CHAPTER 1

GENERAL INTRODUCTION
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Introduction

Infectious diseases of humans have a major public health impact worldwide. Parasitic helminths including trematodes, cestodes and nematodes are amongst the most important causitive agents of these diseases and currently infect one third of the world's human population.\(^1\) Some of the most common and significant helminths infecting humans are the roundworm *Ascaris lumbricoides* (Ascaridida), the hookworms *Necator americanus* and *Ancylostoma duodenale* (Strongylida) and the whipworm *Trichuris trichuria* (Enoplida). These four species are estimated to infect currently more than two billion people globally\(^2,5\) and cause a loss of \(\sim 39\) million years of healthy life.\(^6\) Particularly, in developing countries where rural poverty is extreme and sanitation poor, multiple helminth infections are prevalent.\(^1,2\)

Although light to moderate helminth infections are usually asymptomatic, heavy infections can cause or aggravate mortality and severe morbidity.\(^1\) For instance, infections with *Ascaris* can cause severe haemorrhagic pneumonia due to migrating larvae in the lungs, and/or bowel obstruction associated with the presence of large numbers of adult worms. *Trichuris* infections can lead to diarrhea, dysentery and/or rectal prolapse.\(^7\) The bloodsucking activities of hookworm and *Trichuris* are known to contribute to iron-deficiency anaemia\(^6,8\) and/or a low serum vitamin A level,\(^9\) which can subsequently lead to retarded physical growth and cognitive development.\(^6\) Particularly, infants, children, girls and women of reproductive age are vulnerable because of their relatively high nutrient needs and/or blood loss due to menstruation, pregnancies and lactation.

In the dry sub-sahelian zone of northern Ghana, infection with the soil-transmitted helminths *Oesophagostomum bifurcum* and *N. americanus* are endemic in humans and cause significant morbidity. Currently, hundreds of thousands of people suffer from human oesophagostomiasis and/or hookworm disease in this country. While information on *N. americanus* and hookworm disease is available, there is still a paucity of information on various fundamental aspects of *O. bifurcum* and the disease it causes in humans. This is partly due to serious limitations in identifying the parasite at different developmental stages and in different primate hosts. The purpose of this chapter is to provide the reader with the current state of knowledge of the history, life cycle, pathogenesis and clinical presentation, epidemiology, taxonomy and diagnosis of *O. bifurcum*. Also reviewed are DNA-based methods for the identification and differentiation of parasites. The conclusions drawn from this chapter provide the foundation for the research aims of this thesis.
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1.1. History of human oesophagostomiasis

*Oesophagostomum* species are parasitic nematodes of the order Strongylida. They belong to the subfamily Oesophagostominae within the family Chabertiidae. They are often referred to as 'nodular worms' because the larvae cause small, nodule-like abscesses in the large intestines of the infected animals (characteristically in the submucosa or muscularis) and less frequently in ectopic sites.

In 1803, the first species of *Oesophagostomum* were discovered in pigs (*Oesophagostomum dentatum*) and cattle (*Oesophagostomum radiatum*). Thereafter, nodular worms were also found in a wide range of other host animals, including sheep, goats and monkeys. They appeared to be common and of economic importance. However, the infection of humans with *Oesophagostomum* was considered a rare zoonosis.

The first infection of humans with *Oesophagostomum* was recognized by Brumpt (1902) in southern Ethiopia, and three years later, the description of this case was published. The authors concluded that this parasite was a new species and called it *Oesophagostomum brumpti*. Later, more cases of human oesophagostomiasis were reported, including infections with *O. apiostomum* (Nigeria), *O. stephanostomum* (East Africa), *O. stephanostomum var. thomasi* (Brazil), *O. aculeatum* (Indonesia) and *O. bifurcum* (Ghana). It was believed that some of the species infecting humans were synonymous (Travassos and Vogelsang, 1932) and Chabaud and Larivière (1958) proposed, based on a morphological study, that three main species were responsible for human oesophagostomiasis, namely *O. stephanostomum* (in Brazil and Uganda), *O. aculeatum* (in southeast Asia) and *O. bifurcum* (in Africa). These species have also commonly been found to infect non-human primates, which were thought to be the usual host.

