The biodiversity of gastrointestinal nematodes in South African wildlife

Chalis J. Bird

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The Biodiversity of Gastrointestinal Nematodes in South African Wildlife

Chalis J. Bird

Submitted to the Department of Biology of Smith College in partial fulfillment of the requirements for the degree of Master of Science

Dr. Steven Williams, Faculty Advisor

December 22, 2016
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Abstract

In this study, I investigated the diversity of parasites found in cheetah and wild ungulates in South Africa in collaboration with the Samara Private Game Reserve in Graaff Reinet, South Africa. Scat samples were collected over a period of two months, from September-November, 2014, and DNA was extracted on the preserve for gastrointestinal nematode analysis. Wildlife was tracked using radio telemetry, tracks, and sign, while habitat and host distribution were documented. The aim was to determine the community composition of gastrointestinal nematodes in the wildlife hosts through molecular identification and to examine the role of host habitat preference, geographical distribution and spatial overlap on parasite composition and transmission. Eleven gastrointestinal nematodes were identified to the species level and five were identified to the genus level. Two common parasites were found in two different cases of host spatial overlap, while one parasite was found in two host populations that do not share the same habitat. These are the first steps toward understanding the impacts of parasitic infection on host fitness and population dynamics that may contribute to the management and conservation of African wildlife.
Introduction

South Africa holds a rich diversity of wildlife, a trademark that has become an economic mainstay to the country. The dramatic increase in ecotourism over the past two decades has given South Africa an economic incentive to protect and conserve wildlife and their habitats. Continuous development of private nature reserves, game ranching and game farming has contributed to the population growth of wildlife while the growing ecotourism job market and the influx of travelers has lead to a necessary expansion in livestock production (Junker et al., 2015; Cooper and Van Der Merwe, 2014; Oberem, 2011). As the wildlife and livestock population expands, so too does their distribution, increasing the likelihood for habitat or spatial overlap between wildlife and livestock. This may become a particular concern for the numerous private nature reserves and livestock farms whose properties are adjacent to one other. The growing spatial overlap raises major concerns regarding the management of wildlife and livestock, as well as the possibility of increased pathogen transmission within and between livestock and wildlife populations (van Vuuren and Penzhorn, 2015, Junker et al., 2015). Pathogens of particular concern are gastrointestinal fecal-born parasitic nematodes, as transmission can occur between hosts without direct contact through the ingestion of infective larvae while consuming vegetation (Myers et al., 2013).
Wildlife Helminths: Lifecycle and Infection

Lifecycle

Nematodes (roundworms), of the phylum Nematoda, are a diverse group of worm-like metazoans present in every ecosystem as either free-living organisms or parasites. Parasitic nematodes infect a wide variety of hosts including plants, insects, humans, domestic animals and wildlife. While some nematodes are specialists, adapted to infect only one host species, many are generalists, capable of infecting two or more host species (Walker and Morgan, 2014).

Fecal-borne nematodes, the focus of this study, have a complex lifecycle that involves a parasitic phase within the host and a free-living phase outside of the host (Myers et al., 2013). These phases are comprised of several major life stages: egg, 4 larval stages, and adult (Figure 1). Infectious L3 stage larvae are ingested from feed/vegetation or water and move through the large intestine where they develop into L4 larvae and eventually into male and female reproducing adult worms. Eggs, excreted via feces, hatch and develop into L1, L2 and eventually, L3 larvae, which can then be ingested to continue the cycle of infection (Myers et al., 2013).

The duration of development and survival of free-living larva varies by species and is largely determined by abiotic conditions, mainly precipitation and temperature (Matthee et al., 2004). Research by Okon and Akinpelu (1982) suggests that infective strongyloid larva may survive in the environment for up to four to ten weeks, given sufficient rainfall. The resilience and longevity of nematode eggs and larva have the capability to accumulate and persist in the environment, allowing parasite transmission within and between host populations possible simply due to spatial overlap of hosts.
(Ezenwa, 2003). Thus, parasite-host relationships should not only be examined on an individual level, but on a population and community level.

**Figure 1.** An illustration depicting the direct lifecycle of gastrointestinal nematodes in both the wildlife host and the environment stage.

**Infection**

Nematodes have the most species amongst parasitic helminths that cause mortality in wildlife (Botzler and Brown, 2014), with nearly a third of described nematode genera parasitizing vertebrates (Anderson, 1984; Botzler and Brown, 2014).
Even still, the community composition of wildlife parasites amongst a community of wildlife hosts is not well understood.

Little is known regarding the gastrointestinal nematodes infecting South African wildlife, primarily because the influence of such parasitic infections is often obscure and difficult to detect. Although some parasites have a direct effect on host survival with easily observable symptoms, more often the effects are indirect and sublethal (Gunn and Irvine, 2003). For instance, parasitic infection may increase susceptibility to predation, increase vulnerability to infection by other pathogens (Arneberg, 1996), reduce competitive fitness (Scott, 1988) and reproductive success (Stein et al., 2003), decrease appetite (Arneberg, 1996) and impair growth (Milner et al., 2013). Furthermore, nematodes develop much more slowly than other pathogens, such as viruses and bacteria, therefore diseases that may result arise slowly and are typically chronic in nature.

When parasitic infections do not manifest distinct symptoms, it is often difficult to assess the health of wildlife populations without in-depth examination. Until recent years, the presence of a parasitic disease within a population was determined through morphological identification of helminths via invasive sampling from necropsies or by extracting helminth eggs from stool then allowing the helminths to hatch and mature until identifiable features develop (Budischak et al., 2015; Junker et al., 2015; Horak, 1978; Jacobs et al., 2015). However, morphological characters are frequently difficult to assess even with excellent microscopy, which may lead to erroneous identification (Skerikova et al., 2001). Furthermore, opportunities for invasive sampling are often infrequent and certainly rare with vulnerable or endangered wildlife, particularly in circumstances where signs and symptoms of infection are not easily observable. Understandably, these
methods present limitations on our ability to quantify and understand the impact of helminths on wildlife populations and their evolutionary success. However, recent advances in molecular techniques, such as DNA barcoding, has equipped scientists with the ability to analyze fecal samples for parasites relatively quickly. This enables the collection of many samples, including samples taken from seemingly uninfected hosts, and, in-turn, provides a more complete understanding of wildlife parasite infection (Budischak et al., 2015). Non-invasive sampling coupled with molecular techniques also allows the wildlife host to be observed and monitored which may provide more insight into the impact of parasitic diseases on different host species. Molecular techniques have also improved our potential for species-specific helminth identification from non-invasive samples (Sim et al., 2010). Moreover, species-specific genetic information allows a deeper investigation into the evolution and co-evolution of parasites and their hosts, and for insight into factors driving the diversity and evolutionary success of parasitic lineages (Nieberding et al., 2008).

Survey Locality and Study Site

Samara Private Game Reserve: topology and biodiversity

Samara Private Game Reserve is located near Graaff-Reinet in the Camdeboo region of the Eastern Cape Province in South Africa. At 27,100 hectares, Samara is the largest private nature reserve in the Eastern Cape (Feldon et al., 2013). A portion of the reserve (12,919 ha) is currently fenced in for the purpose of predator management (Figure 2). The reserve is made up of broken, mountainous topography, with upland mountain plateaus in the north and east sections of the reserve, and extensive, flat Karoo
plains in the southeast and southwest of the reserve separated by a deep (perennial drainage) valley in the central section of the reserve (Feldon et al., 2013). Samara’s diverse landscape offers a broad range of environments and climatic conditions, which gives rise to rich habitats that support substantial mammal biodiversity.

The arid climate of Samara is typical of the Nama Karoo Ecoregion; summers are hot, frequently exceeding 35°C, while winters are cold, often below 0°C. Rainfall averages 360 mm/year, and can occur throughout the year with greatest peaks in spring and autumn (Feldon et al., 2013). Snow sometimes falls on the high-elevation mountains.

The vegetation consists of temperate thicket/sweet grassland mosaic, Suurpol/temperate thicket mosaic, sweet grassland, temperate thicket, bosberg thicket, escarpment valley thicket, escarpment spekboom thicket, escarpment spekboomveld, arid thornveld, and pentzia veld (van Cauter, 2004) (See Figure A2: vegetation type map).

Samara encompasses four out of South Africa’s seven biomes: Plateaux Grassland, Nama Karoo, Savanna and Valley Bushveld (Figure 4) (Feldon et al., 2013). The Plateaux Grassland, located on the high elevations of the mountains, such as Kondoa, contain specific grasses that support grazing by Cape mountain zebra, black wildebeest, and blesbok. The Nama Karoo make up the plains, while the Savanna, typified by acacia woodlands, support kudu, rhino and giraffe. The evergreen, drought resistant vegetation, particularly Spekboom which is unique to the Eastern Cape, makes up the Valley Bushveld, and supplies habitat to a wide range of browsers and other herbivores (Feldon et al., 2013).
**Study Animals**

The study animals were chosen based on several factors: ease of collection of scat, habitat and range distribution, feeding habits, and digestion. Table 1 includes a list of hosts, their feeding habits and digestion. Black wildebeest (*Connochaetes gnou*), blesbok (*Damaliscus pygargus phillipsi*) and Cape mountain zebra (*Equus zebra zebra*) were chosen because they are limited to grazing the Sweet grassland vegetation strictly located on Kondoa Mountain (Figure 3). While black wildebeest and blesbok are ruminants, mountain zebra are hindgut fermenters (Alexander, 1999). Cape mountain zebra, listed as endangered by the World Conservation Union, are also an interesting study animal because the species suffered a dramatic bottleneck in the early 1900s from excessive hunting and habitat loss to agriculture. Due to protection and conservation efforts, the Cape mountain zebra numbers have increased from its most critical status of just 80 individuals in the 1950s to roughly 2,800 as of 2009 (Hrabar and Kerley, 2009). Interestingly, Cape mountain zebra rarely occur in sympatry with plains zebra (*Equus quagga*) as Cape mountain zebra have harder, faster growing hooves which are adapted for rugged, mountainous habitat. Therefore, the Cape mountain zebra are unable to inhabit soft, flat plains for extended periods of time (Skinner and Smithers, 1990). This led to my choice in selecting plains zebra as a study animal; plains zebra and mountain zebra are very closely related equids, however, each remain spatially segregated at Samara and elsewhere. Plains zebra are restricted to grazing and consuming tubers on the plains within the escarpment spekboomveld and Penzia veld while Cape mountain zebra remain on the mountain (Figure 3). Host relatedness of the two zebra species coupled with geographical separation presents an ideal system for studying parasite-host interactions.
similarity and spatial overlap. Because of the close relationship of the two zebra species, I hypothesized that the two zebras would share parasites of the same genus but perhaps not the same species.

