

Short Communication

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Corresponding author:
V. Phetla;
Email: v.phetla@sanbi.org.za

Short communication: Exploring gastrointestinal tract nemabiomes in captive ungulates at the SANBI National Zoological Gardens: A next-generation sequencing baseline approach

V. Phetla¹ , K. Masenya¹ and M. Chaisi^{1,2}

¹Foundational Research and Services, South African National Biodiversity Institute, P.O. Box 754, Pretoria 0001, South Africa and ²Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, South Africa

Abstract

Gastrointestinal tract (GIT) nematode infections have a significant negative impact on the well-being and productivity of animals. While it is common for a host to be co-infected with multiple species of nematode parasites simultaneously, there is a lack of effective tools to study the composition of these complex parasite communities. We describe the application of the “nemabiome” amplicon sequencing to study parasitic GIT nematode communities in captive wildlife at the National Zoological Garden, South African National Biodiversity Institute. A total of 13 samples from Roan antelope (n=1), Eland (n=1), Sable antelope (n=9), Arabian oryx (n=1), and Blue duiker (n=1) were analysed for the presence of GIT nematode infections. A total of 256 operational taxonomical units (OTUs) were generated and compared to a curated database with ≥99% identity to sequences in the NCBI database. The OTUs found represented nematode species which are commonly associated with wild ruminants. These belonged to members of the genera *Haemonchus*, *Trichostrongylus*, and *Cooperia*, with a few OTUs classified as unidentified genera detected. These were further classified to species level, with *Haemonchus contortus* and *Trichostrongylus colubriformis* having high relative abundance across all captive ungulate species. Utilizing amplicon sequencing to examine the nemabiome holds great potential for enhancing our basic knowledge of the biology of gastrointestinal nematodes and monitoring infections. This improved understanding could guide the development of more efficacious preventive and control measures against these significant parasites, which negatively impact animal health and productivity on a global scale.

Introduction

Captive ungulates represent diverse species housed in zoological parks, wildlife sanctuaries, and conservation centres worldwide (Naz *et al.*, 2021). Ensuring the optimal health and well-being of these animals is of paramount importance for conservation efforts aimed at maintaining genetic diversity within populations, particularly those under human care and management (Naz *et al.*, 2021). Unfortunately, some gastrointestinal tract (GIT) nematodes can have significant negative impacts on the health of captive ungulate populations (Phetla *et al.*, 2024). These GIT nematode infections lead to diarrhea, behavioral changes affecting feeding and social interactions, severe weight loss, and sometimes mortality, thus significantly constraining ungulate health in captive environments and adversely impacting animal welfare and productivity (Hoberg and Brooks, 2015). As GIT nematodes have evolved to coexist with their hosts, the term “nemabiome” has been coined to describe the intricate ecosystem involving these parasitic nematodes (Avramenko *et al.*, 2015). These complex parasitic communities ultimately play a vital yet understudied role in veterinary health (Beaumelle *et al.*, 2021). Therefore, there is a need to develop improved techniques for assessing the species composition of gastrointestinal nematode populations in wildlife, both in terms of identifying the constituent species and determining their relative abundance (Avramenko *et al.*, 2017). Although these GIT nematode complexes are still poorly understood, strategies utilising next-generation sequencing (NGS) technology, like those used to study the microbiome, demonstrate enormous promise in this respect (Avramenko *et al.*, 2015).

The GIT nematodes that commonly infect ungulates, especially prevalent species like *Haemonchus contortus*, can significantly impact the host’s immune response, nutrient metabolism, and overall physiological fitness (Sulliotti, 2023). Consequently, understanding the composition and dynamics of the intestinal nemabiome is crucial for elucidating the ecological interactions and health implications in ungulate populations (Sulliotti, 2023). Traditional microscopic techniques have limited the comprehensive assessment of nemabiome diversity, composition, and dynamics in captive ungulates due to their low resolution and taxonomic identification capabilities. Leveraging the power of NGS technologies presents a

transformative opportunity to overcome these limitations and gain unprecedented insights into the complex nemabiome communities inhabiting gastrointestinal tracts of captive ungulates.