In 1987, Gigase and others reported 54 cases of human oesophagostomiasis in northern Togo. This relatively large number of clinical cases in northern Togo plus another nine cases found earlier in northern Ghana encouraged Dr Polderman (Laboratory of Parasitology, Leiden) and Dr Gigase (Tropical Institute of Antwerp) to initiate research into the occurrence and prevalence of human oesophagostomiasis in these countries, which were known to be endemic for hookworm disease. During the initial research, it was recognized that many of the so-called 'hookworm eggs' that were excreted in the faeces of people in northern Ghana and Togo were misdiagnosed and actually represented *Oesophagostomum*. Six years later, Blotkamp et al. (1993) proposed, on the basis of a morphological study, that the species of *Oesophagostomum* infecting humans in northern Togo and Ghana represented *O. bifurcum*. In the following years, various epidemiological and clinical studies of *O. bifurcum* were published, and the efficacy of anthelmintic treatment to control the disease they cause was investigated. Furthermore, the life cycle of *O. bifurcum*, which had not been studied previously in humans, received considerable attention.
1.2. State of knowledge on the life cycle and transmission

Details of the life cycle and the transmission patterns of *O. bifurcum* in humans are still lacking. Nevertheless, previous studies have provided important insights into the biology of this parasite. Each adult worm of *O. bifurcum* produces ~5000 eggs per day, which are excreted in the faeces and develop into the first-, second- and third-stage (L1, L2, L3, respectively) larvae outside the host. This development from egg to L3 takes four to seven days, depending on the environmental conditions.

Infection of humans with *O. bifurcum* is believed to occur by ingestion of the L3 in contaminated water or ingestion of contaminated fruit, soil or dust, rather than percutaneous transmission. After ingestion, the L3 of *O. bifurcum* penetrate the intestinal wall and form small nodules, in which they develop into the fourth and subsequent fifth stage larvae (L4 and L5). Finally, the young adults re-enter the intestinal lumen where they grow to become mature adults. After copulation of the males and females, the females start to produce eggs.

The L3 of *Oesophagostomum* species are very resistant to environmental stress. Even after long periods of desiccation (several months) or freezing, some of them (including *O. dentatum* and *O. bifurcum*) can remain viable for long periods of time. During periods of desiccation, the larvae shrink within their sheath. Revival of the dormant larvae occurs after rehydration, which may indicate that periods of rainfall lead to a higher prevalence of infection.

1.3. Pathogenesis and clinical presentation of *Oesophagostomum* infection in humans

Although *O. bifurcum* infection in humans is mostly asymptomatic, two distinct types of clinical disease can be recognized. The uninodular disease, also referred to as the 'Dapaong tumour', presents as a painful, abdominal mass with a diameter of 2-11 cm, formed around a single or a small cluster of encapsulated juvenile worms, frequently adhering to the abdominal wall. Patients generally do not suffer from the effects of this manifestation, but intestinal occlusion and abscessation can occur, leading to considerable discomfort and abdominal pain. In such cases, surgery may be needed to remove the mass in order to avoid rupture and peritonitis. The much less common multinodular disease is characterized by hundreds of pea-sized nodules within the colon wall and other intra-abdominal structures, together with gross thickening and oedema of the colon wall. This type of clinical presentation is often associated with abdominal pain, persistent diarrhoea and weight loss. Multinodular disease can lead to progressive destruction of the colonic wall in which case total or partial colectomy is needed.
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*O. bifurcum* infection in non-human primates may also be pathogenic, particularly in captive primates suffering from stress of confinement and transportation. The lesions caused by the nodular worm are small, dark nodules with a diameter of up to eight mm, located from the submucosa to the serosal surface of the caecum and colon, and occasionally in the colonic mesentery. Like in humans, light to moderate infections seem to be asymptomatic, but heavy infections cause diarrhea, weight loss and abdominal adhesions.

**1.4. State of knowledge of epidemiology of human oesophagostomiasis in northern Togo and Ghana**

*O. bifurcum* infection in humans in northern Togo and Ghana occurs in almost every village, with the highest prevalence (~90%) in the rural areas. This observation may be attributed to poor hygiene and lack of medical assistance in these villages. Krepel et al. (1992) showed that the prevalence in northern Togo and Ghana is usually low in children under the age of five, although heavy infections in children of three or four years old have been demonstrated in a more recent survey. There is a significant increase in prevalence in children between the age of two and ten, which indicates an increased rate of transmission of the parasite in this age group. Females over five years of age are usually more often infected with *O. bifurcum* than males in the same age group. However, the differences in prevalence between human females and males cannot yet be explained satisfactorily; possibly the difference is caused by the daily activities of females, such as cooking, washing and fetching water, which may imply a higher frequency of contact with contaminated water, soil or dust. Alternatively, the higher prevalence in females may be caused by differences in immune response or susceptibility.

