

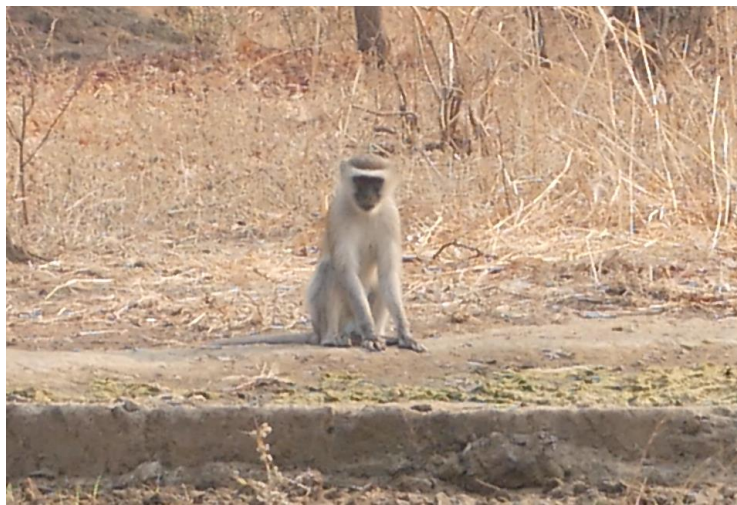


Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine
and Animal Science**

Natural occurrence of *Strongyloides* spp. in vervet monkeys (*Chlorocebus pygerythrus*) in Kuti Wildlife Reserve, Malawi

Assessment of correlation between anthropogenic disturbance
and *Strongyloides* spp. infection



Ana-Marija Camber

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Ana-Marija Camber

Supervisor: Nils Fall, Department of Clinical Sciences

Examiner: Karin Alvåsen, Department of Clinical Sciences

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SUMMARY

The geographic overlap between non-human primates and people is increasing, especially in tropical and subtropical countries. This inevitably creates a potential for anthropogenic and zoonotic transmission of parasites, which could harm both wildlife and human communities. *Strongyloides* spp. are obligate gastrointestinal nematodes that are relatively host specific, but transmission between species can occur. It is estimated that between 30-100 million people are infected by *Strongyloides* spp. worldwide. In this study the ubiquitous non-human primate *Chlorocebus pygerythrus* was used as a model to investigate the relationship between anthropogenic disturbance and *Strongyloides* spp. infection. *Chlorocebus pygerythrus*, more commonly known as vervet monkeys, are medium sized monkeys found in the east and south of Africa. They are highly adaptive to different habitats and are found in semi-deserts, snowy mountains, savannah to more anthropogenic environments such as villages and cities. Vervet monkeys spend a large amount of time foraging on the ground, making them susceptible to soil-transmitted helminths.

The present study investigated the natural occurrence of *Strongyloides* spp. in two vervet monkey troops in Kuti Wildlife Reserve, Malawi. Faecal samples from each troop were analysed (n=60) and the results showed a significantly higher prevalence ($p<0.0001$) in the troop in close proximity to humans (83%) than in the troop with sparse human contact (6.7%). The findings were in line with a previous study performed by Thatcher (2018), where four different localities were included. Vervet monkeys on the sites with higher anthropogenic disturbance had a higher parasite load. This suggests that anthropogenic disturbance indeed does affect the prevalence of *Strongyloides* spp., although since the number of samples was low the results should be cautiously interpreted.

Another interesting finding in the present study was that the troop with higher prevalence had a smaller sized home range, a factor which is linked to increased parasite load in previous studies. For future studies, correction for host density, larger sample size, more troops and molecular analysis to demonstrate host-shifts are suggested.

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ABBREVIATIONS

The following abbreviations are used in the present study:

ST	Sunset troop
VC	Volunteer Centre troop
NHP	Non-human primates
LWT	Lilongwe Wildlife Trust
EPG	Eggs per gram

INTRODUCTION

The human population density is exponentially increasing, especially in developing countries such as Malawi. Paired with climate change, this accelerates habitation loss and degradation for numerous wild species, including non-human primates such as vervet monkeys (*Chlorocebus pygerythrus*). Vervet monkeys are abundant wildlife species in Malawi and are known to carry a numerous of zoonotic pathogens such as *Strongyloides* spp. Contact between human and wild monkey communities are inevitable and creates the potential for zoonotic and anthroponotic transfer of disease. There is consequently strong regional and international interest in establishing baseline health data for vervet populations, assessing the associated risks to human health, and identifying critical control points for risk reduction. At present, little is known about the disease status of wild vervets in Malawi.

According to the World Health Organization (WHO) it is estimated that between 30-100 million people are infected with *Strongyloides* spp. worldwide. However, recent meta-analysis shows that the prevalence in subtropical and tropical countries varies from 10% to 40% (Schär *et al.*, 2013), consequently making the estimated number of cases between 200-370 million people worldwide (de Silva *et al.*, 2003; Schär *et al.*, 2013; Pullan *et al.*, 2010). To the knowledge of the author, no study on gastrointestinal parasitic infection of vervet monkeys in the Salima district, Malawi has been published.

Purpose

The overarching aims of this study were to describe the naturally occurring prevalence of *Strongyloides* spp. in wild vervet monkeys from two troops in Kuti Wildlife Reserve, Malawi and to provide a preliminary assessment to whether a correlation between anthropogenic disturbance and prevalence of *Strongyloides* exist.

In order to put the study in a wider context, a secondary aim was to make a literature review of gastrointestinal parasites, anthropological disturbance and potentially affecting elements including stress and host density, as well as strongyloides in humans. The sources of gastrointestinal parasites in *Chlorocebus pygerythrus* are limited and therefore studies of the close relative *Chlorocebus aetiapis* was included in the assessment of regional prevalence.