This study explores the application of GIT nemabiomes in captive ungulates using an NGS approach in support of SANBI's preventative medicine monitoring program.

Material and methods

A total of 13 samples were from five ungulate species, namely Roan antelope (n=1), Eland (n=1), Sable antelope (n=9), Arabian oryx (n=1) and Blue duiker (n=1) housed in different enclosures at the SANBI/National Zoological Garden. This was a retrospective study using DNA samples collected from a previous study (Mosala, 2017). A 311- to 331-bp fragment of the rDNA ITS-2 region was polymerase chain reaction (PCR)-amplified from the 13 ungulate samples using primers NC1 and NC2 (Gasser *et al.*, 1993). Briefly, the PCR mix consisted of 12.5 μ L AmpliconRed master mix (Lasec, South Africa), 1 μ L (10 μ M) of each primer, 2 μ L template DNA, and 8.5 μ L of nuclease-free water to a total volume of 25 μ L. The PCR cycling conditions were initial denaturation at 95 $^{\circ}$ C for 5 min, 95 $^{\circ}$ C for 30 sec (denaturation); 54 $^{\circ}$ C, 30 sec (annealing); 68 $^{\circ}$ C for 1 min (extension), for 40 cycles, 68 $^{\circ}$ C for 10 min (final extension). The amplicons were submitted to Inqaba Biotechnical Industries (Pretoria, South Africa) where they were barcoded, quantified, and pooled into a single library to generate a normalised library. The library was sequenced on the Illumina NextSeq platform. The paired-end reads raw data were processed using Qiime2 with default settings (Caporaso *et al.*, 2010). The DADA2 plugin was used to remove ambiguous bases, low-quality reads, and chimeras. Sequences were clustered to operational taxonomic units (OTUs) using a cut-off of 97% similarity using VSEARCH (Rognes *et al.*, 2016). The resulting OTUs were compared against the trained nematode ITS2 rDNA (Avramenko *et al.*, 2017) for taxonomic assignments. Exploratory analyses were performed in R v.3.5.1 and Bioconductor v.3.0 (Gentleman *et al.*, 2004).

Results and discussion

The overall analysis of gastrointestinal nemabiome in 13 samples from five species of captive ungulate revealed informative data of sequences of GIT nematode infections classified to species level. The total number of reads ranged between 19254 and 95540 per sample (Supplementary Table 1), with sable antelope having the lowest and eland having the highest number of reads. The results indicated that wild ungulates at the National Zoological Garden are infected by nematodes of three genera, namely *Cooperia*, *Haemonchus*, and *Trichostrongylus*, along with a substantial proportion of sequences that could not be classified at the genus or species level and were categorized as "unidentified" (Figure 1a). The genus *Haemonchus* and *Trichostrongylus* were identified to species level as *Haemonchus contortus* (*H. contortus*), and *Trichostrongylus colubriformis* (*T. colubriformis*), while *Cooperia* was not identified to species (Figure 1b). *Haemonchus contortus* was the most dominant, common nematode species across all samples and species, followed by *Trichostrongylus colubriformis* (Figure 1b).

GIT nematode infections varied among the studied ungulate species. *Haemonchus contortus* was identified from all ungulate host species. A study by Zieger *et al.* (1998) in Zambia indicated the Eland was mostly affected by *H. contortus*, similarly, Phetla *et al.* (2024) supported the findings that *H. contortus* was the most

dominant GIT nematode species infecting wild ruminants, including wild ungulates, across sub-Saharan African countries. *Haemonchus contortus* and *T. colubriformis* have a global distribution and commonly infect various wild ruminants, including Roe deer, Fallow deer, Red deer, and Mouflon (Halvarsson *et al.*, 2022). According to Phetla *et al.* (2024), these nematodes are also common in wild ungulates in sub-Saharan Africa.

