FACULTY OF VETERINARY MEDICINE
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GASTROINTESTINAL PARASITES OF ELEPHANTS IN ZOOLOGICAL GARDENS IN BELGIUM AND THE NETHERLANDS

PART 1: RATIONALE

Charlie Krekels
Student number: 01803531

Supervisor: Prof. dr. Bruno Levecke
Supervisor: Dr. Francis Vercammen

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Gastrointestinal parasites of elephants

I. BACKGROUND INFORMATION & SITUATION EXPLANATION

Elephants are the largest land mammals and play a crucial role in balancing the natural ecosystem. For this reason, elephants are known as 'keystone species' (Chakuya et al., 2016), which can be defined as a species that provides fundamental ecosystem services, necessary for the survival of other species in that particular ecosystem. In addition, keystone species sustain ecological integrity and biodiversity in the habitat they live in (Elsheikha & Obanda, 2010). As a consequence of this, disease management strategies in free-ranging elephants could benefit conservation of biodiversity as a whole.

Nonetheless, most African and Asian elephant species are labelled as either endangered or even critically endangered on the International Union for Conservation of Nature's red list of threatened species (IUCN 2022). Studies have shown that the Asian elephant population has declined with over 50% in just 3 generations (Williams et al., 2020), and according to latest assessments a decrease of 86% in the number of African forest elephants is found over the past three decades (IUCN 2022). The origin of this deterioration is multifactorial, but most significantly consists out of intensified poaching for ivory trade, habitat loss, fragmentation, the ongoing human-elephant conflict and epidemic disease outbreaks (Riddle et al., 2010; Abhijith et al., 2018). Such disturbing reductions in population sizes are globally compelling conservationists, animal organisations and ecologists to act in the search for saving those magnificent creatures. The conservation of any species can be aided by understanding its behaviour, physiology and ecology, as well as the influence of pathogens and climate on those key concepts. Consequently, in order to cease a nearing extinction and rather create a healthy lasting expansion of an endangered species, one must act upon all aspects affecting that particular species. Knowledge of every beneficial and detrimental factor having an effect on individual and population survival, as well as the construct of breeding plus releasing programs will eventually lead to the one plan approach to conservation (OPA). Those influential factors include environment, habitat, human-animal and interspecies interactions as well as pathogens like bacteria, viruses, fungi and parasites. In such a way, surveys on gastrointestinal parasite loads and other burdens in keystone species like elephants will help in the provision of baseline information and guides for future studies (Chakuya & Moyo 2016).

Parasites are the cause of many health-related problems in both humans and animals across the globe. There are different classes of ecto- and endoparasites and a substantial portion of the latter consists of gastrointestinal parasites like protozoa, flukes, round- and tapeworms. Just as in in human medicine, the importance and prevalence of animal parasites is vastly underestimated. Parasites alter and have an enormous impact on host behaviour, health, fertility and facilitation of parasite transmission (Abhijith et al. 2018). Additionally, they can shape community structure through their effects on trophic interactions, food webs, competition, biodiversity, and keystone species (Preston & Johnson, 2010). Furthermore, parasites are known to suppress their host's immune system (Maizels et al., 2012), impede growth (Crompton & Nesheim, 2002) and they can lessen the reproductive success (Akinyi et al., 2019).

Although parasites often live many years in coexistence with their hosts and normally do not lead to mortality (Albery et al., 2018), it has been documented that gastrointestinal parasites might cause death in case of severe infection (Lynsdale et al., 2017). Those are all relevant reasons to include parasitology in the approach of saving an endangered species. However, a potential lack of clinical symptoms, sometimes even despite major parasite burdens, may have partially been responsible for the neglect of parasitic infections in wildlife. Mainly for helminths biologists speculate of a co-evolvement of parasites and their hosts, resulting in subclinical infections rather than disease. Nevertheless, when parasite-host equilibrium is unbalanced, symptoms may become apparent (Elsheikha & Obanda, 2010). Thus, gastrointestinal parasites can affect an individual's fitness (Parker et al., 2019).

I.I. PREVALENCE AND IDENTIFIED SPECIES

I.I.I OVERALL PREVALENCE OF GASTROINTESTINAL PARASITES AND AN OVERVIEW OF THE DIFFERENT PARASITIC GENERA FOUND

Diverse reports, which analyse different elements of the gastrointestinal parasite issue of Asian and African elephants across the world, can be found. The amount of elephants with parasites colonizing their alimentary tract is one of those elements confirmed to be important. Prevalence of parasite propagules, eggs and larvae depends among others on the studied population (King'ori et al., 2020), location (Shrestha, 2018) and husbandry practices (Abeysinghe et al., 2017). Overall prevalence of gastrointestinal parasites of the Asian elephant ranges from 32.2% (Punya et al., 2021) to 100% prevalence in herds of wild elephants of Sri Lanka (Abeysinghe et al., 2017). The prevalence of GI parasites of African elephants stretches from 36.9% (Mbaya et al., 2013) to 100% (Obanda et al., 2011). A significantly higher prevalence of infection was observed in wild elephants compared to semi-captive ones, and the latter on its turn contained a greater percentage of infected individuals than in the group captive elephants (Abeysekara et al., 2018; Shrestha, 2018). Furthermore, the occurrence of parasites in captive elephants is thought to vary according to husbandry practices, disease prophylaxis and treatment ((Fowler 2006; Vanitha et al. 2011). For example, in a comparative study (Abeysekara et al., 2018) involving the captive and semi-captive elephants, unlike the wild ones, received regular deworming (two-three times a year), which was estimated to be the main reason for having a lower prevalence of GI parasites in these two groups. For an overview we refer to Table 1, where a collection of data regarding GI parasites and their prevalence is portrayed.