LITERATURE REVIEW

Vervet monkeys (*Chlorocebus pygerythrus*)

Vervet monkey is the popular name of some non-human primates within the genus of *Chlorocebus*. The taxonomy of the genus is not fully genetically established, but it is commonly described that the *Chlorocebus*-genus consists of 6 different species. The popular term vervet monkey is not yet defined, but arguably only applicable to the species *Chlorocebus pygerythrus* and *Chlorocebus aetiops* (Turner *et al.*, 2019). In this study that definition will be honored and whenever the common term vervet monkey is used, either the species *Chlorocebus pygerythrus* and/or *Chlorocebus aetopis* is alluded.

Chlorocebus pygerythrus are ubiquitous, medium-sized monkeys found in the east and south of Africa. They are highly adaptable, ranging from the forests to the dry, semidesert habitats of eastern Africa and the snowy mountains in South Africa (Turner *et al.*, 2019). Turner *et al.* (2019) stated “*Although not often used as a model for human evolution, like humans, they appear able to adapt to nearly every environment they encounter*”. A proof of their plasticity is the transplanted populations on the islands of St. Kitts, Nevis and Barbados in the Caribbean, that arrived with boats approximately 300 years ago (McGuire, 1974; Chapman & Fedigan, 1984). Due to their adaptive success, vervet monkeys are considered pest animals in some areas. They frequently raid crops and interact with humans, making them a subject for hunting and killing. In some areas, such as the Turkana District in Kenya, vervet monkeys are considered bushmeat (de Jong *et al.*, 2008).

Vervet monkeys are highly social and spend a significant amount of time forming and maintaining social relationships (Dunbar, 1991). They live in multi-male and multi-female groups of up to 38 individuals (Isbell & Jaffe, 2008). Females make up the primary family unit, while males typically leave the group to breed (Seyfarth & Cheney, 1984). Vervets are considered semi-terrestrial omnivores and spend extensive periods of time foraging on the ground. Time spent on the ground increases the exposure to soil transmitted helminths (McFarland *et al.*, 2014). The diet varies with habitat and consists of a wide range of food sources, such as leaves, buds, insects and fruit (Teichroeb *et al.*, 2015). Troop territories range considerably in size and depending factors are accessibility to water, food, competing troops and presence of predators (McFarland *et al.*, 2014).

Gastrointestinal parasites

Parasites are of big importance in the ecosystem, controlling and regulating host population growth, affecting specific interactions and community biodiversity. Today, infectious diseases are emerging at an increasing rate (Jones, 2016). To the individual host, gastrointestinal parasite infections are mostly sub-clinical and non-lethal, although some can be fatal to hosts. Even so, the sub-clinical infections can affect the host by reducing juvenile growth and impair the hosts ability to compete for food and mates due to energy costs (Anthony, 2007; Chapman *et al.*, 2016).

The protective immune response against helminths includes both adaptive and innate components and is generally referred to as a T helper 2 response with production of cytokines,

IgE antibodies, eosinophils and mast cells. Depending on the different sites in the host's body that are affected by parasites, the result will differ. Eosinophils are activated through the *Strongyloides* antigens and IL-5 stimulates further eosinophil growth and activation. The eosinophils stimulate antigen-specific Th-2 cytokine production IL-4 which influences B lymphocytes to upregulate the production of IgE and IgG4 antibodies. IL-8 attract neutrophils to eliminate and eradicate larvae. The production of IgE incite the granulation of mast cells as well as mitigating eosinophil migration. Mast cells are the effectors acting against parasitic adults in the hosts intestines, contributing to eliminate the infection (Anthony, 2007; Weatherhead, 2014; Viney, 2017).

Prevalence of GI-parasites in vervet monkeys

The number of published articles on parasite fauna in vervet monkeys is today relatively small and there is a lack of consistency between the studies in regards of methods of parasite analysis, making comparative work difficult (Gillespie, 2006).

Some of the most commonly reported gastrointestinal parasites include *Strongyloides*, *Trichuris*, *Oesophagostomum*, Hook worm, *Ascaris*, *Entamoeba coli* among others but there are regional differences between sites (Legesse & Erko, 2004; Petrasova *et al.*, 2010; Amenu *et al.*, 2015; Wren *et al.*, 2016; Valenta *et al.*, 2017). For example, *Metastrongylus*, *Toxocara* and *Fasciola* have only been found in one vervet population in Uganda (Valenta *et al.*, 2017).

Table 1. Comparing prevalence of *Strongyloides* spp. in vervets in 7 different studies that included degree of anthropogenic disturbance and habitat type

Reference	No of animals	Country – Area	Habitat type	Degree of anthropogenical disturbance	<i>Strongyloides</i> group prevalence
Wren <i>et al.</i>, 2016 (additional information Valenta <i>et al.</i>, 2017)	38	South Africa – Loskop Dam Nature Reserve	Mixed bushveld and woodland. Protected reserve, tourist resorts and facilities	Moderate	24 %
Legesse and Erko, 2004	25	Ethiopia – Sodore	Anthropogenic – resort	High	0 %
Legesse and Erko, 2004	16	Ethiopia – Wondo Genet	Anthropogenic – resort	High	0 %
Valenta <i>et al.</i>, 2017	24	Uganda – Lake Nabugabo	Anthropogenic landscape, hotels, recreational areas, village, agricultural plots	High	29 %
Amenu <i>et al.</i>, 2015	140	Ethiopia – Lake Hawassa	Anthropogenic – resorts, recreation areas	High	12.9 %
Petrasova <i>et al.</i>, (2010)	Unkown	Tanzania – Rubondo	Mixed forest and human settlement	Moderate	50% (wet season)

