg	x
C34	pos	x	neg	x	neg	x
C35	pos	x	neg	x	neg	x
C36	pos	x	neg	x	neg	x
Total pos	33	x	6	x	5	x

neg: Negative
pos: Positive
x: PCR did not work or did not conduct PCR

Table 3: Avian species microscopy vs PCR

	Trichostrongylus		***Heterakis***		***Capillaria***		***Strongyloides***		***Unknown egg/oocyst***	
Sam	Micro	PCR	Micr	PC	Micr	PC	Micro	PCR	Micr	PCR
B1	pos	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B2	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B3	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B4	neg	*x*	neg	*x*	neg	*x*	neg	*x*	pos	*x*
B5	neg	*x*	neg	*x*	neg	*x*	neg	*x*	pos	*x*
B6	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B7	neg	*x*	pos	*x*	pos	*x*	neg	*x*	neg	*x*
B8	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B9	neg	*x*	neg	*x*	neg	*x*	neg	*x*	pos	*x*
B10	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B11	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B12	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B13	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B14	neg	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
B15	neg	*x*	neg	*x*	neg	*x*	pos	*x*	neg	*x*
B16	pos	*x*	neg	*x*	pos	*x*	neg	*x*	neg	*x*
Total	2	*x*	1	*x*	12	*x*	1	*x*	3	*x*

neg: Negative
pos: Positive
x: PCR did not work or did not conduct PCR