Table 1. Overall prevalence of GI parasites of multiple studies, various management systems and both elephant species.

Study	Elephant species	Management	Prevalence (%)	Total of elephants
Abeyekara et al., 2018	Asian	Wild	93.3	45
		Semi-captive	55	20
		Captive	25	20
Abhijith et al., 2018	Asian	Wild	74.5	55
Hewavithana et al., 2021	Asian	Wild	71	7
Punya et al., 2021	Asian	Captive	32.2	31
Shahi & Gairhe 2019	Asian	Wild	95	40
Shrestha, 2018	Asian	Wild	90	20
		Captive	57	23
Vanitha et al., 2011	Asian	Captive	48	42
		Captive	32	38
		Captive	31	35
Vidya & Sukamar, 2002	Asian	Wild	87	320

Vimalraj & Jayathangaraj, 2013	Asian	Wild	100	
Mbaya et al., 2013	African	Wild	36.9	274
Obanda et al., 2011	African	Wild	100	11

It is important to understand that the prevalence of infection and the parasitic load in elephants throughout studies and populations vary for a cluster of reasons, which go beyond the causes mentioned in the paragraph above. The integrality of influential factors on both percentage infected and parasitic burden of elephants will be discussed in detail in a later section of this dissertation. With regard to the different classes and species, a wide diversity of GI parasites of various phyla have been documented. The most cited genera of protozoa, flukes, roundworms and tapeworms will be discussed in the next passages, but an overview of all the parasites that have been recorded can be found in Table 2. Aside from all the species and classes found on its own, it has become apparent that mixed parasitic infections were common and according to most studies even more so than single infections (Abeysekara et al., 2018; Vimalraj & Jayathangaraj, 2015; Hing et al., 2013).

Table 2: An extensive list of the various gastrointestinal parasite genera and families that are identified throughout different studies in both Asian and African elephants.

Class of Nematodes			
Family	**Genus**	**Elephant species ***	**Study**
Acuaridae	*Parabronema*	Asian & African	Fowler, 2006; Vidya & Sukumar, 2002
Strongylidae	*Equinubria*	Asian & African	Fowler, 2006; Kinsella et al., 2004
Strongylidae	*Decrusia*	Asian & African	Fowler, 2006; Kinsella et al., 2004
Strongylidae	*Chonangium*	Asian & African	Fowler, 2006; Vidya & Sukumar, 2002;
Strongylidae	*Amira*	Asian & African	Fowler, 2006; Vidya & Sukumar, 2002; Muraleedharan, 2016
Strongyloididae	*Strongyloides*	Asian & African	Punya et al., 2021
Cyanthostominae	*Khalilia*	Asian & African	Fowler, 2006
Cyanthostominae	*Quilonia*	Asian & African	Fowler, 2006; Abhijith et al., 2018; Kinsella et al., 2004; Punya et al., 2021
Cyanthostominae	*Murshidia*	Asian & African	Fowler, 2006; Abhijith et al., 2018; Kinsella et al., 2004; Punya et al., 2021
Ancylostomidae	*Bunostomum*	Asian & African	Fowler, 2006; Abhijith et al., 2018; Kinsella et al., 2004; Punya et al., 2021
Ancylostomidae	*Gammocephalus*	Asian & African	Fowler, 2006; Abhijith et al., 2018; Kinsella et