Reference	No of animals	Country – Area	Habitat type	Degree of anthropogenical disturbance	Strongyloides group prevalence
Muriuki et al (1998)		Island National Park			42.7% (dry season)
	123	Kenya – Various locations	Villages	Presumed high	16.3 %
Senst (2017)	51	Malawi – Kasungu	National park	Presumed low	2 %
Senst (2017)	50	Malawi – Lilongwe	Anthropogenic – tourist facilities, wildlife rehabilitation centre	High	32 %

Table 2. *Gastrointestinal parasite prevalence in vervets in different studies*

Reference	No of animals – Country	Trichuris	Oesophagostomum	Hookworm	Ascaris
Wren et al, 2016	38 – South Africa	92 %	84 %	71 %	-
Legesse and Erko, 2004	26 + 15 – Ethiopia	32% resp 43.75%	0% resp 12.5%	-	-
Valenta et al, 2017	24 – Uganda	13 %	8 %	-	33 %
Muriuki et al (1998)	123 – Kenya	47.1 %	-	-	-
Senst (2017)	51 + 50 – Malawi	62% resp 92%	-	29% resp 0%	-
Amenu et al, 2015	149	20.7 %	-	43.7 %	18.6 %

Strongyloides

The genus *Strongyloides* includes approximately 50 obligate gastrointestinal species (Speare, 1989) and infects mammals, birds, reptiles and amphibians. Most of these species are rather host-specific and can infect very few host species. *Strongyloides* have, compared to most other parasitic nematodes, a complicated life cycle. It consists of two adult generations, one parasitic and one environmental. The parasitic adult generation is exclusively female, and the reproduction is done by parthenogenesis, meaning the genetic composition between females and her eggs are identical. The eggs, or the L1s that hatch from the eggs, are excreted from the host through the faeces. The rhabditiform larvae then grow, develop and moult. During the

developmental phase the male eggs/larvae go through four larval stages (L1-L4) and finally moults into free-living adult male worms (Toledo, 2015; Viney, 2017).

The female development phase is more complex and consists of two routes, the direct (homogonic) or the indirect (heterogonic). In the indirect route the female larvae go through the same four larval stages (L1-L4) as their male counterparts and develop into free-living adult female worms. Alternatively, in the direct route, the female larvae develop to third larval stages (L3) which are parasitic, and the development is arrested. The development is then reinitiated once the filariform larvae successfully has penetrated the skin of a new host. During infection the L3 larvae moult into L4 while migrating through the host, aiming for the hosts intestines. Once in the intestines the final stage, parasitic adult females, is reached and the cycle is complete (Toledo, 2015; Viney, 2017).

The free-living adult generation sexually reproduce and the female lays eggs that hatch and moult into parasitic L3s. Ultimately, after a single free-living adult generation all the progeny will be host infective L3s (Toledo, 2015; Viney, 2017). For comparison the close relative species *Parastrongyloides* can have several continuing free-living adult generations (Grant *et al.*, 2006).

The determining factors of the female rhabditiform larvae route (direct or indirect) is not yet established. Studies have shown that factors such as sex-ratio of the free-living generation, immune status of the host, parasite strain differences or environmental conditions possibly could play a role (Grove, 1996; Viney & Lok, 2007).

Strongyloides fuelleborni

Strongyloides fuelleborni is a parasite of old-world apes and monkeys and causes human infection to a lesser extent than *S. stercoralis* (Toledo *et al.*, 2015). Human infections have been reported in both Papua New Guinea and occasionally in Peru (Ashford & Barnish, 1989; Toledo *et al.*, 2015). *S. fuelleborni* is associated with the neonatal disease “swollen belly syndrome” in New Guinea, wherein infants obtain massive internal infections which can develop to disseminated disease (Ashford & Barnish, 1989).

Strongyloides stercoralis

The life cycle of *Strongyloides stercoralis* differs from other *Strongyloides* species in that the non-infective rhabditiform larvae can develop into infective filariform larvae within the human hosts intestine and initiate a new life cycle. Consequently, the parasite load may increase without external reinfections and the infection can become chronic. *S. stercoralis* is primarily a human parasite, however infections have been detected in other primates (chimpanzees, monkeys etc) and domestic dogs (Toledo *et al.*, 2015).

Correlation between stress and parasites

Correlation between stress level and helminth eggs per gram (EPG) has been shown in studies. Whether it is stress that causes increased chance of infection, or if infection causes an internal stress response is not yet fully established (Cizauskas *et al.*, 2015) and studies are contradictory.

Stress responses are prevalent in all animals' lives, and Moberg (2000) defined stress as “*the biological response elicited when an individual perceives a threat to its homeostasis*”. The adaptational response to stress down-regulates the functions of the body which are not of immediate advantage during a fight or flight situation, for example growth regulation, reproduction, thyroid function and immunity. Through the HPA-axis (hypothalamic-pituitary-adrenal-axis), glucocorticoids like cortisol and corticosterone are released from the adrenal cortex in response to disruption of physiological and psychological homeostasis (Black, 1994). Increased serum cortisol levels impair the immune response by inhibiting several functions of lymphocytes, macrophages and leukocytes, consequently making the individual more susceptible to infection and more severe pathologies (Black, 1994; Teixeira *et al.*, 2007). The stress response also includes other neurotransmitters, neuropeptides, neurohormones and neuroendocrine effector molecules which can modulate immune system functions, such as catecholamines, certain opiates and growth hormone (Black, 1994).