In almost all villages endemic for *O. bifurcum*, co-infection with the hookworm *Necator americanus* occurs while other species of gastro-intestinal helminths appear to be rare. Krepel et al. (1992) showed that there was a correlation between the infection with *O. bifurcum* and *N. americanus* in that most patients were either infected with both parasites or not at all, and suggested two possible explanations for this observation. Firstly, there could be a similarity in factors associated with risk of infection, such as poor hygiene, agricultural practice or the relative lack of potable water. Secondly, there could be a similarity in transmission, although this seems to be unlikely as transmission of *N. americanus* occurs percutaneously and *O. bifurcum* transmission is believed to occur via the oral route. In contrast, transmission of the human hookworm *Ancylostoma duodenale* can occur both percutaneously and orally. To date, it is unclear whether *N. americanus* is the only species of hookworm infecting humans in Togo and Ghana or whether infection with *A. duodenale* co-exists. If *A. duodenale* is indeed present...
it would be of importance for control to study the extent and distribution of this hookworm in these countries.

The geographical distribution of human oesophagostomiasis appears to be localised to a well-defined area in northern Togo and Ghana, concentrated in several foci and with a decreasing prevalence toward the South of these countries.\(^{22,23,40}\) In this particular area, the number of non-human primates has decreased significantly during the last decades, which may suggest that humans have become a preferred host for *O. bifurcum* (Polderman, personal communication). To the South, there are locations (e.g. Baobeng-Fiema or Mole National Park) where *O. bifurcum* is commonly found in the Mona monkey (*Cercopithecus mona*), Patas monkey (*Erythrocebus patas*) and Olive baboon (*Papio anubis*) but not in humans, although they live in close association with these non-human primates. These observations raise a number of questions regarding the epidemiology of human oesophagostomiasis. For instance, what prevents the parasite from infecting humans in the South of Togo and Ghana? Is the parasite causing human oesophagostomiasis in northern Togo and Ghana a different species or strain compared with that found in monkeys, thereby having a different host preference and/or geographical distribution?

1.5. State of knowledge of the taxonomy and classification of *Oesophagostomum* species

The classification of members of the genus *Oesophagostomum* is primarily based on morphological features of adult worms. The genus is characterised by having a cervical groove.\(^{42}\) However, the relatively simple body plan and a considerable overlap in morphological parameters (e.g. length, length of tail, and length of sheath) between members of this genus have caused some taxonomic controversies. Between 1905 and the early 1980's, there has been great confusion over the taxonomic status of *Oesophagostomum* species infecting humans (see section 1.1.). For instance, specimens referred to as *O. apiostomum* by Leiper (1911)\(^{15}\) were classified as either *O. bifurcum* or *O. aculeatum*.\(^{12}\) Furthermore, *O. bifurcum* and *O. brumptii* which were considered to be synonymous by Chabaud and Larivière (1958) were described as two different species by Glen and Brooks (1985).\(^{43}\)

Currently, there is controversy over the species status of *O. bifurcum* infecting primates in northern Togo and Ghana. It is unclear whether the parasite causing human oesophagostomiasis and the one infecting non-human primates represent a single species or not. In order to tackle this taxonomic problem, some morphological (Blotkamp, unpublished) and genetic\(^{21}\) studies were conducted but were inconclusive.
1.6. Diagnosis of infection

Traditionally, the specific diagnosis of intestinal nematode infections is based on the detection of eggs or larvae in the faeces of the host. However, the eggs of many species of strongylids are morphologically indistinguishable, causing serious diagnostic problems. For instance, the eggs of *Oesophagostomum* spp., hookworm spp., *Trichostrongylus* spp. and *Ternidens deminutus* are morphologically identical or very similar. Specifically, the eggs of *O. bifurcum* are morphologically very similar to those of hookworms and other strongylid nematodes. To overcome this limitation, coproculture is used to allow eggs to develop into infective L3, which can then be microscopically identified to the genus level. However, coprological diagnosis using this approach is laborious, time consuming and requires skilled personnel.