			al., 2004; Punya et al., 2021
Ancylostomidae	*Batmostomum*	Asian	Fowler, 2006; Vidya & Sukumar, 2002
Atractidae	*Leiperenia*	Asian & African	Fowler, 2006; Kinsella et al., 2004
Trichinelloidea	*Trichuris*	African	Fowler, 2006
Trichostrongylidae	*Haemonchus*	Asian & African	Shrestha, 2018; Mbaya et al., 2013
Trichostrongylidae	*Nematodirus*	Asian	Shrestha, 2018
Trichostrongylidae	*Trichostrongylus*	Asian & African	Fowler, 2006; Shrestha, 2018; Mbaya et al., 2013
Acaridae	*Toxocara*	Asian	Fowler 2006
Acaridae	*Ascaris*	Asian	Shrestha, 2018
Chabertidae	*Chabertia*	Asian	Shrestha, 2018
Class of cestodes			
Family	**Genus**	**Elephant species ***	**Study**
Anoplocephalidae	*Anoplocephala*	Asian & African	Fowler, 2006; Abhijith et al., 2018; Vidya & Sukumar, 2002
Class of Trematodes			
Family	**Genus**	**Elephant species ***	**Study**
Brumptidae	*Brumptia*	African	Fowler, 2006; King'ori et al., 2020
Gastrodiscus	*Gastrodiscus*	Asian & African	Muraleedharan, 2016; Fowler, 2006
Fasciolidae	*Protofasciola*	Asian	Baines et al., 2015; Obanda et al., 2015; Fowler, 2006; Vitovc et al., 1984
Paramphistomoidea	*Pfenderius*	Asian	Fowler, 2006; Firdausy et al., 2019
Paramphistomoidea	*Pseudodiscus*	Asian	Fowler, 2006; Muraleedharan, 2016
Protozoa			
Family	**Genus**	**Elephant species ***	**Study**
Blepharocorythidae	*Raabena*	Asian	Gürelli & Ito, 2014
Bütschlidae	*Blepharosphaera*	African	Anette & van Hoven, 1980
Cryptosporidiidae	*Cryptosporidium*	African & African	Fowler, 2006; Abeysekara et al., 2018; Samra et al., 2011
Cycloposthiidae	*Prototapirella*	African	Kinsella et al., 2004; Anette & van Hoven, 1980
Cycloposthiidae	*Triplumaria*	Asian & African	Kinsella et al., 2004; Gürelli & Ito, 2014; Anette & van Hoven, 1980
Eimeriidae	*Cyclospora*	Asian & African	Abeysekara et al., 2018

Eimeriidae	*Eimeria*	Asian & African	Shrestha, 2018; Abeysekara et al., 2018; Mbaya et al., 2013
Eimeriidae	*Isospora*	Asian & African	Abeysekara et al., 2018
Entamoebidae	*Entamoeba*	Asian & African	Abeysekara et al., 2018; Anette & van Hoven, 1980
Giardiinea	*Giardia*	Asian & African	Majewska et al. 1997
Ophryscolecidae	*Endoralium*	African	Anette & van Hoven, 1980
Paraisotrichidae	*Helicozoster*	Asian & African	Gürelli & Ito, 2014; Anette & van Hoven, 1980
Paraisotrichidae	*Latteuria*	Asian & African	Kinsella et al., 2004; Gürelli & Ito, 2014
Paraisotrichidae	*Paraisotricha*	Asian & African	Gürelli & Ito, 2014; Anette & van Hoven, 1980; Kingsella et al., 2004
Polydiniellidae	*Polydinella*	Asian	Gürelli & Ito, 2014
Polydiniellidae	*Thoracodinium*	African	Kineslla et al.,2004; Anette & van Hoven, 1980
Pseudoentodiniida	*Pseudoentodinium*	Asian	Gürelli & Ito, 2014
Spirodinidae	*Pterodinae*	African	Anette & van Hoven, 1980

* If this parasite is only found in one species, it does not mean that that particular parasite family and genus cannot be found in the other.

I.I.II. PREVALENCE AND IDENTIFIED GENERA OF HELMINTHS

PREVALENCE AND IDENTIFIED GENERA OF NEMATODES

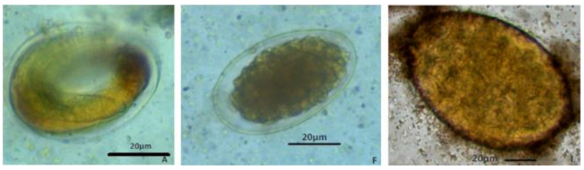

Figure 1. Nematode eggs recorded from wild and captive elephants. (A) *Strongyloides sp.*, (F) *Strongyles,* (I) *Ascaris sp. (Abeysekara et al., 2018)*

A huge variety of helminths is described in both Asian and African elephants and the highlights are summarized in the following paragraphs. First the roundworms, also known as nematodes, will be discussed as this the phylum is most abundantly present in elephants (Baines et al., 2015). The prevalence of nematodes was even determined to be twice as high than the prevalence of flukes (King'ori et al., 2020). Especially the three families of *Strongylidae*, *Strongyloididae* and *Ascaridae* represented the largest portion of roundworms, or even of parasites as a whole in most studies (Abhijith et al., 2018; Abeysekara et al., 2018; Shrestha, 2018), see also Figure 1. Different works (Fowler, 2006; Kinsella et al., 2004; Vidya & Sukumar) have defined the diversity of genera of *Strongyles* detected in elephants, including *Equinubria*, *Decrusia*, *Amira* and *Chonangium*. Other genera of nematodes which were mentioned multiple times belong to the families of *Ancylostimidae* and *Cyanthostominae*. Beside the genera of parasites mentioned in Table 3 and their prevalence, some other species were also detected in Asian and African elephants. However, their prevalence was not determined, but for the names and genera of those parasites we refer to the table above (Table 2).

Table 3: Prevalence of nematodes specified per genera from multiple studies, various management systems and both African and Asian elephants species.