In contrast, I chose white rhino (*Ceratotherium simum*) and black rhino (*Diceros bicornis*) because they are closely related, are hindgut fermenters and have overlapping habitats (Alexander, 1999). However, white rhino are grazers while black rhino are predominantly browsers. I predicted that the two rhino species share some of the same parasite species because their habitats overlap and because they are both hindgut fermenters. However, I expected some differences due to the different feeding habits, specifically, grazer versus browser.

Giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*) and red hartebeest (*Alcelaphus caama*) are ruminants which have overlapping habitats in the escarpment spekboomveld and arid thronveld (Figure 3). While giraffe and kudu are browsers, red hartebeest are grazers. I expected that giraffe and kudu will harbor more similar parasites to one other than to red hartebeest. Eland (*Taurotragus oryx*) was chosen as a study animal because eland are found throughout the preserve, are ruminants and are highly selective mixed feeders (graze and browse). I predicted that eland would have the most cases of shared parasite species with multiple ruminant hosts from either the mountain or the plains. Cheetah (*Acinonyx jubatus*) was chosen because cheetahs are found throughout the reserve and are the main predator of small antelope and young zebra in Samara. Although cheetah were able to repopulate after a severe bottleneck 12,000 years ago, habitat fragmentation, habitat loss to agriculture, elimination by resentful livestock farmers, and out-competition by other predators, has led to their recent
decline placing the cheetah on the ‘Vulnerable’ list (Scantlebury et al., 2014).
Fortunately, Samara values the survival of the cheetah over having larger, more tourist-desirable predators, such as lions and leopards, which may outcompete cheetahs in terms of hunting. On Samara, some cheetahs are also radio-collared and can be tracked via radio telemetry. I hypothesized that cheetah might share some parasite species with their prey due to host switch events or an evolutionary history of repeated predation on infected prey thereby increasing the odds of parasite survival in the cheetah.

Figure 2. The extant and separate units of Samara Private Game Reserve located near Graaff-Reinet, SA (Feldon et al., 2013). The eastern portion, 12,919 ha, is currently fenced for predator management.
Figure 3. Vegetation map of game-fenced area of Samara Private Game Reserve (van Cauter 2004; Elephant management plan)
Figure 4. Photographs representing the four biomes present on Samara Private Game Reserve: (A) Savanna, (B) Plateau Grasslands, (C) Nama Karoo, and (D) Succulent Karoo-bushveld. (September 2014)

Table 1. Wildlife hosts, their digestive system and feeding habits

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<th>Host</th>
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<th>Feeding Habits</th>
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<td>Ruminant</td>
<td>Grazers</td>
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<tr>
<td>Black wildebeest</td>
<td>Ruminant</td>
<td>Grazers</td>
</tr>
<tr>
<td>Eland</td>
<td>Ruminant</td>
<td>Selective mixed feeders</td>
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<td>Giraffe</td>
<td>Ruminant</td>
<td>Browsers</td>
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<td>Kudu</td>
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<td>Red hartebeest</td>
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</tr>
<tr>
<td>Cheetah</td>
<td>Hind Gut Fermenters</td>
<td>Carnivore</td>
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Project Focus and Research Questions

Project Focus

The first aim of this study was to identify the gastrointestinal nematodes infecting blesbok (*Damaliscus pygargus phillipsi*), black wildebeest (*Connochaetes gnou*), eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), red hartebeest (*Alcelaphus caama*), Cape mountain zebra (*Equus zebra zebra*), plains zebra (*Equus burchelli*), white rhino (*Ceratotherium simum*), black rhino (*Diceros bicornis*) and cheetah (*Acinonyx jubatus*). The second aim was to examine the role of habitat preference and geographical distribution of the wildlife host on parasite composition and transmission.

Although many studies have been conducted regarding parasite identification and transmission within and between wildlife host populations (Boomker et al., 1991; Budischak et al., 2015; Ezenwa, 2003; Horak, 1978; Horak et al., 1983; Junker et al., 2015; Krecek et al., 1987b; Round, 1968; van Wyk and Boomker, 2011; Walker, 2014), very few studies have explored the parasites present in a community of wildlife populations. Studying parasites in a wildlife community, as opposed to individual wildlife hosts or single host populations, may reveal a larger, more thorough picture of parasite transmission dynamics. For example, studying gastrointestinal parasites amongst multiple herds of wildebeest, blesbok and eland, which have overlapping habitats within the same game reserve, may provide more insight into the dynamics of parasite transmission than if one were to study gastrointestinal parasites in individual wildebeests within one herd or between multiple herds because parasite transmission may not be limited to the same herd or even the same host species. Therefore, by excluding other
wildlife populations that share the same habitat within the same community, one may unintentionally exclude valuable information regarding the spread of disease in a wildlife community. These findings may then be used as a foundation for studying the impacts of parasitic infection on host fitness and population dynamics in an effort to contribute to the management and conservation of wildlife.

Research Questions:

What are the roles of geographical location and spatial dynamics of host populations on the community composition of gastrointestinal nematodes?

Investigating host spatial dynamics and identifying parasites associated with the host is the first step in understanding how the structure and dynamics of host populations and host communities influence the spread and persistence of infection. It is imperative to investigate the nematode composition not only within and between host populations but also, on a host community scale, as inter-specific and intra-specific interactions can also influence transmission (Hudson et al., 2002; Ezenwa, 2002). A community-level approach is particularly important regarding fecal-borne gastrointestinal parasites that are able to persist in the environment and thus do not require physical contact between hosts (Ezenwa, 2002). Specifically, this study investigates the nematode gastrointestinal parasite community of several wildlife host species that share overlapping habitats, as well as two allopatric hosts (mountain and plains zebra) whose habitats do not overlap. Blesbok, black wildebeest and mountain zebra have overlapping habitats within the sweet grasslands; giraffe, kudu and plains zebra habitats overlap within the escarpment speckboomveld (Figure 4); white and black rhino share a small region of overlap which
cannot be disclosed for protection purposes; eland, cheetah and kudu have been found throughout the preserve.

*Is there an overlap in parasitic community amongst predators and their prey?*

Fecal-borne parasites are intimately linked to their hosts through trophic interaction (Hudson et al., 2002). Most often, this idea is applied to ungulates because ungulates become trophically infected by ingesting infective L3 larva on vegetation while grazing or browsing. However, this study also investigates whether there is a common parasitic trophic interaction between predator-prey. Specifically, does cheetah, the predator of this study, share parasite infections with its common prey (juvenile ungulates such as wildebeest and blesbok)?

*A Note on Nematode Identification Through Barcoding:*

DNA barcoding is a common, inexpensive molecular technique used to identify organisms. The technique depends upon short, unique and highly variable repetitive DNA sequences found in all eukaryotic genomes. Such repeats have been sequenced from thousands of species and can be found in reference databases, such as GENBANK. An organism can be identified by isolating its DNA and sequencing the barcode region. One then only needs to find the closest matching reference record in the database.

Taxonomic or species identification based on DNA isolated from environmental samples, such as soil, water or stool, has been the foundation of many recent ecological studies and is likely to be more common in the future (Damm et al., 2010). Because
non-invasive sampling techniques typically yield degraded or fragmented DNA (Taberlet et al., 1999), DNA barcoding is an ideal system for studying environmental samples since these sequences are short and can be amplified from even degraded DNA samples.

In this study, I used the internal transcribed spacer region (ITS2) within the large subunit of ribosomal DNA (rDNA) as a genetic barcode, or “marker”, for the identification of nematodes (Figure 5a) (Sim et al., 2010). Standard PCR is typically used to amplify barcode sequences by isolating DNA from just a few individual worms. However, standard PCR is not a sensitive enough application for this study as it is often difficult to isolate sufficient DNA from just a few eggs for PCR to amplify the DNA to a detectable range. Here, I used a nested PCR (nPCR) assay developed by Sim et al. (2010) that is able to amplify ITS2 rDNA from a single egg. Nested PCR (Figure 5b) involves two sets of primers with each primer set containing a forward and reverse primer, and two rounds of PCR to ultimately amplify one locus containing the sequence of interest. The first set of primers is applied in the first phase of nPCR (the primary PCR) during which the primers amplify the target sequence as well as non-specific, flanking sequences. The second set of primers are applied in the second phase of nPCR (secondary PCR) during which the primers bind within the primary PCR amplicon which produces a second, final PCR amplicon that is shorter than the first. For example, the first set of ITS2 primers, NC13aF (forward) and NC2 (reverse), designed by Sim et al. (2010), amplify ITS2 rDNA and flanking sequences of the 5.8S and 28S rRNA genes, while the second set of primers, NC14-Fn (forward) and NC2-Rn (reverse), bind internally to primers NC13aF and NC2. The final nPCR barcode product can then be sequenced using
the Sanger Deoxy-sequencing method and compared to a reference database for identification.