Recently, a PCR approach was developed and evaluated for the detection of *O. bifurcum* and *N. americanus* DNA from human faeces. The PCR showed to be effective and rapid to perform, and significantly more sensitive than coproculture. Currently, this specific PCR is used as a diagnostic tool for epidemiological studies of *O. bifurcum* and *N. americanus* in northern Ghana.

1.7. DNA-based methods for the identification and differentiation of parasites

DNA technology can provide tools to overcome limitations of morphological identification.

**DNA target region**

The selection of a suitable genetic target is central to identifying parasites by DNA-based approaches. The choice of the target region depends on the taxonomic level at which the identification should occur, because different DNA regions provide different magnitudes of sequence variation. For the identification to the species level, a DNA region with a significantly lower level of sequence variation within species than between species is required, whereas identification to the strain level requires a significant level of sequence variation within the species. To date, various DNA target regions (i.e., single loci) have been employed for species and/or strain identification, including DNA regions of nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA).

Nuclear rDNA evolves in a concerted fashion rather than independently, resulting in greater sequence similarity (i.e., homogeneity) within species than among species, and it contains sequence regions of varying evolutionary rates (i.e., sequence variation). Therefore, rDNA can provide different appropriate targets for the identification of eukaryotes at various taxonomic levels. Particularly, the non-coding first and second internal transcribed spacers
(ITS-1 and ITS-2) provide genetic markers for the identification of various species of parasitic nematodes.\textsuperscript{53-57}

The mitochondrial genome is small, maternally inherited, self-replicating and evolves at a rate faster than the nuclear DNA.\textsuperscript{58,59} This makes the mitochondrial genome more applicable for identification and/or differentiation of parasites below the species level (i.e., strains, isolates or cryptic species).\textsuperscript{47,60,61} Particularly, the cytochrome c oxidase subunit I (\textit{cox1}) gene and the nicotinamide dehydrogenase subunit 4 gene (\textit{nad4}) of mtDNA have proven to be appropriate targets for identification and differentiation of parasites within the species.\textsuperscript{62-66}

\textit{PCR-linked restriction fragment length polymorphism (PCR-RFLP)}

PCR-RFLP\textsuperscript{67} is a technique in which enzymatically amplified DNA fragments are digested with one or more specific restriction endonucleases and are subsequently separated by agarose gel electrophoresis. The restriction fragments are detected by ultraviolet transillumination of ethidium-bromide stained gels and recorded by photography. PCR-RFLP has been used effectively to identify different groups of parasites to the species or/and strain levels.\textsuperscript{56,68,69,70,71} For instance, Gasser and Hoste (1995)\textsuperscript{72} used PCR-RFLP to characterise seven closely-related nematode parasites. They showed that the rDNA region spanning the ITS-1 and ITS-2 as well as the 5.8S rDNA gene provided genetic markers for the specific identification of these parasites and also indicated that the spacer sequences would be of use to study their systematics. Although PCR-RFLP is a simple and rapid DNA fingerprinting technique a limitation of this method is that genetic variation in restriction fragments of the same size can remain undetected. Fragments of the same size but different sequence co-migrate as one band during gel electrophoresis and may be misinterpreted as one molecule.\textsuperscript{73}

\textit{DNA sequencing}

The most commonly used method of sequencing DNA is the enzymatic or dideoxy chain termination method of Sanger.\textsuperscript{74} A DNA polymerase is used to synthesize a complementary copy of a single-stranded DNA template. The method relies on the incorporation of dideoxynucleotides (ddNTPs) into the elongated strand in the same way as the conventional deoxynucleotides (dNTPs). ddNTPs differ from dNTPs by lacking the 3'-OH group necessary for chain elongation. When a ddNTP is incorporated at the 3'-end of the new strand, chain elongation is terminated at one of the four nucleotides (A, C, G, T) because the absence of the hydroxyl-group prevents the formation of a phospho-diester bond with the dNTP. By using the correct ratio of the four conventional dNTPs and one of the four ddNTPs, DNA fragments of varying lengths are produced. To acquire the complete sequence of the DNA fragment analysed, four different ddNTPs are used either in four separate reactions using radioactive labels (manual sequencing) or in a single reaction using fluorescent labels (automated
sequencing). The nucleotides are separated by denaturing polyacrylamide gel electrophoresis and can be read directly from an autoradiograph of the gel (manual sequencing) or an electropherogram (automated sequencing).