Study	Elephant species	Management	Parasite	Prevalence (%)	Total of elephants
Abeyekara et al., 2018	Asian	Wild	*Strongyles*	55.6	45
			Strongyloides	31.1	
			Ascaris	37.8	
		Semi- captive	*Strongyles*	20	20
			Ascaris	10	
Abeysinghe et al., 2017	Asian	Captive	Overall nematodes	38	47
		Captive	Overall nematodes	90	94
		Wild	Overall nematodes	100	50
Abhijith et al., 2018	Asian	Wild	*Strongyloides*	53	55
			Ancylostoma	1.82	
Hing et al., 2013	Asian	Wild	*Strongyle*	70.2	104
Shrestha, 2018	Asian	Wild	*Strongyloides*	85	20
			Ascaris	45	
			Dromeostrogylus	30	
			Haemonchus	25	
			Chabertia	15	
			Nematodirus	10	
			Bunostomum	10	
			Trichostrongylus	10	
		Captive	*Strongyloides*	53	23
			Ascaris	28	
			Haemonchus	8	
			Bunostomum	3	
Vimalraj & Jayathangaraj, 2013	Asian	Wild	*Strongyles*	64	50
			Strongyloides	16	
Baines et al., 2015	African	Wild	Overall nematodes	77	
Dibakou et al., 2021:	African	Wild	Oesophagostomum	67	3
			Ancylostoma	33	
			Strongyloides	100	
King'ori et al., 2020	African	Wild	Overall nematodes	96.3	
Obanda et al., 2011	African	Wild	*Strongyles*	100	11
Shahi & Gairhe, 2019	Asian	Wild	*Strongyles*	61	38
			Strongyloides	45	
			Oesophagostomum	45	
			Chabertia	26	

PREVALENCE AND IDENTIFIED GENERA OF TREMATODES

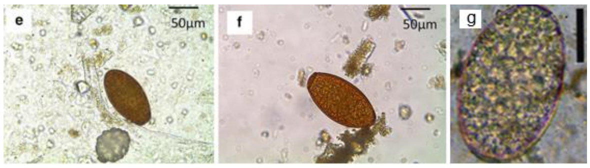

Figure 2. Trematode eggs recorded in elephants, (e) *Protofasciola*, (f), *Brumptia* and (g) *Paramphistomum spp.* (King'ori et al., 2020; Dibakou et al., 2021)

Secondly, the phylum of trematodes has several times been reported in rather substantial prevalence, for instance 24% (Baines et al., 2015) and 39.1% trematode prevalence (King'ori et al., 2020) has been found in groups of African elephants. Those numbers may not be as high as those of nematodes, yet still more than significant enough to consider when reviewing parasites of the alimentary tract. This, however, is in contrast with other studies who had not detected a single trematode (Abhijith et al., 2018; Punya et al., 2021; Shrestha, 2018). This very well might be the result of the life cycle of trematodes, predominantly due to the fact that in order to develop into the infectious phase of the fluke an asexual reproduction in an intermediate host is fundamental. The presence of this intermediate host, mostly various species of snails, is crucial for these parasites to flourish and because of certain habitat requirements of those snails, distribution might be patchy. It also might be a reason why nematode prevalence peaks so much higher, for their life cycle is direct and as such knows no intermediate hosts, as well as for having a much faster completion of their life cycle compared to that one of flukes. Furthermore, trematodes have quantitively been examined in elephants and their prevalence can be found in Table 4. The most frequent identified trematodes are *Paraphistomum spp* and *Protofasciola*, with predominantly *Protofasciola robusta*, see Figure 2. However, besides those more abundant families, Amphistomes of the families *Gastrodiscus* and *Brumptidae* have been described too, see the data above (Table 2).

Table 4. Prevalence of trematodes identified in multiple studies, various management systems and both elephant species.

Study	Elephant species	Management	Parasite	Prevalence (%)	Total of elephants
Abeyekara et al., 2018	Asian	Wild	*Paramphistomum spp*	6.7	85
Pathak & Chhabra, 2012	Asian	Wild	Overall trematodes	33.78	-
		Captive	Overall trematodes	18.18 – 62.28	-
Saseendran et al., 2003	Asian	Wild	Amphistomes	7	99
Shahi & Gairhe, 2019	Asian	Wild	*Paramphistomum spp*	29	38
Baines et al., 2015	African	Wild	Overall trematodes	24	458
Dibakou et al., 2021:	African	Wild	*Paramphistomum*	33	3
King'ori et al., 2020	African	Wild	Overall trematodes	39.1	243
Kinsella et al., 2004	African	Wild	*Protofaciola robusta*	67	6
Obanda et al., 2011	African	Wild	*Protofaciola robusta*	45	11

PREVALENCE AND IDENTIFIED GENERA OF CESTODES

Thirdly, the phylum of cestodes, vernacularly known as tapeworms, have been described in elephants, but only species belonging to the family of *Anoplocephalidae* are published to be present (Fowler, 2006), see Figure 3. The elephant specific tapeworm species named *Anoplocephala manubriata* infects both Asian and African elephants through ingestion of infected oribatid mites. Thus, just like the various trematodes, the elephant tapeworm knows an indirect lifecycle, which might have an impact on their distribution. Prevalence differs across studies, yet a recurrent finding is that prevalence of cestodes does not reach as high those of nematodes (Abhijith et al., 2018; Shrestha, 2018) nor that of trematodes (Shahi & Gairhe, 2019). Even though cestodes have been found in both African and Asian elephants (Fowler, 2006), the prevalence studies have only been conducted on Asian elephants, see Table 5.