Stress behavior in chronically stressed, recently translocated vervet monkeys (*C. pygerythrus*) shows a clear relationship with higher infection densities, compared to non-stressed, wild vervet monkeys. The vervet monkeys showing stress behavior had recently been held captive, leading to the discussion that wildlife living in close proximity to humans might suffer from higher rates of infection due to stress, but are also more susceptible to anthroponotic pathogens (Senst, 2017). Studies on chimpanzees (*Pan troglodytes*) showed that both testosterone and cortisol were positively associated with parasite richness, but only significantly when both testosterone and cortisol were included, since both can inhibit immune function. By manipulating the immune response, it is possible that multiple infections become more resistant (Muehlenbein, 2006).

Chapman *et al.* (2007) followed a group of Colobus monkeys (*Colobus*) during a period of 23 months, measuring faecal cortisol and intestinal parasites. The study showed no correlation between indices of parasite infection and cortisol level in a given month, but multiple infections and more highly pathogenic parasites (such as *Oesophagostomum*) led to a greater stress response the subsequent month. Cortisol levels did, however, not increase when the previous month's prevalence of the less-pathogenic *Trichuris* increased. This showcases that parasite infections themselves causes internal stress in the monkeys, leading to postponed cortisol elevations (Chapman *et al.*, 2007).

Host cortisol mimics the effect of worm ecdysteroids and can directly influence parasites growth (Romano *et al.*, 2015). In human studies it has been shown that increased levels of endogenous corticosteroids enhance the fecundity of *S. stercoralis* parthenogenetic females and enhances the transformation of rhabditiform larvae into infective filariform larvae, thus leading to autoinfection (Silva *et al.*, 2016).

Environmental variation

Anthropogenic disturbance

The rapidly changing habitats for primates due to human modification is expected to alter the patterns of parasitic infection in such way that primates in anthropogenically-disturbed areas may be exposed to a higher diversity of parasites than primates in secluded areas. In addition to that, an increased human activity may cause chronic stress due to repeated conflicts with humans (Chapman, 2006). However, one of the benefits of proximity to human settlement is the access to nutritional food, which in turn decreases the nutritional stress and increases resistance to parasitic infection (Wallis, 2000; Hahn *et al.*, 2003; Valenta *et al.*, 2017).

High level of human disturbance has been shown to impact body size, coat condition and endoparasite diversity in primates (Borg, 2015). Poor coat appearance can indicate skin infection, ectoparasites, over-grooming, physiological stress, endoparasites, nutrient deficiencies and general illness (Beisner, 2009; Jolly, 2009). Previous studies done on macaques (*Macaca sinica*) showed a greater prevalence of *Strongyloides* sp., *Trichuris* sp., strongyle-type eggs, *Entamoeba coli* and *Entamoeba histolytica/dispar* in the troops that ranged in areas used by humans. Other species such as gray langurs (*Semnopithecus priam*) and purple faced langurs (*Trachypithecus vetulus*) had a higher prevalence of *Cryptosporidium* sp. infections in areas used by humans than in areas that lacked human presence (Ekanayake *et al.*, 2006). Similarly, red colobus (*Procolobus rufomitratus*) with a higher dietary intake of crops had greater parasite infections than its counterparts in old-growth forest (Chapman, 2006).

Thatcher *et al.* (2018) also showcased that increased anthropogenic disturbance in fact does increase parasite load in vervet monkeys, comparing 10 troops in 4 different sites. The anthropogenic disturbance was standardised using human density per km² and anthropogenic structure per km² to create a fixed effect. The sites included private reserve (with 2 human density/km² and 2 anthropogenic structure/km²), industrial land (48 human density/km² and 4 anthropogenic structure/km²), gated estate (2970 human density/km² and 275 human density/km²) and city centre (3100 human density/km² and 352 anthropogenic structures/km²). The study aimed for an equal number of samples from each troop, to allow more accurate interpretation. The parasites identified in the study were *Strongyloides* sp., *Tricuris* sp., *Ascaris* sp., *Oesophagostomum* sp. and *Coccidia* sp. However, the paper does not provide any information regarding the differences in parasite species between the troops or even between the different sites (Thatcher *et al.*, 2018).

Comparing anthropogenic disturbance in relation to parasite infection in several studies shows that living in a highly disturbed habitat does not alone explain a higher parasite species richness. Several other elements must be attributed such as habitat-specific factors (e.g. soil moisture, frequency of intra-group interactions), regional parasite pool and condition of the host (Valenta *et al.*, 2017). Genetic analysis of parasites is required to determine if host shifts are occurring (de Gruijter *et al.*, 2005).

Internal risk factors

Published studies of wild vervet monkeys are predominantly based on non-identified individuals for logistical reasons. However, some researchers have managed to determine individuals within a troop and could therefore assess internal risk factors such as age and sex in relation to gastrointestinal parasites. In these studies, neither age nor sex seem to correlate with richness nor intensity of gastrointestinal parasites (Wren *et al.*, 2015; Valenta *et al.*, 2017; Bliersch *et al.*, 2019).

Clinical signs in NHPs

The symptoms of strongyloides have been detailly described in gibbons where diarrhoea, which may be bloody or mucoid, is most common. Other clinical signs include dermatitis, urticaria, anorexia, depression, vomiting, emaciation, cough and dyspnoea (Toft & Eberhard, 1998; Dufour *et al.*, 2006). Fatal strongyloidiasis has been described in lowland gorillas, chimpanzees (Penner, 1981), gibbon, orangutan, patas monkey and woolly monkey (Blacklock & Adler, 1922; Pillers & Southwell, 1929; Depaoli & Johnsen, 1978; Johansen *et al.*, 1970; Orihel & Seboil, 1972; McClure *et al.*, 1973; Harper *et al.*, 1982).