Figure 5. (A) Loci of the internal transcribed spacer region (ITS2) within the large subunit of ribosomal DNA and (B) nested PCR of ITS2 DNA barcode.
**Survey Methods and Lab Protocols**

*Overview of Methods*

This study was conducted at Samara Private Game Reserve in Graaff-Reinet, South Africa from September to November, 2014 and at Smith College, Northampton, MA, USA from December 2014 to May 2016. A schematic overview of the workflow for the survey methods and lab protocols are illustrated in Figure 6. Vegetation and wildlife surveys, wildlife tracking, stool sample collection, GPS data collection and genomic DNA extraction took place at Samara. Extracted DNA samples were transferred to the Center of Molecular Biology (CMB) at Smith College where analysis for the identification of nematode parasite species took place. Analyses included nested polymerase chain reaction (nPCR) amplification of nematode ribosomal DNA (rDNA), gel extraction of amplified PCR products, capillary sequencing to obtain nematode ITS2 sequence data and creating consensus sequences. The ITS2 consensus sequences were compared to the closest matching reference sequences on Genbank, a genomic reference database, to determine the identification of a given nematode parasite.
Located wildlife & collected stool samples

Extracted genomic DNA

Nested PCR amplification of ITS2 rDNA

Gel extraction of PCR product

Capillary Sequencing & Comparison with reference database

**Figure 6.** Schematic overview of experimental workflow
Required Permits and Applications

The following permits were approved by the Director of Environmental Affairs, Alan Southwood, from the South African Government’s Department of Environmental Affairs and Tourism (DEAT) in order to conduct research and collect samples from South Africa: the “Bioprospecting, Access and Benefit Sharing (BABS)” permit to undertake research and collect biological material on privately owned land in the eastern Cape Province, and the “Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)” permit to obtain biological samples from endangered species.

In the event that DNA isolation was not possible and stool samples had to be exported to the United States, the following permits were obtained: the “Threatened or Protected Species (TOPS)” export permit approved by the Manager of Environmental Affairs, Dylan Govender, from South African Government’s Department of Environmental Affairs and Tourism (DEAT) and the “United States Veterinary Permit for Importation and Transportation of Controlled Materials and Organisms or Vectors” approved through the Centers for Disease Control and Prevention (CDC).

In order to work with samples in the Center for Molecular Biology at Smith College, a Biosafety protocol and a BSL-2 manual for work with possible zoonotic pathogens were prepared for review. Both were approved.

Noninvasive Fecal Sampling

Fecal samples used in this study were taken from eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), mountain zebra (*Equus quagga*), plains zebra (*Equus*...
zebra zebra), cheetah (Acinonyx jubatus), kudu (Tragelaphus strepsiceros), white rhino (Ceratotherium simum), red hartebeest (Alcelaphus caama), black rhino (Diceros bicornis), and blesbok (Damaliscus pygargus) at Samara Private Game reserve in Graaff-Reinet, South Africa from September to November, 2014.

Wildlife stool samples were collected under the guidance of a wildlife tracker and a certified game ranger with over 10 years of field experience and four years of tertiary education in wildlife biology. Target host species were located with radio telemetry or by tracking sign and spoor, which is any kind of mark or disturbance left by an animal (footprints, hair, scent markings). We used our knowledge of the host’s natural history, such as preferred diet, typical habitat and behavior to narrow the tracking area. Once found, the individuals were observed for as long as the animals remained in the area. The majority of samples were collected after observing an individual deposit the scat. However, unobserved, freshly deposited scat samples were also collected while walking through the animal’s previously occupied locality. The tracker positively identified the scat of the target animal based on the spoor surrounding the scat and the physical appearance of the scat as well as the undigested contents within, such as vegetation type or prey remains. GPS coordinates of each sample were recorded and the host’s common name, species name, date, and time of collection were documented. When witnessed, the gender and age class (adult vs juvenile) of the individual was also recorded.

During collection, gloves were worn and changed between samples; hands were cleansed between glove changes using disinfectant wipes and hand sanitizer. One hundred and fifteen samples of scat were collected in 50 mL leak-proof specimen containers using sterile tongue depressors or sterile, disposable tweezers and placed in a
cooler with ice packs. The specimen collection containers were wrapped with Parafilm around the lid and, upon returning to Samara housing, were placed in the freezer at -20°C until DNA could be extracted on-site.

**Genomic DNA Isolations**

Genomic DNA (gDNA) was extracted in the field at Samara Private Game Reserve from wildlife fecal samples. Isolations were performed using the QIAamp Fast DNA Stool Mini Kit (Qiagen Catalog # 51604), following the manufacturer’s protocol for “Isolation of DNA from Stool for Pathogen Detection”. A converter was used to run the centrifuge, vortex and hot plate due to the difference in voltage output between the United States and South Africa.

Approximately 200 mg of each frozen fecal sample was suspended in 1 mL of InhibitEX buffer and vortexed continuously for one minute to homogenize the sample. The samples were heated in a water bath to 90°C for five minutes to lyse cells. The samples were next centrifuged for one minute to pellet stool particles. All centrifugation steps were carried out at 16,000 x g (approximately 14,800 rpm). The samples were centrifuged for three minutes to pellet inhibitors bound to the InhibitEX matrix. 200 µL of supernatant were incubated with 15 µL Proteinase K for 10 minutes at 70°C to allow for digestion of contaminating proteins. The samples were purified with 500 µL of ethanol-based wash buffers, AW1 and AW2. Genomic DNA was eluted in 200 µL Buffer AE and wrapped with Parafilm. The samples were placed in the freezer at Samara until packaged for transfer to the Williams Laboratory at Smith College where further downstream applications took place.
The quantity and purity of extracted DNA was assessed before DNA amplification. The DNA concentration and purity was measured with a NanoDrop™ 1000 spectrophotometer (Thermo-Fisher). DNA concentrations were determined using the Qubit® 2.0 Fluorometer quantification assay (Life Technologies).

**Nested ITS2 PCR Amplification and Sequencing**

Nested PCR primers and cycling conditions were performed using a modified version of a protocol developed by Sim et al. (2010). All PCR reactions were run on a Veriti® 96 Well Thermal Cycler (Applied Biosystems). Please refer to Appendix A, Figures A3 and A4 for primary and secondary nPCR primer sequences.

**ITS2 Primary PCR Amplification**

Amplicon primary PCR reactions were performed using a modified version of a protocol used in Sim et al. (2010). The 25 µl PCR reaction mix is based on standard Phusion® master mix ratios. All PCR reactions were run on a Veriti® 96 Well Thermal Cycler (Applied Biosystems) using PCR cycling conditions from Sim et al. (2010) with the exception of a modified annealing temperature (Table 2).

The second internal transcribed spacer (ITS2) of ribosomal DNA (rDNA) is commonly used as a genetic marker (barcode) to identify nematode species. The first set of primers, NC13aF (forward) and NC2 (reverse) (see Figure A3 for primer sequences) amplify ITS2 rDNA and flanking sequences of the 5.8S and 28S rRNA genes (Sim et al., 2010). Two microliters of DNA template was added to a 23 µL aliquot of ITS2 primer master mix (all reagents and quantities are shown in table 3).
Table 2. The 24 µl master mix for Primary ITS2 Amplicon PCR reactions.

<table>
<thead>
<tr>
<th>PCR Reagent</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease Free H₂O</td>
<td>16.5</td>
</tr>
<tr>
<td>HF Buffer</td>
<td>5.0</td>
</tr>
<tr>
<td>dNTPs (10 mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>NC13aF Primer (Forward) (10 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>NC2 Primer (Reverse) (10 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.75</td>
</tr>
<tr>
<td>Phusion® High-fidelity DNA polymerase</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 3. Primary PCR cycling conditions for the ITS2 Amplicon PCR reactions.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>55</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>1 minute</td>
<td>30</td>
</tr>
<tr>
<td>Final Extension</td>
<td>72</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4</td>
<td>∞</td>
<td>1</td>
</tr>
</tbody>
</table>

**PCR Purification**

To remove unused nucleotides, primers, and small DNA fragments before secondary PCR, the primary PCR products were column purified in accordance with the Qiagen QIAquick® PCR Purification Kit (Cat. # 28106, Qiagen) protocol with the use of a vacuum manifold.

To confirm that the pH of PB buffer was less than or equal to 7.5 (the pH in which DNA binds to the silica membrane most effectively), 1 µL of pH indicator was
added to 250 µL PB buffer. To bind the rDNA to the membrane, 125 µL PB buffer was added to 25 µL primary PCR product and mixed by pipetting up and down. The tips of the columns were placed into the vacuum manifold and the entire volume of the PCR product/PB buffer mix was loaded onto the column. A vacuum was applied to draw the solution through the column to bind the rDNA to the membrane. The membrane was washed with 750 L PE and a vacuum was reapplied. The vacuum was broken and the columns were transferred to 1.5 microcentrifuge tubes. The columns were centrifuged for 1 minute at 17,900 x g to remove any residual PE wash buffer. The columns were transferred to a new 1.5 mL centrifuge tube for rDNA collection. Thirty-five microliters of nuclease free water was applied to the membrane and was allowed to sit for 5 minutes. The column was centrifuged for 60 seconds at 17,900 x g to elute the rDNA. The purified rDNA was saved for secondary (nested) PCR amplification.

**ITS2 Secondary PCR Amplification**

Secondary PCR reactions were performed using a modified version of a protocol used in Sim et al. (2010). The 25 µl PCR reaction mix is based on the standard Phusion® master mix ratios shown in Table 4. All PCR reactions were run on a Veriti® 96 Well Thermal Cycler (Applied Biosystems) using PCR cycling conditions from Sim et al. (2010) with the exception of a modified annealing temperature (Table 5).

The second set of primers, NC14-Fn (forward) and NC2-Rn (reverse) (see Figure A4 for primer sequences) amplify interior in the primary nPCR amplicons, including the ITS2 rDNA and a small portion of the flanking sequences of 5.8S and 28S rRNA genes (Sim et al., 2010).
Table 4. The 24 µl master mix for nested ITS2 Amplicon PCR reactions.