Figure 3. Cestode eggs recorded from captive and wild elephants (B,C) *Anoplocephala* spp. (Abeysekara et al., 2018)

Table 5. Prevalence of cestodes identified in multiple studies and various management systems

Study	Elephant species	Management	Parasite	Prevalence (%)	Total of elephants
Abeyekara et al., 2018	Asian	Captive	*Anoplocephala*	1.2	20
Abhijith et al., 2018	Asian	Wild	*Anoplocephala*	2	55
Hing et al., 2013	Asian	Wild	*Anoplocephala*	50	104
Shrestha, 2018	Asian	Wild	*Anoplocephala*	10	20
Vimalraj & Jayathangaraj, 2013	Asian	Wild	*Anoplocephala*	46	50

I.I.III. PREVALENCE AND IDENTIFIED GENERA OF PROTOZOA

Lastly, besides helminths, various phyla of the subkingdom of protozoa are known to colonize the gastrointestinal tract of many mammals, including elephants, see Figure 4 and Table 6. As for those unicellular parasites, multiple genera belonging to the subclass of intestinal coccidia were recorded, for instance *Eimeria* spp, (Shrestha, 2018), *Cryptosporidium*, *Cyclospora* and *Isospora* (Abeysekara et al., 2018). *Eimeria* and *Cryptosporidium* spp. have been diagnosed in both wild and captive elephants, whilst *Cyclospora* and *Isospora* have mainly been documented in captive African and Asian elephants (Majewska et al. 1997). Furthermore, African elephants are host to a complex assortment of intestinal ciliate fauna (Anette & van Hoven, 1980), and similar findings are documented for the Asian

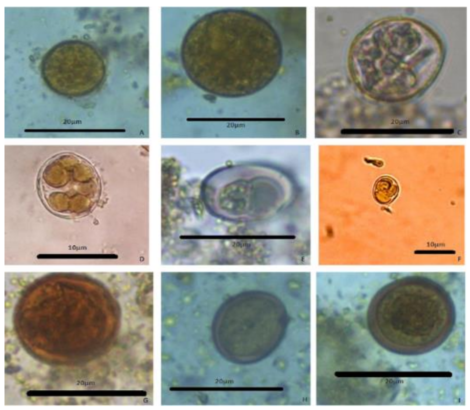

Figure 4. Types of protozoan cysts recorded during fecal analysis of wild (B, D, F, H, I), Semi-captive (A, G), and Captive (E, C) elephants, using modified salt flotation (A, B, E, F, G, I) and direct iodine smear (C, D, H) methods. A, B, C - Amoeboid parasites (*Entamoeba* sp.) D, E, F - Coccidian cysts G, H,I - Unknown cysts – (Abeysekara et al., 2018)

counter species. Namely, intestinal ciliates from the genera *Prototapirella*, *Triplumaria* and *Lateuria* were common in a study of the African forest elephant (Kinsella et al., 2004). The latter two ciliate genera plus *Raabena*, *Helicozos ter*, *Paraisotricha* and *Polydinella* have been thoroughly studied and identified in the Asian elephant (*Elephas maximus*). However, it is apparent that many unknown ciliate species in elephant remain to yet be detected (Gürelli & Ito, 2014).

Table 6. Prevalence of protozoa identified in multiple studies, various management systems and both elephant species.

Study	Elephant species	Management	Parasite	Prevalence (%)	Total of elephants
Abeyekara et al., 2018	Asian	Captive	*Entamoeba*	10	20
			Coccidia	10	
		Semi-captive	*Entamoeba*	20	20
		Wild	*Entamoeba*	22,2	45
			Coccidia	24,4	
Shrestha; 2018	Asian	Wild	*Eimeria*	15	20
		Captive	*Eimeria*	15	23
Baines et al., 2015	African	Wild	*Coccidia*	51	458
Kingsella et al. 2004	African	Wild	Intestinal ciliates	100	6
			Triplumaria	100	
			Prototapirella	84	
			Latteuria	67	
			Thoracodinium	17	
			Paraisotricha	17	
Samra et al., 2011	African	Wild	*Cryptosporidium*	25,8	93

I.II. CLINICAL SIGNS RELATED TO GI PARASITE INFECTION

As reported above, GI parasites typically do not result in fatality, but they frequently establish long-term coexistence with their hosts resulting in subclinical infections. For instance Fowler (2006) suggests that tapeworms are doubted to be a cause of clinical disease in elephants. They also state that roundworms can cause peracute, acute and chronic illnesses of the digestive tract, predominantly leading to a debilitation of the individual in varying degrees making them more susceptible to secondary infectious agents. Symptoms of emaciation, anorexia, diarrhoea and even anaemia due to nematodes have been described (Fowler., 2006; Obanda et al., 2010). As for protozoa, including coccidia, one can deduce that they are commonly found in free-ranging and captive elephants and rarely cause disease (Abeysekara et al., 2018). Finally, the phylum of trematodes has also been assessed for their pathological importance. From the handful of different genera of flukes known to infect elephants, some have proven to be associated with substantial lesions in starving animals (King'ori et al., 2020) and even fatality of a young African elephant calf (Vitovc et al., 1984). Furthermore, most of these pathologies, including the fatal case were recorded to be the elephant specific species of *Protofasciola robusta*, being an intestinal trematode of the family of *Fasciolidae*. The lesions *P. robusta* creates are associated with digestive tract tissue damage and haemorrhagic colitis

(Baines et al., 2015; Fowler, 2006). Moreover, Amphistomes of the families *Gastrodiscus*, *Paramphistomoidea* and *Brumptidae* create petechia and ulcers of the cecal mucosa. They also lead to focal necrosis of the villi of the large intestine (Fowler,2006). Thus, it can be decided that trematodes can be the cause of severe problems in elephants and for this reason may not be neglected.