ITS-2 rDNA metabarcoding has previously been used to determine the nemabiome of wild and domestic ruminants in other parts of the world. According to the study by Queiroz *et al.* (2020), analysis of the sheep nemabiome from more than 90 flocks across western Canada indicated that *H. contortus* was the predominant gastrointestinal nematode species, followed by *Teladorsagia circumcincta* and *T. colubriformis*. Many of the surveyed flocks had high intensities of these parasitic infections. This is the case with the current study as four ungulates (two Sable antelopes, one Eland, and one Arabian Oryx) were co-infected by *T. colubriformis* and *H. contortus*. This is not surprising as these species have a global distribution and have been reported in different domestic and wild hosts (Abuessailla *et al.*, 2013).

Cooperia infection was detected to genus level in only one Sable antelope (Sable antelope 3, Figure 1(a)). *Cooperia* are usually low in both domestic and wildlife (Roos and Grant, 1993). Furthermore, the study by Avramenko *et al.* (2017) indicated that one of the *Cooperia* spp. (*Cooperia punctata*) was generally found in lower intensities in cattle; however, they still formed a significant parasite burden in several eastern Canadian herds. The infection patterns in this study reveal a complex interplay of single and multiple parasitic species across the ungulate populations. This diversity of GIT nematode infections among and within ungulate species underscores the intricacy of wildlife parasitology and its potential implications for animal health and conservation.

In terms of GIT nematode infections, free-ranging wild animals typically exhibit higher parasite species richness than their captive counterparts (Phetla *et al.*, 2024). This increased diversity in free-ranging animals can be attributed to their unrestricted movement across various habitats, diverse feeding patterns, frequent contact with different host species, and natural exposure to the environmental stages of parasites. In contrast, captive animals generally show lower parasite species diversity due to controlled conditions, including restricted habitat exposure, managed diets, limited animal contact, regular antiparasitic treatments, and routine veterinary care. This is evident in this study as only three genera (*Haemonchus*, *Trichostrongylus*, and *Cooperia*) were detected across the captive ungulate species housed at the SANBI Zoological Garden. The controlled environment and preventive health measures in captivity effectively reduce the likelihood of animals encountering multiple parasite species, resulting in lower overall parasite species richness.

The distribution of genera in our study could be influenced by various factors, such as the host species, environmental conditions, and management practices in the captive setting (Chaudhari *et al.*, 2022).

According to Phetla *et al.* (2024), nematodes are the most diverse and widespread GIT parasites found across different species of wild ungulates and geographic regions. Wild ungulates can therefore play an important role in the transmission of GIT nematodes, especially *Haemonchus*, *Trichostrongylus*, and *Cooperia* species to domestic animals, particularly where they share grazing areas. These genera have a global presence in Europe and South Africa (Boomker *et al.*, 1996) and are distinguished by elevated temperatures and humidity which provided favorable conditions for the presence and spread of species including