<table>
<thead>
<tr>
<th>PCR Reagent</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease Free H₂O</td>
<td>16.5</td>
</tr>
<tr>
<td>HF Buffer</td>
<td>5.0</td>
</tr>
<tr>
<td>dNTPs (10 mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>NC14-Fn Primer (Forward) (10 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>NC2-Rn Primer (Reverse) (10 µM)</td>
<td>0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.75</td>
</tr>
<tr>
<td>Phusion® High-fidelity DNA polymerase</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 5. Nested PCR cycling conditions for the ITS2 Amplicon nested PCR reactions.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>61</td>
<td>1 minute</td>
<td>30</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>1 minute</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72</td>
<td>5 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4</td>
<td>∞</td>
<td>1</td>
</tr>
</tbody>
</table>

**Gel Extraction of ITS2 Product**

To separate and visualize PCR amplicons for gel extraction, a volume of 15.0 µl of each PCR product was run on a 2% agarose gel in 1X TAE running buffer at 64 volts for 80 minutes. A 100 bp DNA ladder was run with the samples to estimate amplicon length (Cat. # N3231L, New England Biolabs). Bands were visualized with long wavelength (>300nm) UV light then excised quickly (to limit exposure of the rDNA to
UV light) from the agarose gel using clean, sterile razor blades and flat forceps. The agarose gel slice was transferred to a 1.5 mL tube and weighed to the nearest milligram. The amplified rDNA was extracted from the gel using Promega Wizard® PCR Preps DNA Purification System (Promega, catalog # A2180) according to the manufacturer’s protocol for purification of DNA from high-melting-temperature agarose with the use of a vacuum manifold. A volume of 1 mL PCR Preps resin was added to each excised gel slice and heated to 65°C for 5 minutes, or until the gel was completely melted. One Wizard® Minicolumn was prepared for each gel extraction; a syringe barrel was attached to the Luer-Lok® extension of each Minicolumn, then the tip of the Minicolumn was inserted into the vacuum manifold. The resin/DNA mix was loaded into the syringe barrel and a vacuum was applied to draw the mixture through the membrane of the Minicolumn. The vacuum to the Minicolumn was broken and the membrane was washed with 2 mL of 80% isopropanol. A vacuum was applied to draw the solution through the Minicolumn and continued for another 30 seconds to dry the resin. The Minicolumns were transferred to 1.5 mL microcentrifuge tubes and the syringe barrels were removed. The Minicolumns were centrifuged for 2 minutes at 10,000 x g to remove any residual isopropanol. The Minicolumns were transferred to a new 1.5 mL centrifuge tube. A volume of 50 µL nuclease free water was applied to the Minicolumn and was allowed to sit for 3 minutes to increase rDNA yield. The Minicolumns were centrifuged for 20 seconds at 10,000 x g to elute the rDNA PCR fragments. The Minicolumns were discarded and the purified rDNA PCR products were retained for sequencing.
**ITS2 Cycle Sequencing**

Ribosomal DNA templates were sequenced using forward and reverse primers in separate reactions (in duplicate) for Sanger dideoxy sequencing. The forward and reverse ITS2 primers were each diluted to 0.8 pM by adding 8 µL of 10 µM primer to 92 µL nuclease free water (n/f H₂O). PCR for sequencing was conducted in 10 µL volumes (Table 6) using BigDye® standard cycling conditions (Table 7). Once sequencing was complete, the samples were briefly centrifuged and placed on ice while purification for downstream sequencing was prepared.

**Table 6.** The 10 µl BigDye® master mix for cycle sequencing ITS2 nPCR amplicons

<table>
<thead>
<tr>
<th>PCR Reagent</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease Free Water</td>
<td>2.0</td>
</tr>
<tr>
<td>Primer Forward or Reverse (0.8 pm/µl)</td>
<td>2.0</td>
</tr>
<tr>
<td>BigDye® terminator mix</td>
<td>4.0</td>
</tr>
<tr>
<td>nPCR Product</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Table 7.** Cycling conditions for sequencing of ITS2 rDNA amplicons

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>96</td>
<td>1 minute</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>96</td>
<td>10 seconds</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>50</td>
<td>5 seconds</td>
<td>25</td>
</tr>
<tr>
<td>Extension</td>
<td>60</td>
<td>4 minutes</td>
<td></td>
</tr>
<tr>
<td>Hold</td>
<td>8</td>
<td>∞</td>
<td>1</td>
</tr>
</tbody>
</table>
Purification of the Sequencing Reactions

Performa® DTR Gel Filtration Cartridges (EdgeBio catalog # 42453) were used to purify the sequenced products in accordance with the manufacturer’s protocol. The gel filtration matrix removes unincorporated BigDye® Terminator v3.1, dNTPs, salts, DNA primers and fragments up to 20 bases, buffers, and other low molecular weight materials. The spin columns are pre-packaged with a fully hydrated matrix to maintain the integrity of the gel during storage for optimal performance. To remove the hydrating solution from the column, the gel filtration cartridges were spun down for 3 minutes at 900 x g and the flow-through was discarded. The spin columns were removed and transferred to the provided 1.5 mL microcentrifuge tubes. The entire sequenced reaction sample, 10 µL, was carefully added to the center of each column, avoiding the sides of the column. The columns were centrifuged for 3 minutes at 900 x g to elute the DNA. The column was discarded and the eluate was retained for capillary sequencing by Lou Ann Beirwert in the Center for Molecular Biology.

Capillary Sequencing

The products from the cycle sequencing reaction were injected into capillaries filled with polymer. During capillary electrophoresis, the DNA fragments are separated by size as the negatively charged DNA fragments move through the polymer within the capillaries toward the positive electrode. Before reaching the positive electrode, fluorescently labeled nucleotides move through the path of the laser beam, causing the dye to fluoresce. The fluorescence is detected by an optical detection device and the signal is converted into digital data. Each of the four different fluorescent labels are
specific for each of the four nucleotides, thus different wavelengths of light are emitted for each nucleotide.

The sequence data was recorded in ABI format and visualized on FinchTV DNA analysis software.

**Data Analysis**

FinchTV was used to visualize the chromatogram files of the DNA sequences and to discern sequence quality and edit the sequences. Low quality basecalls within the first 24 nucleotides at the start of the sequence were removed. The sequences were then exported as FASTA files for sequence alignment.

Lasergene’s SeqMan Pro program was used to assemble, analyze and create a consensus sequence from the two forward and two reverse cycle sequencing replicates from each nested PCR reaction. The consensus sequence was then compared to the closest matching reference sequence on Genbank using BLASTn.
Results

Sample Collection and DNA Isolations

DNA yields were inconsistent using the DNA extraction method

Over the duration of six weeks, a total of 115 fecal samples were collected from wildlife hosts located within the fenced portion of Samara Private Game Reserve in Graaff-Reinet South Africa (see Figure 2). Because the aim of this study was to conduct a survey of wildlife gastrointestinal parasites present across a broad sample of mammals within a preserve, a robust sample collection size was not necessary. Of the 115 fecal samples collected, 2 were collected from blesbok, 2 from black rhino, 1 from cheetah, 31 from eland, 15 from giraffe, 4 from kudu, 14 from mountain zebra, 9 from plains zebra, 3 from red hartebeest, 27 from black wildebeest, and 7 from white rhino.

Total genomic DNA extraction at Samara proved to be difficult due to fecal contaminants causing the DNA concentration and quality to vary widely amongst individual samples. DNA yields ranged from 11 ng/µL to 84 ng/µL (Figure 7). Nonetheless, every sample underwent nested PCR for ITS2 amplification, as nested PCR is extremely sensitive and is able to amplify even very low concentrations of DNA.
A. NanoDrop™ 1000 Spectrophotometer screenshot of genomic DNA extracted from two eland fecal samples on November 22, 2014, at Samara Private Game Reserve. (A) Eland sample ELD 14. (B) Eland sample ELD 18.

**Figure 7.**

**PCR Amplification and Gel Extraction**

*Nested PCR was able to amplify low concentrations of ITS2*

ITS2 rDNA of the positive control, *Strongyloides stercoralis*, using the primer set NC13aF, NC2, NC14-Fn, and NC2-Rn, designed by Sim et al. (2010) and the suggested DNA polymerase (Taq® PCR), failed to amplify. However, the positive control was
successfully amplified when the primer sets designed by Sim et al. (2010) were coupled with Phusion® high fidelity DNA polymerase and standard Phusion reaction mix and ratios (Figure 8). Several annealing temperatures were used in order to determine the optimum annealing temperature (Figure 8b). The annealing temperature of 55°C for primary PCR and 62°C for nested PCR yielded the strongest, clearest bands indicated by gel electrophoresis.

As expected, PCR products ranged from ~300-400 bp. The nested PCR primers amplify the ITS2 target region which ranges from approximately 250 to 350 bp depending on the parasite species. All ITS2 PCR reactions were run on 2% agarose gels and PCR products were visually identified using UV illumination. Products were excised with sterile forceps (Figure 9).

Although standard (primary) PCR was not able to sufficiently amplify low concentrations of parasitic nematode ITS2 rDNA to a detectable level, the nested (secondary) PCR was able to amplify ITS2 rDNA to a visibly observable range on gels. For example, after standard PCR only 6 out of 11 samples showed visually detectable bands after gel electrophoresis and 4 of the 6 bands were very faint. However, after nested PCR, all 11 amplicons were visually detectable as indicated by strong gel bands (Figure 10).

Figure 9. Photograph of UV illuminated amplified ITS2 nested PCR products on a 2% agarose gel run at 65 V for three hours (lane 6: 100 bp DNA Ladder). Confirmation of ~450 bp product in lane 1 and ~350 bp product in lanes 2 and 3. Note absence of product of the right size in lanes 4 and 5.