I.III FACTORS INFLUENCING THE GASTROINTESTINAL LOAD OF PARASITES

Multiple studies have been published handling the subject of GI parasites of free-ranging and (semi-) captive elephants. Both the Asian and African species as well as captive and free-ranging elephants are discussed in prevalence, as shown in the collected data above. However, prevalence as well as detected GI parasite species are strongly dependent on a vast variety of influences. Those influences can be subdivided in different groups, being individual dependent, environment dependent and socially reliant factors. Hence, due to their impact, it is important to analyse the various factors which determine how heavily an individual elephant or a population of elephants is infected with (GI) parasites. The same studies mentioned before and additional ones have been conducted to define those individual, environmental and social factors. They found that the following factors had significant effect on extensiveness of parasite infection: husbandry and management, anti-parasitic treatment, location, season, sex, age, and group size and composition (Vidya & Sukumar, 2002; Mbaya et al., 2013; Thurber et al., 2011; Punya et al., 2021 Abhijith et al., 2018; Parker et al., 2020; Baines et al., 2015). Yet, in spite of those significantly proven elements of influence, it is vital to emphasize that among those and other surveys many conflicting statements have been portrayed. In this section the factors and their contradictions will be thoroughly analysed and represented. Firstly, the individual factors, subsequently the social ones and finally the environment ones.

I.III.I. INFLUENCE OF INDIVIDUAL FACTORS ON GI PARASITES

To start with, the individual dependent influences on parasitic burden of the digestive tract of elephants are to be disclosed. Many characteristics have been analysed for their effect and the three most researched traits were age, sex and body condition score. A consistent finding throughout studies is that body condition score is a factor that could not be related to how heavy an individual is infected (Vidya & Sukumar., 2002; Chakuya & Moyo., 2016). This is on the contrary with both age and sex, where the results happened to be less unified, see Table 7 . As for the factor age, many conflicting statements have been made, ranging from no influence to a significant increasement of GI parasite load due to either youthfulness or maturity. Regarding sex, a similar disagreement can be found throughout various research sources of the Asian and African elephant, leading to inconclusive results about male or female bias. So the influence of the factors ‘age’ and ‘sex’ on GI parasite burden remain equivocal. The dissension about those individual dependent factors might find their cause in the variety of population size, intra-individual variation, ages investigated and other intertwined aspects influencing parasitology, but should ideally be analysed further. Moreover, up to date no comparative study of GI parasites covering the similarities and contrasts between both elephant species is composed. As species could have a vast effect on this matter, it would be advised to perform such a study.

Table 7. Conclusions about individual factors that have been examined for their influence regarding gastrointestinal parasites of African and Asian elephants in different studies.

Factor	Conclusion of influence according to the study	Elephant species	Management	Study
Age	No influence	Asian	Wild	Abhijith et al., 2018
				Vidya & Sukumar., 2002
	Younger elephants have a higher load of parasites	African	Wild	Mbaya et al., 2013
				Parker et al., 2020
	Older elephants have a higher a load of parasites	African	Wild	Baines et al., 2015
				Thurber et al., 2011*
Sex	No influence	Asian	Wild	Vidya & Sukumar., 2002
	Male elephants have a higher load of parasites	Asian	Wild	Mbaya et al., 2013
		-	-	Poulin, 1996
	Female elephants have a higher load of parasites	African	Wild	Thurber et al., 2011
				Parker et al., 2020
				King'ori et al., 2020
	Female elephants have higher prevalence of being infected	Asian	Wild	Abhijith et al., 2018
		African	Wild	Baines et al., 2015
Body Condition Score	No influence	Asian	Wild	Vidya & Sukumar., 2002
		African	Wild	Chakuya & Moyo., 2016

* in family situations, not in solitary bulls

I.III.II. INFLUENCE OF SOCIAL FACTORS ON GI PARASITES

Social factors are proven to be of significant influence regarding GI parasitic burden and the analysed factors are listed in Table 8. Population size, for instance, can be associated with occurrence of helminths since every additional elephant added to a herd resulted into a greater likelihood of finding worm eggs (Baines et al., 2015). Social constructure inside a flock was examined too. Whilst some say that no associations between helminth infection and elephant social groups could be noticed (King'ori et al., 2020), others proclaim that sociality has an eminent impact on infection rate (Parker et al., 2020; Baines et al., 2015). Namely that less socially integrated or solitary individuals excreted smaller amounts of worm eggs than the more dominant and socially merged elephants. Baines et al (2015) also discovered that all female herds or herds with bulls under the age of 15 were host to a more substantial number of nematodes than male groups aged above 15. Lastly, as can be expected, orphaned elephant calves left by their families shed less parasitic propagules than their peers growing up in families (Parker et al., 2020). Thus, similarly as the individual factors, no certain assertions can be made about the social factors of African elephants and definitely not of Asian elephants as they have not yet been described in those studies. However, when consulting less species-specified articles, their results uncover the effectiveness of spreading of parasites through faecal-oral transmission in socially structured populations (Nunn et al., 2011).