Pathogen transmission between humans and primates

The importance of zoonoses are increasing, especially in rural areas of developing countries where people live in close proximity to both wildlife and domestic animals, and under conditions of poor hygiene (Schantz, 1991). Recent events with devastating impacts such as the Ebola outbreaks and the now widely spread HIV illustrate how immense the effects, on both human and wildlife communities, primate zoonoses can be (Chapman, 2005a).

Pathogen sharing among host species has two broad explanations. The first one being that hosts have a common ancestor from which the pathogen communities are inherited. The second one includes “host shifts”, where pathogens develop affinity with new hosts (Antonovics *et al.*, 2002) that have similar immunological responses. The closer host species are related, the more pathogens are shared. For example, humans have more pathogens in common with chimpanzees (*Pan troglodytes*) than with colobus monkeys (*Colobus*) (Pedersen, 2009).

A successful host shift that results in disease emergence is a process involving three fundamental stages. For a transmission to take place, the spatial opportunity is the first crucial step, i.e. a geographical overlap must occur so that the naïve host can be exposed to the pathogen. The second stage is the actual transmission, which is limited by evolutionary and ecological barriers such as host immunological response and pathogen plasticity. Establishment of the disease is the third and last step, which incorporates contact rate, demography and population density (Pedersen, 2009). Following a host shift, naïve hosts may lack appropriate immune responses resulting in a high pathogenicity and rapid spread of disease (Osterhaus, 2001).

Host switching, although sometimes necessary in some conditions, can be costly for parasites. Infecting alternative hosts might decrease the number of eggs and reduce larvae survival. This since the relationship between the undeveloped parasite and host is delicate and highly specialized. The host exhibits defence mechanisms against infections and maturing parasites

need to be resilient to or avoid the immune system. This extraordinary specialization limits the host suitability for parasites (Jones *et al.*, 2015). Due to this effect, the number of pathogen transmissions occurring in nature is finite (Cable *et al.*, 2017). Nonetheless, after a few generations within the new host species, parasites seem to adapt and the initial cost that followed the host shift is rewarded (Jones *et al.*, 2015).

Human strongyloidiasis

Two species of the *Strongyloides* genus are known to affect humans, *Strongyloides stercoralis* and *Strongyloides fuelleborni*. Strongyloides are one of the soil-transmitted helminthiases (STH) and a WHO-recognized neglected tropical disease (NTD).

Epidemiology

Currently there are several knowledge gaps in the epidemiology of strongyloidiasis, such as undetermined population at risk, morbidity and the global burden of disease (Toledo *et al.*, 2015). It is estimated that between 30-100 million people are infected worldwide (Genta, 1989; Jorgensen *et al.*, 1996; Bethony *et al.*, 2006), however this estimated number is likely to be low (Viney & Lok, 2007; Olsen *et al.*, 2009; Schär *et al.*, 2014). This is partly due to the low sensitivity and difficult parasitological diagnostic methods, shortage of parasitological surveys, prevalence difference between areas and large proportion of asymptomatic hosts.

Endemic areas of strongyloidiasis include sub-Saharan Africa, the West Indies, Latin and South America, Southeast Asia, Bangladesh, southern Pakistan among others (Toledo *et al.*, 2015). Strongyloidiasis is mainly presented in tropical and subtropical climates, and sporadically in temperate regions (Vadlamudi *et al.*, 2006; Puthiyakunnon *et al.*, 2014). Although, in recent years there has been an increase of cases in developed countries, most likely due to the growing number of immigrants and travellers. An overview paper on the global prevalence of *S. stercoralis* showed a heterogeneous range of infection rates from 0.1% in the Central African Republic to up to 91.8% in Gabon (Izquierdo *et al.*, 2013).

Clinical Signs

Strongyloides is the only pathogenic helminth parasite that can complete its entire lifecycle within human hosts. This enables autoinfection and development of persistent infections. Human infection ranges from asymptomatic light infections to chronic symptomatic strongyloidiasis. Hyperinfection and dissemination of larvae to all internal organs in immunocompromised patients causes mortality in up to 85% of patients (Grove, 1996)

Classic symptoms are related to the skin or the gastrointestinal system and includes diarrhoea, abdominal pain and urticaria, but a variety of presentations including neurological and pulmonary has been seen (Grove, 1996).

The second troop, Sunset troop (ST), consisted of 30 individuals during the study period (infants not included, but subadults included). The troop ranged in an area without permanent human residents, but with one large building with a round-the-clock park guard and regular usage by tourists.

Home range analysis

Troops were followed by foot from dawn, approximately 06.00 in the morning, until midday or until sunset when the monkeys chose their sleeping sites. During the observation of the troops, a minimum distance of 10 meters between the vervets and the researcher was always kept to reduce the anthropological impact. The GPS coordinates (Universal Transverse Mercator coordinates) were noted every 30 minutes. Using Google Earth Pro 7.3.2.5776, all coordinates were mapped and outlined to calculate the home range area.

The anthropologic disturbance was quantified and standardised by measuring human density per km² and anthropogenic structure per km². An anthropologic structure was defined by a building of man-made material with a roof. Similarly, the vervet density was measured per km².

Sample collection

Sixty faecal samples were collected non-invasively and opportunistically during the 8-week study period between August and October. Samples were mostly collected in the morning and was often found in close proximity to the chosen sleeping site. Collection from one identified troop was made during two consecutive days, before rotating to the second identified troop for two days and then back to the first troop again. The individual troop members were not identified, consequently enabling the possibility of multiple samples from one single individual.

Due to logistical reasons and the potential health hazard, formalin solution was not used to preserve samples immediately upon collection, instead samples were placed in plastic containers and analysed on the same day they were collected.