Capillary Sequencing and Data Analysis

Low-quality sequences may indicate multiple infections

Obtaining clean, high-quality sequences proved difficult from both zebra and rhino species. Often, chromatogram files visualized on FinchTV indicated low quality and low confidence base calls. Sequences of low quality were discounted and the DNA sample which produced the low-quality sequence was rerun beginning with primary PCR. If the sequencing results yielded low-quality sequences a second time, a different aliquot of genomic DNA was used for PCR amplification and downstream analysis. Samples that repeatedly produced low-quality sequence results may indicate multiple parasitic infections which yield similar size ITS2 regions. During gel electrophoresis, ITS2
amplicons of multiple parasites that have the same or similar size ITS2 regions may not separate sufficiently for each fragment to be visually distinct, therefore, a single UV-illuminated band containing multiple sequences (i.e. multiple parasites from the same host) would have been excised and co-purified from the gel. The two DNA fragments would have been extracted from the gel and then sequenced as one sequence/sample. Therefore, it is likely that ITS2 rDNA from multiple parasites would produce low quality ITS2 sequences.

Sequences that resulted in high quality and high confidence base calls were analyzed and assembled to create a consensus sequence on Lasergene’s SeqMan Pro software. The consensus sequence was compared to the closest matching reference sequence on Genbank using BLASTn.

Parasite Identification

_Sixteen strongyle parasites were identified from 10 host species_

In total, 12 nematode parasites species and four nematode genera were identified from 10 host species (Table 8). All nematodes identified belong to the order Strongylida, of which five parasites belong to the family Trichostrongyloidea, ten belong to the family Strongyloidea and one belongs to the family Ancylostomoidea. Eland and plains zebra each harbored four parasites, mountain zebra and white rhino each harbored three parasite, blesbok and cheetah each harbored two parasites, and black rhino, kudu, and wildebeest harbored one parasite each. No parasites could be identified from red hartebeest.
Host and Parasite Distribution

Parasite species were shared between overlapping hosts

Across much of Samara, multiple ungulate species were found within the same location due to overlapping habitats and overlapping geographical host range. Although five parasite species were detected across two or more hosts, there were only two cases where one parasite species was shared between two spatially overlapping wildlife hosts. *Trichostrongylus colubriformis* was found in both eland and giraffe, while *Haemonchus contortus* was found in both black wildebeest and blesbok. Interestingly, *Cylicostephanus bidentatus* was found in plains zebra and blesbok, which do not share overlapping habitats. However, *C. bidentatus* was also found in cheetah (found throughout the reserve). The distribution of wildlife hosts and the associated parasites documented during the September-November 2014 survey are illustrated in Figure 11.
**Table 8.** Molecularly identified nematodes found in stool and the wildlife hosts infected. Red squares depict parasite species shared between hosts.

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Ruminant (n=2)</th>
<th>Eland (n=7)</th>
<th>Giraffe (n=3)</th>
<th>Kudu (n=15)</th>
<th>Wildebeest (n=15)</th>
<th>Hindgut fermenter (n=4)</th>
<th>P. zebra (n=6)</th>
<th>B. rhino (n=2)</th>
<th>W. rhino (n=6)</th>
<th>Cheetah (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coopera curticet</em></td>
<td>X</td>
<td></td>
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<tr>
<td><em>Coopera hueleborni</em></td>
<td></td>
<td></td>
<td>X</td>
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<tr>
<td><em>Coopera sp.</em></td>
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<tr>
<td><em>Haemonchus contortus</em></td>
<td>X</td>
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<tr>
<td><em>Trichostrongylus colubriformis</em></td>
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<tr>
<td><em>Cyathostomum terracanthum</em></td>
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<tr>
<td><em>Cyclocycus radiatus</em></td>
<td></td>
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<td>X</td>
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<tr>
<td><em>Cyclocycus sp.</em></td>
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<td>X</td>
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<tr>
<td><em>Cyclostomum bidentatus</em></td>
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<tr>
<td><em>Kilulama solitaria</em></td>
<td></td>
<td></td>
<td>X</td>
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<td><em>Kilulama sp.</em></td>
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<tr>
<td><em>Murshidia linstowi</em></td>
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<td>X</td>
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<tr>
<td><em>Parapoteriostomum euruchus</em></td>
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<tr>
<td><em>Triodontophorus nipponicus</em></td>
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<td><em>Triodontophorus serratus</em></td>
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<td><strong>Ancylostoma sp.</strong></td>
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* Carnivore
** Family = Ancylostomatoide (hookworms)
Figure 11. Map of the fenced portion of Samara Private Game Reserve showing host geographical ranges from GPS locations and observations recorded during a September-November 2014 survey of the study site. A. Eland (yellow) and giraffe (blue) have overlapping habitats (teal) and both harbored *Trichostrongylus colubriformis*. B. Black wildebeest (pink) and blesbok (teal) have overlapping habitats (light pink) and both harbored *Haemonchus contortus*. C. Eland (yellow) and kudu (orange) have overlapping habitats (orange) and both harbored *Cooperia* sp. D. Blesbok (teal) and plains zebra (green) do not share overlapping habitats, however cheetah have a range which includes the entire fenced portion of Samara (distribution not shown); *Cylicostephanus bidentatus* was identified from all three hosts.
Discussion

This is the first comparative study conducted on gastrointestinal nematode parasites amongst a broad range of wild ungulate species and a carnivore from the same preserve in South Africa. The objective of this study was to molecularly identify parasite species within a wildlife reserve and to better understand the role of host distribution on parasite composition and transmission. Overall, I identified 12 nematode parasite species and four nematode genera from 10 wildlife host species. I found that geographic location and spatial dynamics of host populations may influence community composition and transmission of gastrointestinal nematodes and that parasite superfamilies may be more prominent amongst hosts with a specific type of digestive system.

Nematode Identification

Trichostrongyloidea Superfamily

Cooperia

The family Trichostrongylidae is widespread amongst all wild antelope, with Cooperia being a common genus (Boomker et al., 1991). In the present study, C. curticei, C. fuelleborni and a Cooperia species that could not be categorized to species level was identified from the scat of eland. For ease of discussion, we will call this species Cooperia X. Cooperia X was also identified from the scat of kudu. Only one helminth species (Cooperia X) was recovered from kudu. This is in agreement with Boomker et al. (1991) who found similar results on a study of helminths in kudu. Boomker found that
the majority of kudu individuals harbored no helminths and the parasite load of those infected was low and were of the genus *Cooperia*.

*Haemonchus*

In this study, we identified *Haemonchus contortus* from the scat of black wildebeest and blesbok. *Haemonchus contortus* is a well-documented parasite of both blesbok and wildebeest (Horak, 1979; Horak et al., 1982; Horak et al., 1983; van Wyk et al., 2011). In fact, many *Haemonchus* species occur in the abomasum of various antelope and livestock, thus *Haemonchus* does not appear to be a host specific parasite genus. However, *Haemonchus* species may be specific to various antelope relative to host feeding habits and digestive type. In fact, surveys done in Kruger National Park (Boomker, 2014) found that certain host groups were associated with certain *Haemonchus* species. For example, in Kruger, *H. vegliai* is associated with browsing antelope, but in areas where antelopes and domestic livestock graze the same pastures, the wildlife has been found to harbor *H. contortus*, which is the predominant *Haemonchus* of sheep.

The ability of *Haemonchus contortus* to infect both wildlife and domestic livestock has negative implications for the South African economy and for wildlife and livestock management, since the wild ruminants can act as reservoir hosts for resistant *H. contortus*. Furthermore, resistant *H. contortus* infections have led to the deaths of various antelopes, such as sable and kudu (Boomker, 2014).
Trichostrongylus

In the present study, Trichostrongylus colubriformis was identified from the scat of eland and giraffe. This is consistent with a study by Garijo et al. (2004) that also found T. colubriformis in giraffe and a study by Vadlejch et al. (2015) that found Trichostrongylus sp. in farmed eland. Eland can be infected with nematodes that are commonly found in cattle and small ruminants (Vadlejch et al., 2015)

Trichostrongylus colubriformis has been recovered from various antelope, moreover, Horak (1979) was able to artificially infect sheep, goats and cattle with larvae cultured from the feces of impala, Aepyceros melampus.

Strongyloidea Superfamily

Cyathostominae

In this study, Cyathostomum tetracanthum, Cylicocyclus radiatus, and Cylicostephanus bidentatus were identified from the scat of plains zebra. Cyathostomins, such as Cyathostomum, Cylicocyclus, and Cylicostephanus are well-documented parasites of zebra (Krecek et al, 1994). In this study, Cylicocyclus sp., which could not be categorized to the species level, was recovered from the scat of Cape mountain zebra and Cheetah.

Parapoteriostomum

Consistent with the results of others studies, Parapoteriostomum euproctus (formerly Cylicodontophorus euproctus) was identified from the scat of plains zebra. Interestingly, the genus Cylicodontophorus is commonly recorded in plains and
Hartmann’s zebra but is absent in Cape mountain zebra (Scialdo et al., 1982; Scialdo-Krecek et al., 1983; Krecek et al., 1987a, 1987b, 1994; Junker et al., 2015).

**Kiluluma**

*Kiluluma* is a genus that commonly infects rhinoceroses (Round, 1968; Penzhorn, 1994). In this study, *Kiluluma solitaria* was identified from the dung of black rhino and a *Kiluluma* sp., which could not be categorized to the species level, was recovered from the dung of white rhino.

**Murshidia**

In a review by Penzhorn et al. (1994) that compiled a list of parasites infecting black and while rhino, *Murshidia* and *Kiluluma* were the predominant nematode genera recorded. In this study, *M. linstowi* was identified in the dung of white rhino.