DNA sequence analysis is a powerful tool for the identification of parasites and to study their genetic variation.\textsuperscript{47,49,75-78} For instance, sequencing of the ITS-2 of rDNA from \textit{O. bifurcum} and \textit{N. americanus} has shown that this region contains genetic markers for the identification of both species.\textsuperscript{79} Furthermore, sequencing has proven to be a useful tool to study the systematics of a range of parasite groups at different taxonomic levels.\textsuperscript{80-88} Advantages of DNA sequencing over PCR-RFLP are its high resolution, the quantifiable nature of sequence data and the ability to design specific primers based on the sequence differences among taxa for species or strain identification. Also, direct sequencing of PCR products allows polymorphisms to be identified. However, although the introduction of capillary electrophoresis (CE) has made Sanger sequencing faster and more accurate, the method can still be laborious, time-consuming and costly (particularly when a large number of samples require analysis).\textsuperscript{sec89} Moreover, direct sequencing does not always allow the number of alleles of a gene to be determined directly and it may be difficult or impossible to read a sequence if significant levels of sequence heterogeneity exist within the DNA fragment analysed.\textsuperscript{72}

\textit{Mutation scanning methods}

Mutation scanning methods provide useful alternatives for the detection of genetic variation and can overcome limitations of conventional methods. Unlike standard electrophoretic procedures that separate DNA fragments according to size, mutation scanning methods rely on the separation of DNA fragments based on their sequence. The first mutation scanning method (detection of mutations by S1 nuclease) was described by Shenk et al. (1975)\textsuperscript{90} followed by an increasing number of other approaches.\textsuperscript{89,91,92,93} In the last decade, these methods have complemented DNA sequencing to screen for genetic variation and are now widely used in biomedical research. A commonly used mutation scanning method is single-strand conformation polymorphism (SSCP).

The technique of SSCP\textsuperscript{94} is based on the principle that the electrophoretic mobility of a single-stranded DNA molecule is dependent on its structure (i.e., conformation) and size. When single-stranded DNA is placed in a non-denaturing solution it will take on sequence-dependent secondary and tertiary conformations as a result of intra-strand base pairing. Even a point mutation can influence the conformation of single-stranded DNA, resulting in an altered electrophoretic mobility. Theoretically, SSCP is relatively simple. PCR amplified DNA is denatured and separated as single-stranded molecules by electrophoresis in a non-denaturing polyacrylamide gel.

Provided an appropriate target region is used, PCR-SSCP can be employed effectively to study genetic variation in parasites and to identify them to species or strain.\textsuperscript{47,73,95} For
instance, SSCP-based mutation scanning approaches were effectively employed to fingerprint sequence variation in the ITS-1 rDNA of *Ascaris* from humans and pigs, in the ITS-2 rDNA of ascaridoid nematodes and the lungworm *Dictyocaulus*, and in the NADH dehydrogenase 1 gene and *cox1* mtDNA of *Schistosoma japonicum*. SSCP has also been employed successfully to screen for sequence variation in the ITS-2 of rDNA of *O. bifurcum* from human and non-human primates.

**DNA fingerprinting**

Sometimes the genetic variability within a single locus is not sufficient to fulfill the research aim (i.e., identification or differentiation of parasites). In those cases DNA fingerprinting methods based on screening of multiple genetic loci that are distributed throughout the nuclear and/or mitochondrial genome may be useful. In this section, the principles of two commonly used DNA fingerprinting methods, namely random amplification of polymorphic DNA (RAPD) and AFLP are described, which have been used successfully to study the genetic diversity in a range of organisms.