Parasitological analysis

The parasitological analysis was made using a combination of methods to assess the prevalence of *Strongyloides* spp. guidelines set up by Gillespie (2006) were followed, with some modification for logistical purposes. The methods used in this study included, in the following order, direct wet mount, direct floatation procedure, faecal sedimentation and McMaster egg count procedure for detection of eggs and larvae. Direct wet mount is a simple test to discover helminths and protozoa, but it is only of value when concentrations of egg, larvae or cyst are high. Some samples with lots of debris can interfere with the identification of the eggs and larvae. By performing faecal flotation debris is separated from the eggs which enables easier identification. To identify eggs that are too large and heavy to float, the sedimentation method was used. Baermanns technique was used in order to recover and identify live nematode larvae.

A standard electron phase microscope was operated in all tests and *Strongyloides* sp. eggs and larvae were identified based on size and morphology, i.e. a thin shell and an embryonated egg. For initial identification of eventual infection, faecal material with saline was smeared on a microscope slide for direct wet mount analysis.

Faecal floatation was performed with sugar solution, where 454 g granulated sugar and 355 ml tap water (Dryden *et al.*, 2006) was gently heated and then filtered to a clear solution. The faecalizer was 1/2 filled with sugar solution, fresh faeces were homogenised and additional sugar solution was poured to the brim. A microscope slip was placed on lip of tube for 7 minutes, and then placed on a slide for analysis with $\text{\AA} \sim 10$ and $\text{\AA} \sim 40$ objective lenses. Samples with positive results on the sugar floatation were prepared and pipetted into chambers of a McMaster slide. The slide was assessed using standard methods to assess eggs per gram (EPG), with an $\text{\AA} \sim 10$ objective lens.

For faecal sedimentation, faeces were homogenised and the suspension was filtered before allowed to settle until sediment appeared, approximately 5 minutes. The supernatant was removed and 3 drops of 5% methylene blue was added to the sediment. The stained sediment was examined with a $\text{\AA} \sim 10$ and 40 objective lens.

Recovery of live nematode larvae was used with Baermann technique. A gauze containing faeces was placed in a funnel with an attached rubber tube. The funnel was then filled with water and left for 24 hours. Water from the bottom of the tube was collected and placed on a slide with 1 drop of iodine under a coverslip, a $\text{\AA} \sim 10$ objective lens was used for identification of nematodes.

Any slide positive for parasites was washed with 70% ethanol into microtube and labelled, for future molecular analysis.

Statistical analysis

Data was stored and managed in Microsoft Excel. To determine if the proportion of positive samples was significantly different in the study groups, a Wilcoxon Rank Sums test was performed using JMP \textregistered Pro 14.0.0. The proportion of positive samples of *Strongyloides* spp. in each troop was assessed using the number of positive samples divided with the number of samples from the specific troop (n=30).

RESULTS

Prevalence of *Strongyloides* spp.

In total, 45% (27/60) of the faecal samples were positive for *Strongyloides* spp. on one or more of the parasitology analyses performed. Blood or mucus were not observed in any faecal sample, nor did colour differ significantly between masses. The VC troop had a point prevalence of 83.33% (25/30), whereas in the ST only 2 positive samples out of 30 were found positive (6.67%). The difference between the two troops was statistically significant ($P < 0.0001$).

Comparing the mean *Strongyloides* spp. EPG per sample, the VC troop also showcased a substantially higher number (96.67) than the ST (3.33).

Table 3. Results from present study, comparing the two troops

Troop	Number of samples	Number of positive samples	Percent of positive samples	Mean Strongyloides spp. per sample
Sunset troop (ST)	30	2	6.67%	3.33
Volunteer centre troop (VC)	30	25	83.33%	96.67

Home range analysis

The ST ranged over an area of 260 645m², which included one anthropogenic structure and one present human at any given time (park guard). Human density and anthropogenic structure per km² were both calculated to 3.8.

The VC troops home range stretched over an area of 78 923m² which included 50 anthropogenic structures and 22 permanent inhabitants.