**Triodontophorus**

*Triodontophorus* is a common helminth found to infect multiple equids including horse, donkey, and zebra (Penzhorn, 1984; Mathee et al., 2004). In the present study, *T. nipponicus* and *T. serratus* were identified from the dung of Cape mountain zebra. These findings are consistent with a study by Krecek et al. (1994) in which helminth parasites were collected and documented from 13 Cape mountain zebra. Krecek et al. (1994) found between 40-50 individual *T. nipponicus* and *T. serratus* larva in the ventral colon of Cape mountain zebra. *Triodontophorus* nematodes tend to be gregarious and, when large groups feed, create large ulcers within the host’s colon (Jacobs, 2015). Although
sublethal, these ulcers could have an indirect health effect on the already endangered Cape mountain zebra, possibly making the zebra more vulnerable to predation or susceptible to infection by other pathogens. Furthermore, because domestic equids can also be infected by *Triodontophorus* nematodes, wild and domestic *Equus* hosts could potentially share and transmit *Triodontophorus* species in areas of spatial overlap.

*Ancylostomatoidea Superfamily*

A hookworm, belonging to the family Ancylostomatoidea, was identified from the dung of white rhino, however the parasite could not be categorized to species. Multiple DNA extractions yielded results from the reference database, BLASTn, which found *Ancylostoma ceylanicum* to be the closest match with a query coverage of 100% and an identification match of 81%. However, because the percent identification was low, a qPCR *Ancylostoma ceylanicum*-specific assay was performed and the results concluded that the parasite was not specifically *Ancylostoma ceylanicum*. *Ancylostoma* is a hookworm genus that is a natural parasite of carnivores. Although *Ancylostoma* has not previously been recovered or documented in white rhino, hookworms have been found in rhinoceros (Rookmaaker et al., 1998). Our findings indicate that the species of parasite in question may belong to the *Ancylostoma* genus or that of a very similar genus but has either not yet been identified or not entered into the reference database. One such hookworm possibility is *Grammocephalus intermedius*, a hookworm (Ancylostomatoidea) that specifically infects the large intestine of rhinoceros and the DNA sequence of which is not available on NCBI BLASTn database (Zumpt, 1964; Penzhorn, 1994). Even more significantly, the dung sample containing the hookworm
was taken from a male white rhino (the only male) who was newly introduced to Samara at the time of this study. Because this significant finding of the hookworm was discovered toward the end of this study, the specific identification of the parasite was unable to be investigated any further. However, because serious hookworm infestation causes blood loss leading to anemia, as well as to protein loss, hookworm infestation may have a negative impact on already critically endangered rhinoceros populations (CDC, 2013). Thus, it is imperative that the identification of the unknown hookworm be studied further.

**Predator and Prey Parasite Correlation**

It is difficult to determine whether an identified nematode species is infecting a predator, such as cheetah via molecular analysis alone. Although *Cylicostephanus bidentatus* and another *Cylicocyclus* sp. were identified in one cheetah from Samara, it is difficult to determine whether the nematodes identified are actually parasitizing the cheetah, or if the cheetah merely consumed prey species harboring *C. bidentatus* and *Cylicocyclus* sp. For example, cheetah on Samara are known to prey on blesbok, young plains zebra and young mountain zebra. Blesbok and plains zebra were found to harbor *C. bidentatus*, while Cape mountain zebra were found to harbor the unidentified *Cylicocyclus* sp. Therefore, it is plausible that the *C. bidentatus* and *Cylicocyclus* sp. ITS2 rDNA amplified from cheetah scat may be a product of a kill digested by the cheetah rather than a true infection. However, it is important to note that *Cylicocyclus* sp. have been recorded in nodules in the stomach’s of lions and leopards, which may suggest that *Cylicocyclus* spp. can, in fact, parasitize other wild, South African felids such as the
The theory of parasite overlap between predator and prey is supported by Strona’s (2015) study, which found that host trophic ecology could affect predator-prey co-evolutionary history and/or transmission ecology. Furthermore, constant occurrences of predation may give way to the colonization of parasites among predators which gives rise to a larger parasite distribution through the expansion of host range (Strona, 2015). Thus, it would be evolutionarily advantageous for a parasite to have the ability to parasitize both predator and prey species in order to reduce the chance of parasite extinction. For example, predator and prey populations could contribute to the persistence of a shared parasite by spreading the parasite between prey metapopulations, thereby reducing the parasite’s risk of going locally extinct (Strona, 2015).

**Host Influences on Parasite Community Composition**

**Host Distribution and Spatial Dynamics**

Geographical location and spatial dynamics of host populations may influence community composition and transmission of gastrointestinal nematodes.

Across much of Samara, multiple ungulate species were found within the same location due to overlapping habitats and overlapping geographical host range. *Trichostrongylus colubriformis* was identified from the stool of both eland and giraffe. Although eland are found throughout the preserve, giraffe are typically found only in the escarpment speckboomveld and arid thronveld, which make up the savanna (see Figure 3 for the vegetation map). Interestingly, both eland and giraffe are browsing ruminants; however, eland may also graze, as eland are considered highly selective mixed feeders (browser and grazer). *T. colubriformis* has been recovered from various antelope and in a
study by Horak (1979) sheep, goats and cattle were able to be artificially infected with \textit{T. colubriformis} larvae cultured from the feces of impala \textit{(Aepyceros melampus)}. Horak’s (1979) experiment supports our findings, which indicate that \textit{T. colubriformis} may infect multiple host antelope species that spatially overlap, and furthermore, that wild and domestic ungulates may transmit parasites to one another.

\textit{Haemonchus contortus} was found in the stool of both black wildebeest and blesbok. Black wildebeest are grazing ruminants that inhabit the sweet grasslands (see Figure 3 for the vegetation map) that makes up part of the Plateaux Grasslands located on Kondoa Mountain.

\textit{Cylicostephanus bidentatus} was identified from blesbok, plains zebra and cheetah stool samples. Although \textit{C. bidentatus} has been well documented in equids, including zebra, \textit{C. bidentatus} has not been well document in antelope species, such as blesbok, nor carnivores, such as cheetah (Boomker, 2014; Junker et al., 2015; Krecek et al., 1987b). Interestingly, plains zebra and blesbok do not have overlapping distributions nor do they possess the same type of digestive system. While plains zebra are grazing hindgut fermenters found within the escarpment speckboomveld and arid thronveld, blesbok are grazing ruminants found inhabiting sweet grasslands (see Figure 3 for the vegetation map) on Kondoa Mountain. However, cheetah are found throughout the preserve and often move from the mountains to the plains hunting small antelopes, including blesbok, young zebra and wildebeest. Our findings regarding parasite overlap amongst multiple hosts may suggest that \textit{C. bidentatus} may not be as specific to equids as previously thought. However, our results of \textit{C. bidentatus} within the stool blesbok and cheetah should be investigated further before any conclusions can be drawn.
The family Trichostrongylidae is widespread amongst all wild antelope, with *Cooperia* being a common genus. In this study, both eland and kudu were found to harbor a *Cooperia* sp. that could not be classified to the species level. Similarly, Matthee et al. (2004) found that closely related sympatric host lineages often possess more similar parasite communities.

In this study, despite the fact that plains and mountain zebra are closely related, the two equids did not harbor the same parasite species. However, previous studies have indicated that both mountain zebra and plains zebra are capable of harboring many of the same parasite species (Matthee et al., 2004). For example, in this study, *Cylicostephanus bidentatus* was present in plains zebra but absent in mountain zebra. Similarly, a study by Matthee et al. (2004) found that *C. bidentatus* was present in plains zebra and absent from mountain zebra when the two zebra did not share sites; however, when mountain and plains zebra overlapped in habitat use, both zebra species harbored *C. bidentatus*. Furthermore, our findings, which indicate plains zebra harbored more parasites (n=4) than mountain zebra did (n=3), are also supported by Matthee et al. (2004) study, which indicate that when mountain zebra occurred alongside plains zebra, the two hosts tended to share more helminth species and mountain zebra tended to host more parasite species than when the two equids did not share the same site. This may suggest that total spatial segregation of two closely related hosts may limit the number of shared parasite species.
Host Digestive System

Parasite superfamilies may be more prominent amongst hosts with a specific type of digestive system.

The composition of gastrointestinal nematode parasites in wild ungulates may be largely determined by feeding habits, such as whether the animal is a grazer, browser or mixed feeder. Other studies have indicated that host diet and digestive systems may be a driving determinant in shaping the microbiome communities of the gut (Godoy-Vitorino et al., 2008; Tajima et al., 2001). However, it may not only be the type of vegetation or diet which influences the microbiome of the gut. Perhaps it is also the location of vegetation, with regard to height above ground, that influences the parasite diversity and composition of the gut. For instance, grazers, which usually feed on grasses or forbs close to the ground, may be more susceptible to infection than browsers, which usually feed on tender twigs and leaves further from the ground, simply due to the contingencies of the nematode’s direct lifecycle. In other words, because animals typically defecate on the ground, the ingestion of infective nematode larva may be more likely when vegetation is consumed close to the ground. In the present study, browsers were infected with an average of one parasite species, grazers were infected with an average of almost three parasite species and the one mixed feeder of the study, eland, was infected with four parasite species. Although these findings support my hypothesis that grazers and mixed feeders may have a greater parasite load than browsers, a larger, more robust sample size of wildlife browsers, grazers and mixed feeders must be examined.

Parasite composition may also be impacted by the host digestive system, such as whether the animal is a ruminant or a hindgut fermenter (Junker et al., 2015).
Interestingly, all nematodes identified belonging to the superfamily Trichostrongyloidea exclusively infected ruminants as opposed to hindgut fermenters or carnivores, while nearly all nematodes of the superfamily Strongyloidea exclusively infected hindgut fermenters, with the exception of *Cylicostephanus bidentatus*, which also infected blesbok (ruminant) and cheetah (carnivore), and *Cylicocyclus* sp., which also infected cheetah. Based on these results, parasite superfamilies may be correlated to host digestive pathways. In fact, Godoy-Vitorino et al. (2008) found that the organ function is a stronger determinant of microbial community structure than is host phylogeny in a study that compared the gut microbiome of foregut (ruminants) and hindgut fermenters. Therefore, when studying parasite transmission amongst wildlife communities and between livestock, host digestive type may be an indicator of the parasite species that colonize that host.