*Random amplified polymorphic DNA (RAPD)*

RAPD or arbitrarily primed-polymerase chain reaction (AP-PCR) is a DNA fingerprinting method by which complex and informative genomic fingerprints can be readily produced without prior sequence information. The technique is based on the amplification of DNA fragments of genomic DNA using (usually) single primers (~10-mers) of arbitrary sequence, and subsequent separation of the amplicons by (agarose) gel electrophoresis. Although this method has the advantage of being rapid and easy to perform, the reproducibility of the RAPD banding patterns can be influenced by a number of factors. These include DNA quality, primer sensitivity, template concentration, co-migration of non-homologous fragments and/or the use of different thermal cyclers. Despite this limitation, studies have shown that RAPD markers can be useful for the identification and differentiation of species and strains of a range of parasite groups, including nematodes, trematodes, cestodes, and protozoans, particularly if high stringency conditions are used in the PCR.

*AFLP*™

AFLP™ is another DNA fingerprinting method and has been widely applied as a tool for investigating the genetics of plants and animals, diagnosis of diseases, forensic science and parentage analysis. The principle of the method relies on (i) digestion of genomic DNA with a chosen set of two restriction enzymes (ii) ligation of restriction halfsite specific adapters to the 3'- and 5'-end of the restriction fragments (iii) amplification of the restriction fragments
using primers which anneal to the sequences of the adapters, and (iv) analysis of the restriction fragments by polyacrylamide gel electrophoresis.

The main advantages of AFLP are that it requires no sequence information prior to analysis, is performed under high stringency conditions and has a high discriminatory power. Furthermore, it can, in principle, be applied to complex DNA of any origin. A drawback may be that it is relatively time consuming to perform compared with other DNA fingerprinting methods such as RAPD, particularly when organisms with complex genomes have to be analysed, which require two steps of amplifications (i.e., pre-selective and selective PCR). Also, the high level of variation and limited number of homologous markers detected by AFLP can become a limitation. To date, AFLP has been used successfully to identify and genotype a range of organisms, including vertebrate animals, plants, fungi and bacteria. Remarkably, AFLP has not yet found wide application within the field of parasitology and the number of published studies on AFLP of individual parasitic nematodes is limited. However, it is likely that this method will find important applications to the identification of a broad range of other parasitic nematodes of veterinary and/or human health importance. At the commencement of this project, there was no application of AFLP to nodule worms or hookworms.

1.8. Conclusions and aims of this thesis

Human oesophagostomiasis is recognised as a parasitic disease of major socio-economic significance in northern Togo and Ghana. However, in spite of the serious health problems caused by *O. bifurcum* in these countries there are currently serious gaps in the knowledge of the biology and transmission of the parasite. For instance, it has been suggested that non-human primates can act as a zoonotic reservoir for human infection with *O. bifurcum*. However, it is unclear to what extent non-human primates in Ghana are infected with *O. bifurcum* and whether the parasite infecting humans represent the same species as the one found in non-human primates. Also, it is uncertain whether, besides *N. americanus*, other species of hookworm (or strongylid nematodes with hookworm-like eggs) co-exist with *O. bifurcum* in northern Ghana. To fill such gaps in our knowledge, accurate identification of the parasite(s) involved is crucial.

Over the last decade, DNA technology has provided useful tools to overcome limitations of traditional approaches for parasite identification. For instance, PCR-linked restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing have been used effectively to identify different groups of parasites to the species and/or strain level. However, these techniques do not always accurately resolve sequence variation, and some of them can be relatively laborious, time-consuming and costly, particularly when large numbers of samples
require analysis. These limitations may be overcome by employing high-resolution mutation scanning methods, such as single-strand conformation polymorphism (SSCP) or DNA fingerprinting methods, such as RAPD or AFLP.

The principal goal of the research conducted in this thesis is to provide biological evidence that *O. bifurcum* from human and non-human primates in Ghana are biologically the same or distinct. Therefore, the specific research aims are (i) to estimate the prevalence of *O. bifurcum* infection in non-human primates in Mole National Park and Baobeng-Fiema (chapter 2), (ii) to conduct a morphological study of a relatively large number of adult *O. bifurcum* from human and non-human primates in Ghana in order to assess the extent of morphological variability within the species (chapter 3) (iii) to study the genetic make up of *O. bifurcum* from different host species and locations in Ghana (chapter 4-7).

An additional aim of this thesis was to establish whether the hookworm *Ancylostoma duodenale* occurs in sympatry with *O. bifurcum* and impacts seriously on human health in northern Ghana (chapter 8). Achieving these four research aims would have important implications in relation to the diagnosis, prevention and control of human oesophagostomiasis and hookworm disease.