Table 8. **Conclusions about social factors that have been examined for their influence regarding gastrointestinal parasites of elephants in different studies**.

Factor	Conclusion of influence according to the study	Elephant species	Management	Study
Population size	Higher loads of GI parasites with increasing herd size	African	Wild	Baines et al., 2015
Social constructure	No association between parasite load & sociality	African	Wild	King'ori et al., 2020
	Sociality has a significant impact on parasite burden	African	Wild	Baines et al., 2015
				Parker et al., 2020
	All-female herds or herds with bulls under 15 years old have a higher parasite load	African	Wild	Baines et al., 2015
	Orphaned and left by the herd, isolated	African	Wild	Parker et al., 2020

I.III.III. INFLUENCE OF ENVIRONMENTAL FACTORS AND MANAGEMENT ON GI PARASITES

Finally, yet equally important, is the impact of the environment and management on the occurrence of parasites. A much explored and pivotal factor determining parasite concentration is season, yet anew, disagreements among studies have been published. Since one study claims the dry season as peak in parasite load and the others state that infection rates are higher in the rainy season, see Table 9. The seasonal variation can find its roots in the fact that water availability simultaneously varies which is not only a cause of stress to elephants, but also a determinant in the life cycles of the parasites and therefore altering progression in their development (Chakuya & Moyo, 2016). Furthermore, most of these GI parasites are obtained through close interaction with infectious stages in vegetation, soil or faeces. This implies that ranging behaviour and home range overlap will have a major impact on their spread (Nunn et al., 2011), meaning that habitat and surroundings revealed to be a relevant factor of influence (Shrestha, 2018). Though not as pertinent as management conditions and associated actions. Since a significantly greater prevalence of helminth infection was observed in wild elephants compared to both captive and semi-captive ones (Abeysekara et al., 2018). It is believed that this variance in occurrence of GI parasites is principally linked to anthelmintic treatment, as a vital reducer of parasite load, followed by management methods (Abeysinghe et al., 2017; Punya et al., 2021). Thus, as a final observation, it may be said that the influence of seasonality is controversial, but the impact of habitat and husbandry practices is definitely present.

Table 9. **Conclusions about environmental factors and management that have been examined for their influence regarding gastrointestinal parasites of African and Asian elephants in different studies**.

Factor	Conclusion of influence according to the study	Elephant species	Management	Study
Season	Parasite loads peak during the dry season	Asian	Wild	Vidya et Sukamar., 2002
	Parasite loads peak during the rainy season	African	Wild	Mbaya et al., 2013
				Baines et al., 2015
		Asian	Captive	Chichilichi et al., 2019
Habitat and surroundings	Locational variation is influential to parasitic load	Asian	Wild & captive	Shrestha, 2018)

Management conditions	Husbandry (wild > semi-captive > captive elephants) has an impact on parasitic load	Asian	Wild, semi-captive & captive	Abeysekara et al., 2018
	Treatment results in lower parasitic load	Asian	Wild & captive	Abeysinghe et al., 2017
			Captive	Punya et al., 2021

II. PROBLEM

Even though the GI parasitic situation of free-ranging elephants has been reasonably researched, the amount of studies conducted on GI parasites of elephants in zoological gardens and their treatment is scarce. This is peculiar, considering the importance of health and knowledge of pathogens in zoological gardens. Studbooks and management protocols alike have been designed and revised to maintain a vigorous population of elephants, which simultaneously serves as a metapopulation and genetic backup for elephants as a species across the globe. Today, zoological gardens periodically deworm their elephants to ensure parasitic worms do not affect the health of the herd. Regular screening of the herds indicated that the worm infections have now become sparse or even absent, suggesting that deworming may have become unnecessary. Hence, the issue this thesis wants to address and the knowledge gap it strives to minimize will be done through assessment of GI parasites of elephants in zoological gardens in order to answer the question: is there still a need for deworming?

III. GOAL

The overall aim of this project is to verify whether it is possible to reduce or even stop the current deworming scheme. The specific objectives are to:

(i) Conduct an intensified survey to accurately assess the worm burden in 6 zoological gardens in Belgium and the Netherlands.
(ii) Summarize historical data available at the zoological gardens on GI worms and treatment schemes.

If GI worms are considered prevalent, despite ongoing deworming efforts, we will also:

(iii) Assess the therapeutic efficacy of current treatment schemes.
(iv) Assess a pooled sample strategy as a cost-saving strategy to monitor GI worms in elephant herds.