**Ecological Importance**

In many areas of South Africa, domestic livestock such as sheep, goats and cattle, graze the same pastures as various wild antelope species. The Karoo’s fertile valleys, semi-arid deserts and majestic mountains converge to generate a major agricultural heartland with a flourishing ecotourism industry. Increased interest in ecotourism, biodiversity conservation, and growth in livestock production may increase the risk for transmission of parasites between wildlife and livestock due to their expanding spatial overlap (van Vuuren and Penzhorn, 2015, Junker et al., 2015). Moreover, because of the economic benefits of ecotourism, private game reserves are often developed from old domestic livestock farms (van Vuuren and Penzhorn, 2015). However, because
gastrointestinal nematode eggs can persist for long periods of time in the soil, relocated wildlife may be at risk of becoming infected by parasites from livestock which previously occupied the old farm.

Wildlife relocation and wildlife stocking is constantly taking place and requires the creation of thorough management plans in order to estimate the stocking rate and carrying capacity of a reserve (van Vuuren and Penzhorn, 2015). Determining wildlife stocking rates is complex and requires extensive knowledge regarding the diet, habitat and average weight of each wildlife species as well as the characteristics of the land, vegetation and soil within the management area in order to estimate the wildlife carrying capacity of the reserve (De Fortier et al., 2014). The carrying capacity is the number of herbivores and/or carnivores a game reserve can support for long-term persistence (De Fortier et al., 2014). Although vegetation type and spatial extent is the limiting factor in determining the carrying capacity of a reserve, the density dependence of parasitic infection should not be ignored when creating a management plan. Because parasite infection increases on a per capita basis with increasing host population densities, wildlife population abundance may influence parasite prevalence and transmission (Matthee et al., 2004). Thus, parasitic infection should also be a factor evaluated and addressed when creating management plans for determining the wildlife-stocking rate or carrying capacity of game reserves.

With the progression of climate change, which is predicted to decrease precipitation in most of South Africa, the number and size of water sources will decrease (Hewitson et al., 2005). More animals, both livestock and wildlife, will congregate to limited water resources, likely leading to higher rates of disease transmission. Thus, the
potential influence of helminth infections and other diseases found in a wildlife
community could increase with climate change and should be incorporated in the
development of new conservation programs.

Cheetah populations may be particularly vulnerable to the threats of increased
parasite transmission and infection, as a mass extinction 12,000 years ago wiped out the
majority of the cheetah population, resulting in a population bottleneck. Today, the
cheetah population’s low genetic diversity has resulted in low fecundity, poor sperm
quality, and susceptibility to epidemics (O’Brien et al., 1985). Such low genetic diversity
may make cheetah uniquely susceptible to the threat of parasite infections and other
diseases.

Although significant effort has been made to keep wildlife and livestock free of
parasite infection in zoos and national parks, this has not always been the case with
private game farms/ranches and livestock farms, where many factors allow helminths to
persist in the environment. Over-crowding of animals due to human urbanization, the
concentrated location of resources that limit hosts to a small range, wilderness
fragmentation, translocation, and high stocking rates of animals are all factors that
contribute to parasite persistence (Matthee et al., 2004). Furthermore, fecal-born
nematode eggs and worms are able to accumulate and persist in the environment,
sometimes for very long periods of time. Collective latrines, or middens, often used by
some ungulates, may also play a part in parasite persistence (Walker, 1996). Parasitic
infection and disease may also have an additional effect on population dynamics because
even non-pathogenic parasites may pose a risk to wildlife populations when the host is
stressed or malnourished and may predispose the host to other population pressures (van Wyk and Boomker, 2011).

The presence of disease and parasites is often difficult to assess in wild populations, but with the use of non-invasive molecular techniques such as scat collection and DNA sequence analysis/barcoding, parasites can be matched to their host and the influence of infection on the individual host and the host population can be better understood. This thesis makes progress toward the goal of understanding the role of host habitat preference and the role of geographical distribution and spatial overlap on parasite composition and transmission so that we may gain further insight into the impacts of gastrointestinal parasitic infection on host fitness and population dynamics. Such knowledge will contribute to improved management and conservation of wildlife in game preserves and parks.
Challenges and Limitations

Field Collections

Sample collection from wildlife is often the limiting factor

The present study was a survey of wildlife gastrointestinal parasites present amongst a broad sample of ungulates and cheetah. It was not designed as a study to quantify parasite burdens or to determine host infection rates. Because under-sampling results in incomplete species representation, a sample size of at least 15 fecal samples per host species was planned, however, many factors, including weather, wildlife behavior, and host prevalence, made attaining this goal for sample size difficult. For example, plains zebra and kudu typically fled upon seeing a vehicle, resulting in reduced observation time and fewer scat collections. The plains zebra’s and kudu’s easily excitable behavior is likely due to hunting practices for population control on Samara Private Game Reserve. Collecting cheetah scat was also difficult for a few reasons: cheetah are constantly moving throughout the reserve, are fairly elusive, and only three cheetahs were radio-collared for radio telemetry tracking. A small number of “trackable” individuals resulted in a low probability of fresh cheetah fecal collection. However, collecting numerous fecal samples per host species was not essential, as the present study was a survey of gastrointestinal nematodes within a specific reserve, therefore, sample sizes obtained for each host were considered adequate for the purpose of this study.

Although the methods used for sample collections and DNA isolation in the present study were adequate, I would have preferred to use a method suggested to me at the end of my field collections by Graham Kerley, Ph.D, Professor of Zoology & Director of the Centre for African Conservation Ecology at the Nelson Mandela
Metropolitan University. In the present study, scat was collected in the field and, upon returning to base camp, was immediately placed in the freezer until DNA isolations could be done. Although extracting DNA in the field facilitated the legal transportation of materials to the United States because the samples were no longer infectious, DNA extractions performed in the field also presented several problems. For instance, the power was lost at the reserve during every rain and wind storm which interrupted DNA extractions for minutes to days. Because of this, DNA isolations often had to be repeated. DNA extraction also took away time that could have been spent tracking and collecting samples in the field. In order to avoid the aforementioned troubles of DNA extraction associated with fieldwork in very rural locations where electricity is not dependable, samples should be treated to make them non-infectious and easy to ship. To transport biological samples across borders, the samples must be noninfectious. Additionally, it is easier to transport samples that are light and dry. As suggested by Dr. Kerley, I propose for future studies that samples be collected, saturated with 95% ethyl alcohol for 24 hours and then dried with silica beads for 1-4 days. Saturating the biological sample with 95% ethyl alcohol renders the sample non-infectious, and dehydrating the sample with silica beads removes the alcohol for legal transportation on an aircraft while preserving the integrity of the DNA within the stool sample. Furthermore, the dried samples are light with no chance of leaking during transportation. Most importantly, this method has been shown to be effective for several of Dr. Kerley’s studies regarding herbivore diet and is the primary method for sample preparation prior to DNA extraction in the Metabarcoding School, which is a series of lectures and practicals introducing different aspects of DNA metabarcoding taught in South Africa.
DNA Sequencing Analysis

Obtaining clean, high-quality DNA sequences proved difficult from many scat samples

Often, chromatogram files repeatedly indicated low quality and low confidence base calls, regardless of multiple DNA extractions, PCR amplifications and sequencing reruns. This was a consistent problem amongst plains zebra, mountain zebra, white rhino and black rhino. Such results may be a consequence of multiple parasite infections where the ITS2 rDNA amplicons of closely related parasite species may be so similar in size that the amplicons of each species could not be separated during gel electrophoresis. Moreover, the hypothesis of multi-infections is supported by several studies by Krecek (1984), Buckle et al. (1996), Matthee et al. (2004), and Boomker (2014) who showed that infections by multiple parasite species is more common than single parasite infections. This is particularly true for Equus species who commonly harbor species of both genera Cylicocyclus and Cylicostephanus. On the other hand, this study had numerous singleton infections, compared to studies which sampled animals from necropsies, likely because of the DNA extraction aliquot size (scat size) and because only stool was examined for parasites. Although multiple DNA extractions, amplifications and sequencing were performed on each sample, the fact that DNA isolation required only small aliquots of stool decreased the probability of procuring eggs from different nematode species in a single, small fecal sample, particularly if the infection intensity of a parasite is low. Thus, the inability to separate ITS2 rDNA amplicons from multiple parasite species prior to sequencing and the use of small scat aliquots for DNA isolation, may have combined to result in the underrepresentation of parasite diversity in this study. Despite these
limitations, the results obtained from the large number of scat samples from Samara provide valuable insight into the diversity of parasite fauna found on the reserve.

**DNA Barcoding to Identify Parasite Species**

*Identification is limited by the availability of DNA sequences in reference databases*

DNA Barcoding is both inexpensive and commonly used for species identification, thus, many sequences are available in reference DNA sequence databases. One limitation, however, is that the identity of an unknown organism can only be determined if the same genetic sequence from the same species is available in the reference database. Thus, DNA Barcodes must first be taken from a taxonomically identified specimen in order for DNA Barcoding to be effective. Understandably, this is an arduous task, not only due to the sheer abundance of species that need to be referenced, but also because many morphologically identified species are not easily available for researchers to isolate DNA for sequencing. Furthermore, many species of parasites are yet to be discovered. The lack of referenced genetic information, particularly regarding wildlife parasites, may have been the issue regarding our inability to identify the specific species of hookworm found in the dung of a male white rhino. Although our next step would have been to morphologically identify the parasite in question, this course of action was not an option. Because total DNA was isolated in the field and dung samples could not be taken back to the lab for analysis, the alternate possibility of isolating parasitic eggs from stool then allowing the larva to hatch and mature until identifiable features developed was not possible. The combination of morphological characteristics and genetic information would have been used to identify
the hookworm species. Parasitic DNA sequences could then be added to a database to be used for future research.