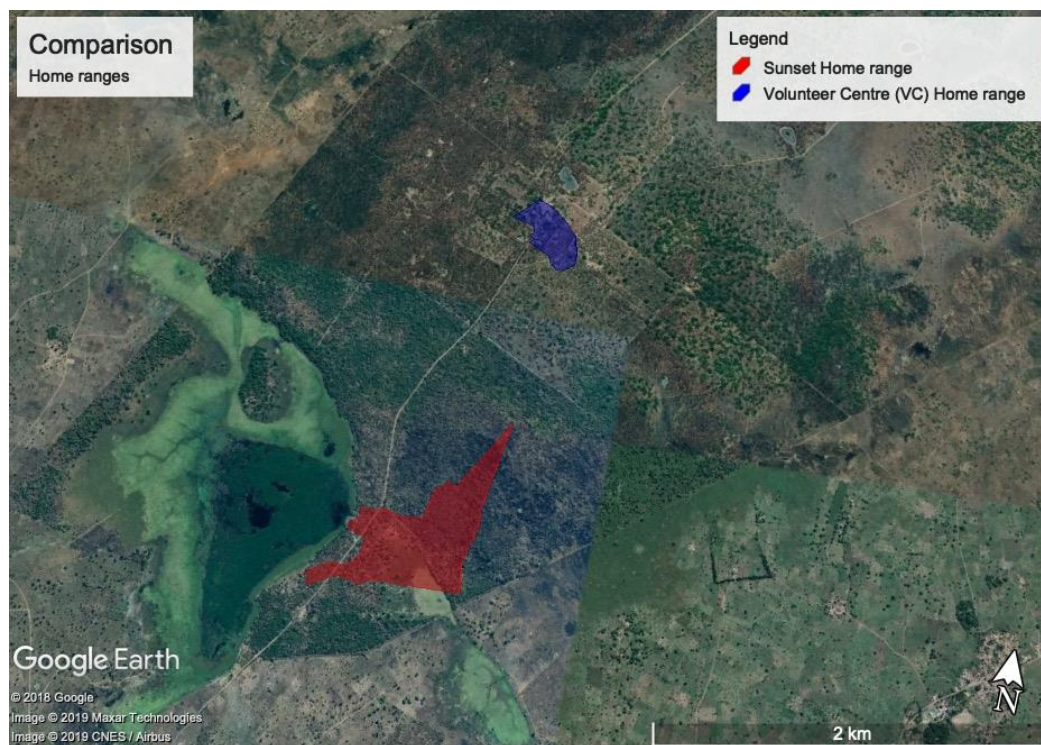


Figure 3. Comparison between home ranges, see legend.

Table 4. Home range analysis of the two vervet troops

Troop	Home range, m ²	Human density per km ²	Anthropogenic structure per km ²	Vervet density per km ²
Sunset troop	260 645	3.8	3.8	115.1
VC troop	78 923	278.8	631.1	304.1

DISCUSSION

In this study the *Strongyloides* spp. point prevalence was 6.7% for the vervet troop with low anthropogenic disturbance (ST) and 83.3% for the troop with high anthropogenic disturbance (VC). The difference between the groups was significant ($p < 0.0001$). This was in line with previous results on anthropogenic disturbance from Thatcher et al. (2018). During the field study and the home range analysis it was discovered that the VC troop had a substantially smaller home range than ST. Since the VC troop had both the highest anthropogenic disturbance and host density, distinguishing each factor's individual role in this particular study was not possible.

Compared to the results of 7 other studies (9 different troops), the VC troop had the largest percentile of positive samples. Across all studies the mean prevalence of *Strongyloides* spp. in highly anthropogenic disturbed areas was 22.49% (SD: 25.9).

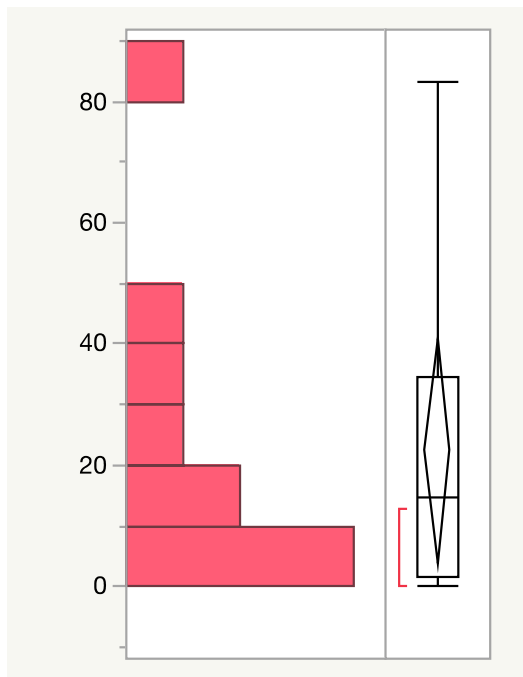


Figure 2. *Strongyloides* spp. prevalence (percentage on y-axis) in vervets from 9 different troops with high anthropogenic disturbance.

Anthropogenic disturbance

When discussing anthropogenic disturbance, in this case particularly urbanization and farming intensification, and breaking down the concept to biological compartments within the affected animals, stress plays a key role. Human presence inevitably increases stress levels, which is known to impair the immune response. Whether this transfers into an actual decline in the defence against helminths, however, is still subject for research since evidence has been contradictory. During field observation, the VC troop seemed to be more reluctant to stay in one area for an extended period of time, than the ST. This behaviour can be explained by a persistent higher stress level in the VC troop. The VC troop regularly stole food from villagers

and the research facility, leading to frequent conflicts with humans and they therefore had to be more aware of outside threats. Kuti Wildlife Reserve does not host any carnivores or other land-living predators, making the environment rather safe for the vervets. The theory about stress is supported by the previous study on vervets done by Senst (2017), where individuals displaying behaviour linked to stress showed a much higher EPG than less stressed counterparts. Data for this, i.e. time and behaviour tracking, was not collected during the present field observation and therefore the hypothesis cannot be statistically evaluated.

When discussing human disturbance neither climate change nor pollution has been taken into account, this since the two troops studied are assumed to be equally affected by those factors.

Host density and troop size

Host density has been shown to be positively correlated with the prevalence of parasite with direct transmission cycles (Morand & Poulin, 1998). This since both contact rates and durations between hosts and transmission stages increases with population density (Anderson & May, 1978; Dobson, 1990). Few studies have shown the effect of host density in correlation with *Strongyloides* prevalence specifically. Chapman (2005b) conducted a study on red colobus (*Piliocolobus tephrosceles*) and black-and-white colobus (*Colobus guereza*) that migrated from a larger home range to a smaller one. After the migration the density had almost doubled (from 358 colobus/km² to 657 colobus/km²). In both colobus species the prevalence of *Trichuris* sp., the only gastrointestinal parasite that frequently occurred in the troops, increased initially. The long-term effect, however, differed between the species (Chapman, 2005b).

Nevertheless, the results showcased in this study is most probably a combination between the two factors, i.e. host density and anthropogenic disturbance. Proximity to humans provides greater access to food, thus reducing the need for a large home range. This results in smaller territories and higher host density. In addition to that, the VC troop shared much of its territory with a yellow baboon troop. Yellow baboons are also susceptible to *Strongyloides* spp., consequently adding even more potential hosts to an already host dense area.