In the following paragraphs the applied methodologies for each specific objective will be discussed:

CONDUCT AN INTENSIFIED SURVEY TO ACCURATELY ASSESS THE WORM BURDEN IN 6 ZOOLOGICAL GARDENS IN BELGIUM AND THE NETHERLANDS.

To accurately assess the worm burden, we will conduct a survey during which we will intensify both the sampling (multiple samples of the same animal over time) and the diagnostic efforts (deploying different and more sensitive diagnostic methods). Figure 5 provides a schematic overview of this intensified survey. In short, we will periodically (every 2 months) screen individual faecal samples with multiple diagnostic methods (e.g. Mini-FLOTAC) across a period of 6 months. At each time slot, we will determine the (i) genera of parasites (up to species whenever possible) and (ii) the worm burden by means of eggs/(oo)cysts per gram of stool (EPG, CPG and OPG).

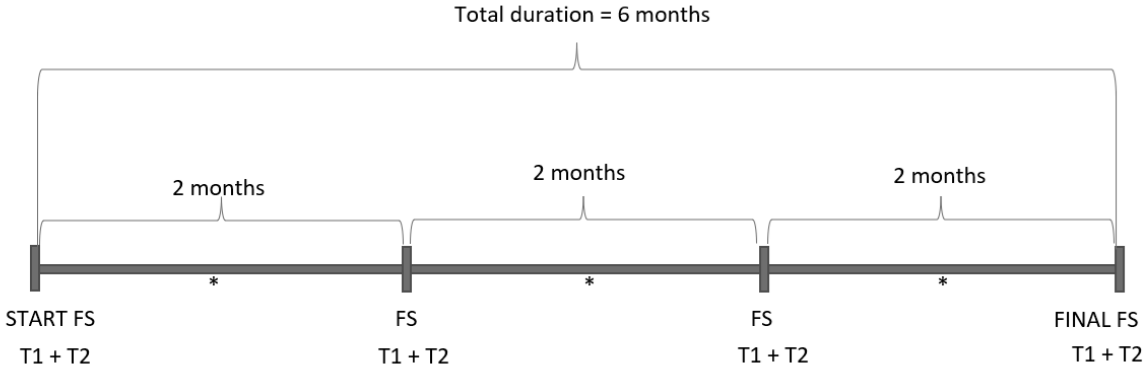

Figure 5. A schematic overview of the intensified survey to assess GI infections in elephants in zoological gardens.

Table 10 provides an overview of the different elephant herds across 12 zoological gardens in Belgium (n = 4) and the Netherlands (n = 8). We will focus on all four zoological gardens in Belgium and Dierenrijk Europa and Beekse Bergen in the Netherlands, resulting in 43 animals (10 African elephants; 33 Asian elephants). The selection is mainly based on the geographical distribution of the zoological gardens (nearby Ghent, Belgium). If zoological gardens that are not selected would be interested, we can offer the screening service if samples are sent to the Laboratory of Parasitology.

Table 10. An overview of the different elephant herds across 12 zoological gardens in Belgium (BE) and the Netherlands (NL). The zoological gardens in italic are those that are selected for this study.

Zoological garden	Total number of elephants	Number of African elephants	Number of Asian elephants
Antwerp Zoo (BE)	***2***	***-***	***2***
Pairi Daiza (BE)	***21***	***2***	***19***
Planckendael (BE)	***6***	***-***	***6***
Pakawi Park (BE)	***1***	***-***	***1***
Dierenpark Amersfoort (NL)	4	-	4
Artis (NL)	4	-	4
Burgers Zoo (NL)	2	-	2
Wildlands Zoo (NL)	12	-	12
Dierenrijk Europa (NL)	***5***	***-***	***5***
Diergaarde Blijdorp (NL)	5	-	5
Beekse Bergen (NL)	***8***	***8***	***-***
Ouwehands zoo (NL)	4	4	-

SUMMARIZE HISTORICAL DATA AVAILABLE AT THE ZOOLOGICAL GARDENS ON GI WORMS AND TREATMENT SCHEMES

We will analyse available historical data on GI worms and treatment schemes that is both available and can be made accessible at the different zoological gardens. This information will be summarized into a chronological profile (retrospective study) and will be used to both supplement and interpret (e.g. Are more GI parasites observed? Are the burdens higher than initially anticipated?) the findings of the intensified survey.

ASSESS THE THERAPEUTIC EFFICACY OF CURRENT TREATMENT SCHEMES

In consultation with the zoo veterinarian, we will assess the therapeutic efficacy (by means of faecal egg count reduction) of the currently deployed treatment scheme. In brief, we will collect individual faecal samples before and three weeks following administration of anthelminthic drugs. We will determine the reduction in faecal egg counts and the corresponding 95% confidence intervals as per recommendation by the World Association for Advances in Parasitology (2023).

ASSESS A POOLED SAMPLE STRATEGY AS A COST-SAVING STRATEGY TO MONITOR GI WORMS IN ELEPHANT HERDS

At each time point, we will also pool all individual samples of the same herd into one sample. Subsequently, we will verify (i) whether the faecal egg counts based on a pooled sample strategy correlate with the mean faecal egg counts across the individual samples and (ii) whether these faecal egg counts are significantly different.

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