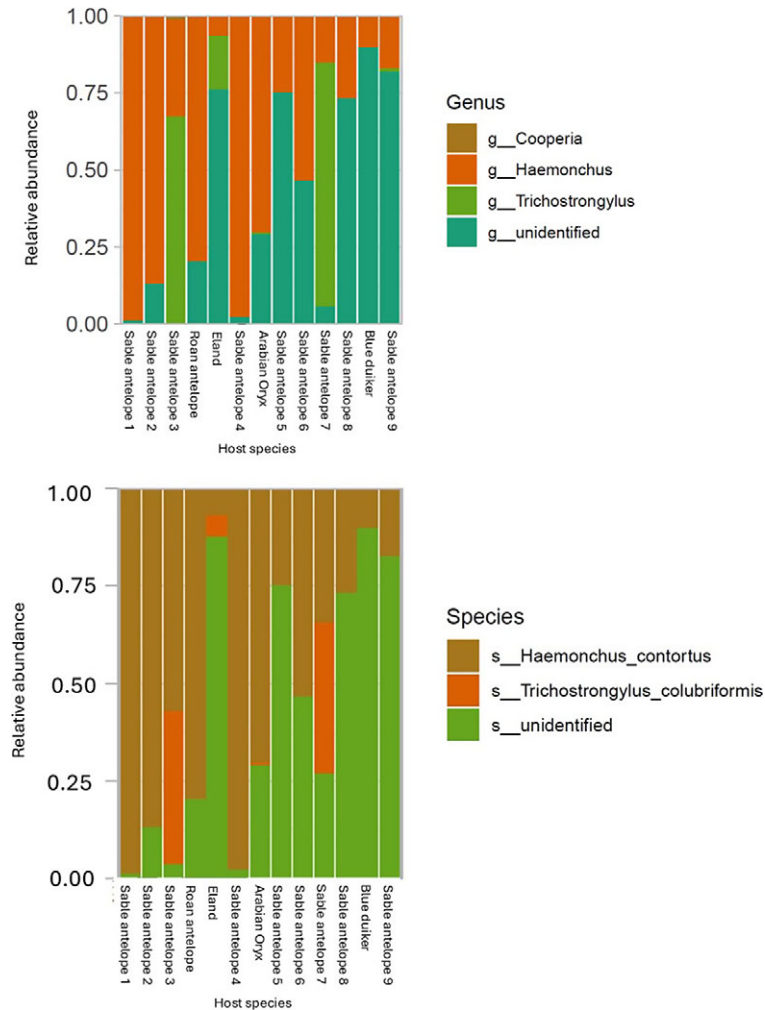


Figure 1. (a) Species diversity and composition of gastrointestinal nematodes to genus level of captive wild ungulates from the SANBI National Zoological Gardens. (b) Species diversity and composition of gastrointestinal nematodes to species level of wild ungulates from the SANBI National Zoological Gardens.

Haemonchus contortus, *Trichostrongylus colubriformis*, and *Cooperia* spp. The manifestation of these GIT nematodes is due to their well-adapted life cycle involving free-living larval stages in the environment (e.g., grass, soil), increasing their chances of GIT parasite transmission to grazing host animals.

Understanding the diversity and composition of GIT nematodes in captive wildlife is crucial for management, as some nematode species are potentially virulent and infections can result in severe disease or mortality (Hoberg *et al.*, 2015; Pittman *et al.*, 2020). These infections can be transmitted to susceptible animals in shared enclosures. Additionally, the high proportion of unidentified nematode sequences underscores the need for more extensive reference nematode databases for accurate taxonomic identification of nematodes to species level. This could facilitate a better understanding of the nemabiome dynamics and potentially pathogenic or commensal relationships between nematodes and their ungulate hosts (Avramenko *et al.*, 2015). Further investigations, incorporating analysis of larger sample size and additional molecular markers, could provide more insights into the nemabiome composition and the functional roles of the identified and unidentified nematode species in captive ungulate populations (Hoberg *et al.*, 2015; Chaudhari *et al.*, 2022).

Conclusion

This study investigated the gastrointestinal nematode communities (nemabiomes) in captive ungulates in the SANBI National Zoological Garden in South Africa. The study revealed a limited diversity of GIT nematode infections, identifying only three nematode genera: *Haemonchus*, *Trichostrongylus*, and *Cooperia*. Among these, *Haemonchus contortus* showed the highest prevalence, followed by *Trichostrongylus colubriformis*, while *Cooperia* was the least abundant. However, these infections can be transmitted to susceptible animals in the zoo, leading to mortality. These findings highlight the need for more comprehensive research to fully characterise the nemabiome composition in captive and free-ranging ungulate populations in South Africa and to determine the risk of transmission to domestic livestock. Moreover, the high proportion of unidentified nematode sequences emphasizes the importance of expanding and improving reference databases and molecular techniques for accurate taxonomic identification of nematodes. This would facilitate a better understanding of the nemabiome dynamics and the potential pathogenic or commensal relationships between nematodes and their ungulate hosts.

Supplementary material. The supplementary material for this article can be found at <http://doi.org/10.1017/S0022149X24000804>.

Availability of data. The nucleotide sequence data reported are available in the NCBI GenBank databases under the BioSample accessions number (SAMN42574826 – SAMN42574838) and SRA accession number PRJNA1137257.

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Competing interest. None.

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