Pathogen transmission

The potential for a pathogenic transmission between species is especially interesting in this case. *S. stercoralis* is classically associated with human infection, and *S. fuelleborni* with primate infection, but both species can infect both hosts. Reviewing the principles required for transmission, molecular typing of *Strongyloides* from both human and vervet samples in Kuti Wildlife Reserve is needed in order to conclude whether a zoonotic or anthroponotic transmission has occurred.

Strongyloides diagnosis

Literature on human strongyloidiasis frequently reports difficulties with diagnosing Strongyloidiasis in patients due to low test sensitivity (Siddiqui & Berk 2001), a discussion which is completely lacking in studies on vervet monkeys. The life cycle of *Strongyloides* spp. includes an intermittent excretion of both eggs and larvae into faeces and consequently not all samples from infected individuals will be positive. Currently, the preferred technique for recovering *Strongyloides* larvae in faecal samples are Baermann technique or Agar plate culture, both of

which are time-consuming and labor intensive (de Kaminsky 1993; Requena-Méndez *et al.* 2013). The low sensitivity paired with a low number of samples in this particular study inevitably adds an uncertainty factor to the results and begs for careful interpretation.

Reviewing the seven studies in Table 1, none used either of the two recommended methods for recovering *Strongyloides* larvae. Indeed, none of the studies focused on *Strongyloides* spp. in particular, but rather on gastrointestinal parasites in general. This broad approach is valuable for establishing baseline data on regional parasite fauna, but the sensitivity decreases substantially. This is due to usage of non-optimal testing methods and thus increasing the likelihood of missing infected individuals. Gillespie (2006) has provided guidelines for the community to follow, since currently the methods in primate studies differ significantly, making comparative analyses difficult.

Regional differences

We already know that multiple factors, not only human presence and host density, affects the parasite prevalence in hosts and environment. In nine other vervet troops that were investigated for *Strongyloides* spp. (Table 1.), six were categorized as being in close proximity to humans. Out of these, two reported a point prevalence of 0% (Legesse & Erko, 2004). As previously mentioned, there is a lack of consistency between studies regarding sampling effort and parasitological methods, but even so there are regional differences in parasite fauna that also need to be accounted for. Strongyloidiasis occurs in higher prevalence in tropical and subtropical countries (Toledo, 2005) and natural variances can be attributed to difference in regions and climates. During hot and dry months, the number of parasite eggs in the environment declines, compared to months with more rain and environmental moisture (Appleton & Henzi, 1993; Appleton & Brain, 1995; Larsen & Roepstorff, 1999; Forrer *et al.*, 2019).

Other sources of error

As previously discussed, this study had low number of samples from two troops and was conducted over a limited period of time. A degree of selection bias was also present, since remote and unhabituated troops were not included, but rather two relatively spatially close troops. Samples were collected from non-identified individuals which means several samples could potentially be from one individual only, while some other individuals might not have been sampled at all.

Conclusion

In conclusion, there was a difference in occurrence between the two vervet troops in the study and it is suggested that this variance is due to a combination of both anthropogenic disturbance and host density, although these results should be interpreted carefully due to the low sample number and the insensitive nature of *Strongyloides* spp. diagnosis. Future studies including correction for host density, larger sample size, more troops and molecular analysis to demonstrate host-shifts is suggested.

POPULAR SCIENCE SUMMARY

The geographic overlap between non-human primates and people is increasing, especially in tropical and subtropical countries. This could cause conflicts over territories and resources which potentially can affect the health of primates and humans alike. In the present study, the relationship between human proximity and the natural occurrence of a gastrointestinal parasite called *Strongyloides* spp. in the ubiquitous vervet monkey was assessed.

Strongyloides spp. consists of 50 different species, out of which two can infect both humans and primates (*Strongyloides stercoralis* and *Strongyloides fuelleborni*). Strongyloidiasis is classified as a neglected tropical disease by the World Health Organization and affects an estimate of 30-100 million people worldwide. The parasite lives in soil and enters its host through intact skin or by being ingested, and then travels to the intestines. Eggs and hatched larvae are then excreted through faeces and the cycle continues. The parasite rarely causes symptoms in most people but can be fatal if the immune system is compromised, for example in HIV patients or if certain drugs such as glucocorticoids are administered. Common symptoms include skin lesions, diarrhoea and abdominal pain.

Vervet monkeys are adaptable medium-sized monkeys and can be found in urban areas as well as savannah, mountains and grassland. They live in multi-male and multi-female groups and spend an extensive amount of time foraging on the ground. Proximity to human settlement offers nutritional resources which might increase fitness and the ability to fight off infections. However, it additionally enables pathogens such as parasites to spread between communities. Parasites that classically are referred to as human parasite can spread to and infect primates, called anthropogenic transmission, and vice versa, parasites of primates can infect humans, called zoonotic transmission.

Stool samples were collected during 8 weeks from two vervet troops, one with extensive contact with humans and another with infrequent contact with humans. The results showed that the troop that lived close to humans had a proportion of positive samples of *Strongyloides* spp. of over 80%, while their counterparts had below 7%. However, another factor that differed between the two troops was home range size. It is known that a lot of animals in a smaller area are more prone to infectious diseases, since the diseases can spread faster. This is also true for *Strongyloides* spp. because the troop that lived in close proximity to humans also had the smallest home range, it is not possible to distinguish the role each factor plays in this study but most likely it is a combination of the two.

Future research to confirm the relationship between anthropogenic disturbance and prevalence of *Strongyloides* spp. is needed.

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