However, the availability of morphologically identified reference samples does not constitute a flawless method for barcoding an entire species. For example, phenotypic convergence of unrelated species may cause discordance between morphological identification and molecular analysis. DNA barcoding also presents limitations as a tool for species identification in cases where genetic divergence takes place more rapidly than morphological divergence (Collins and Cruickshank, 2012). Nonetheless, DNA barcoding has proven to be a powerful tool for biodiversity assessment. Newly identified organisms can often be categorized as to their genus and closely related species can be identified by similarity in DNA sequences.
**Future Directions**

This study represents an initial effort to understand the influence of host distribution on helminth transmission and, ultimately, the influence of helminth infection on the population dynamics of the host. How could this work be improved and expanded? First, additional PCR primers should be used in future studies to help identify individual nematodes that share highly similar ITS2 rDNA. Such additional barcode sequences include 18s (SSU) ribosomal DNA gene and COXI mitochondrial DNA gene (Floyd et al., 2005; McLean et al., 2002). The use of multiple sequences in DNA barcoding will improve species identification and enhance the usable data obtained from studies such as this one.

Methods need to be developed to separate and identify nematodes species from hosts infected with multiple nematode species. This is complicated because multiple parasite species will give multiple PCR amplicons that can be difficult to separate and sequence from a complex mixture such as stool. However, methods do exist to aid in such separation and these methods will give additional insight into the parasite community composition of each host species, which can then be used to investigate host distribution and parasite transmission more thoroughly. Another strategy would be to use Next Gen Sequencing (NGS) to obtain massive amounts of DNA sequence data from each stool sample. Although costly, NGS would give a broader understanding of the community composition of parasitic nematodes because multiple nematode species could be identified and the nematode abundance could be determined within a single stool sample.
DNA sequence data from a study such as this one could be used to do a parasite-host co-phylogenetic analysis. Deciphering the evolutionary relationships between parasites and their hosts is particularly important for understanding the current and potential virulence of a given parasite (Penczykowski et al., 2016). Because the long-term goal of this kind of analysis is to understand the impact of parasite infections on the health of African wildlife, determining phylogenetic congruencies, parallel speciation and host specificity may help to define the delicate balance between innocuous parasite infections and pathogenic infections.

In terms of wildlife management, it would also be interesting to study how host distribution may influence parasite persistence in a population. Specifically, how do natural or artificial barriers influence parasite persistence in wildlife and livestock populations? Such studies would be particularly pertinent regarding the resilience of wildlife and the persistence of pathogens in the face of climate change, where resources such as water and food, may become more limited than they are today.
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* Samara Private Game Reserve management plan authors: (Feldon et al., 2013) Ken Coetzee (vegetation survey 2002), Angela Gaylard (Regional, Ecologist: South African National Park), Alan Feldon (compiler Samara private game reserve).
Appendices

Appendix A: Supplemental Figures and Tables

Figure A1. Topographic map of fenced portion of Samara Private Game Reserve (roads, water points, infrastructure, camps).
Table A1. Host species information

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<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Game numbers (2013)</th>
<th>Habitat (refer to Figure A2)</th>
<th>Feeding habits</th>
<th>Digestive system</th>
<th>NCBI taxonomic ID</th>
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<td>Sweet grassland</td>
<td>Grazers</td>
<td>Ruminant</td>
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<tr>
<td>Cheetah</td>
<td><em>Acinonyx jubatus</em></td>
<td>10 (2012)</td>
<td>Throughout</td>
<td>Carnivore</td>
<td>N/A</td>
<td>32536</td>
</tr>
<tr>
<td>Eland</td>
<td><em>Taurotragus oryx</em></td>
<td>160</td>
<td>Sweet grassland, escarpment speckboomveld</td>
<td>Highly selective mixed feeders</td>
<td>Ruminant</td>
<td>303929</td>
</tr>
<tr>
<td>Giraffe</td>
<td><em>Giraffa camelopardalis</em></td>
<td>30</td>
<td>Escarpment speckboomveld/thicket</td>
<td>Browsers</td>
<td>Ruminant</td>
<td>9894</td>
</tr>
<tr>
<td>Hartebeest-red</td>
<td><em>Alcelaphus caama</em></td>
<td>168</td>
<td>Escarpment speckboomveld</td>
<td>Grazers</td>
<td>Ruminant</td>
<td>59519</td>
</tr>
<tr>
<td>Kudu</td>
<td><em>Tragelaphus strepsiceros</em></td>
<td>621</td>
<td>Arid thornveld, Escarpment speckboomveld/thicket</td>
<td>Browsers</td>
<td>Ruminant</td>
<td>9946</td>
</tr>
<tr>
<td>Rhinoceros-black</td>
<td><em>Diceros bicornis</em></td>
<td>5</td>
<td>Escarpment riverine, Temperate thicket mosaic</td>
<td>Browsers</td>
<td>Hindgut fermenter</td>
<td>9805</td>
</tr>
<tr>
<td>Rhinoceros-white</td>
<td><em>Ceratotherium simum</em></td>
<td>4</td>
<td>Arid thronveld, Escarpment speckboomveld, Penzia thicket</td>
<td>Grazers</td>
<td>Hindgut fermenter</td>
<td>9807</td>
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<tr>
<td>Wildebeest-black</td>
<td><em>Connochaetes gnou</em></td>
<td>110</td>
<td>Sweet grassland</td>
<td>Grazers</td>
<td>Ruminant</td>
<td>59528</td>
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<tr>
<td>Zebra-mountain</td>
<td><em>Equus zebra zebra</em></td>
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<td>Sweet grassland</td>
<td>Grazers</td>
<td>Hindgut fermenter</td>
<td>301482</td>
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<td>Zebra-plains</td>
<td><em>Equus quagga</em></td>
<td>50</td>
<td>Escarpment speckboomveld, Penzia thicket</td>
<td>Grazers</td>
<td>Hindgut fermenter</td>
<td>9790</td>
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</tbody>
</table>
1. NC13aF: 5′-ATCGATGAAAAACGCAGC-3′
2. NC2: 5′-TTAGTTTCTTTTCCTCCGCT-3′

Figure A2. 1. Forward and 2. Reverse primer sequences for primary PCR amplification of ITS2 rDNA (Sim et al., 2010).

1. NC14-Fn: 5′-GAACGCATAGCGCCGTTGGT3′
2. NC2-Rn: 5′-TGATATGCTTAAGTTCAGCGGG-3′

Figure A3. 1. Forward and 2. Reverse primer sequences for secondary PCR amplification of ITS2 rDNA (Sim et al., 2010).
### Appendix B: Glossary of Terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
<td>The ruminant’s fourth and true, glandular stomach. (Bowen, 2003)</td>
</tr>
<tr>
<td>Abundance</td>
<td>The total number of individuals, or biomass, of a species present in a specific area. (Molles, 2013)</td>
</tr>
<tr>
<td>Allopatric</td>
<td>Describes the condition in which populations or species have nonoverlapping geographic ranges. (Molles, 2013)</td>
</tr>
<tr>
<td>Biome</td>
<td>Distinguished primarily by their predominant plants and are associated with particular climates. (Molles, 2013)</td>
</tr>
<tr>
<td>Browser</td>
<td>Herbivore that consumes leaves, bark, and stems from plants. (Godoy-Vitorino et al., 2008)</td>
</tr>
<tr>
<td>Carrying capacity</td>
<td>The maximum population of a species that a particular ecosystem can sustain. (Molles, 2013)</td>
</tr>
<tr>
<td>Community</td>
<td>An association of interacting species living in a particular area; all the organisms living in a particular area. (Molles, 2013)</td>
</tr>
<tr>
<td>Density-dependent factor</td>
<td>Biotic factors of the environment (disease, competition) which effects are related to, or dependent upon, local population density. (Molles, 2013)</td>
</tr>
<tr>
<td>Distribution</td>
<td>The geographic range of an organism or the spatial arrangement of individuals in a local population. (Molles, 2013)</td>
</tr>
</tbody>
</table>
Fecundity  The number of eggs or seeds produced by an organism. (Molles, 2013)

Grazer  Herbivore that consumes grasses. (Godoy-Vitorino et al., 2008)

Hindgut fermenter  Has enlarged fermentation compartments in the cecum and/or colon. (Steven and Hume, 1998)

Nematode  Simple roundworms; colorless, un-segmented, and lacking appendages, nematodes may be free-living, predaceous, or parasitic. Many parasitic species cause important diseases of plants, animals, and humans. Other species are beneficial in attacking insect pests, mostly sterilizing or otherwise debilitating their hosts. (www.biocontrol.entomology.cornell.edu/)

Population  A group of individuals of a single species inhabiting a specific area defined by natural boundaries (i.e. mountaintop, river basin, island) or artificial boundaries (i.e. country, county, national park). (Molles, 2013)

Ruminant  Also known as a foregut fermenter; has a pre-gastric fermentation chamber. (Steven and Hume, 1998)

Spoor  A collective term for tracks and sign, or any type of mark or disturbance left by an animal; footprints, hair, evidence of feeding, scent marking, etc.

Strongylida  An order of nematodes that represents one of the major radiations of nematode parasites of vertebrates. Their distinguishing features include a copulatory bursa (in males) and a buccal capsule of variable shape and size. (Durette-Desset et al., 1994)
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sympatric</td>
<td>Describes the condition in which populations or species have overlapping geographic ranges. (Molles, 2013)</td>
</tr>
<tr>
<td>Ungulate</td>
<td>Hoofed mammals</td>
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