CHAPTER 8

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Several studies have demonstrated that non-human primates can maintain and promote transmission of parasitic helminth infections, particularly in the rural areas of Africa where humans and non-human primates live in close proximity. For *O. bifurcum*, it has been suggested that non-human primates can represent a zoonotic reservoir for human infection. Although in the area endemic for human oesophagostomiasis in northern Ghana the number of non-human primates has reduced significantly over the last decades. The results presented in chapter 2 demonstrated that there are currently areas south of the human endemic area where infected Mona monkeys, Patas monkeys and Olive baboons range freely in human habitats and interact frequently with local people. Furthermore, Ghana's wildlife is attracting an increasing number of international travellers. In particular the 'eco-tourism' sector is becoming more popular, focusing on wilderness adventures and safaris through National Parks and/or other natural habitats of wildlife. Mole National park (Ghana's largest National Park) and Baobeng-Fiema Monkey sanctuary are two major attractions where tourists can be exposed to *O. bifurcum* from droppings of non-human primates. Thus, it was of public health importance to assess the zoonotic potential of *O. bifurcum* in Ghana.

While human infections with *O. bifurcum* are restricted to northern Togo and Ghana, infections of non-human primates proved to be prevalent elsewhere in Ghana (chapter 2). Detailed morphological examination of adult *O. bifurcum* showed significant differences in morphological characters (including parasite length, maximum width, length of the oesophagus, and length of the spicules) between worms from humans, the Patas, Mona or Green monkey, and the Olive baboon from Ghana (chapter 3). Furthermore, the results achieved in chapters 4 to 6 indicated the existence of genetically distinct 'strains' of *O. bifurcum* in different primate host species. Taken together, the epidemiological, morphological and molecular data (chapters 3 to 6) obtained in this thesis provide strong support that the 'strain' infecting humans in northern Ghana has a different transmission cycle compared with *O. bifurcum* from different species of non-human primates. This information suggests that non-human primates are not a zoonotic reservoir for *O. bifurcum* infection of humans in Ghana and thus are not a threat to the public health of local people and international travellers in this country.

Why human infections with *O. bifurcum* are restricted to the north of Togo and Ghana remains unclear. It is possible that the development of the eggs of the human variant of *O. bifurcum* into infective third stage larvae (L3) requires environmental (ecological) conditions that are only present in the north of these countries. However, we cannot exclude that the human variant of the parasite is also present elsewhere in the world but is overlooked or misdiagnosed as hookworm infection. Indeed, this is precisely what happened in Togo and Ghana before it was discovered that a significant number of human hookworm infections in
those countries were actually *O. bifurcum* infections. Hence, it would be important to spread information on the clinical presentation of human oesophagostomiasis and the morphology of larvae to countries endemic for hookworm and/or other hookworm-like infections.

To further prove that *O. bifurcum* from non-human primates in Ghana is not transmissible to humans reciprocal cross-infection could be conducted. In the past, such an experimental cross-infection study was performed. In that study, L3s of *O. bifurcum* (cultured from human stool from northern Ghana) were first used to establish a patent infection in a rhesus monkey (*Macaca mulatta*). Subsequently, a number of additional monkeys (i.e., *Macaca mulatta* and *Macaca fascicularis*) were inoculated with L3s generated from that monkey. The results showed that less than 50% of the monkeys established a patent infection. In addition, the pre-patent period for these monkeys was long (88 to 134 days), and egg production was both sporadic and temporary. Thus, it was concluded that the monkeys used in that study were poorly susceptible to *O. bifurcum* from humans. However, future experimental cross-infection studies of *O. bifurcum* should (also) include the species of non-human primates present and infected with *O. bifurcum* in Ghana (i.e., Olive baboon, Mona monkey or Patas monkey). In addition, it would be important to investigate the reproductive fitness of the F2 generation. Finally, it would be interesting to perform reciprocal cross-mating experiments to determine the extent of mating between adult *O. bifurcum* from human and non-human primates and, if produced, to assess the reproducibility of any F1 generation. DNA fingerprinting could be used to type the F1 and to investigate their genetic make up. The method of AFLP would be applicable for this study, as it allows computerized analysis of DNA fingerprints and the development of databases, such that new data can be readily compared with previous data (see chapter 6). However, experimental cross-infection/mating studies are very time-consuming, laborious and costly to perform. Moreover, they cause ethical problems.

Helminth control aims at reducing the mortality and morbidity caused or induced by the parasites. Given that there is a positive correlation between the morbidity and the intensity of helminth infection (i.e., disease is mostly caused by heavy infections), it can be important to have a quantitative diagnostic tool for monitoring the effectiveness of intervention programs. Currently, the intensity of *O. bifurcum* and hookworm infections is estimated by coproculture. However, this method is relatively time-consuming. Also, the results obtained can vary considerably due to differences in culture conditions, the species of parasites cultured, fungal growth and/or the development of maggots in the culture. Furthermore, the L3s of *O. bifurcum*, *N. americanus* and *A. duodenale* are morphologically similar to each other and to those of a range of other strongylid nematodes (i.e., other *Oesophagostomum* spp., *Ternidens deminutus* and *Trichostrongylus* spp.). These factors can cause diagnostic problems and can lead to under- or over-detection of the intensity of infection. As PCR has proven to be a valuable complementary tool for the diagnosis of *O. bifurcum* and hookworm infection (chapters 2 and 7), future studies should focus on developing a (multi-plex) real-time PCR
to assess the intensity of these helminth infections. Such quantitative PCR would have important implications for monitoring the effectiveness of mass treatment with albendazole in north Ghana. The PCR approaches used in the present thesis to differentiate *O. bifurcum*, *N. americanus* and/or *A. duodenale* (chapters 2 and 7) represent a foundation for the development of such a tool.

In the last decade, advances in molecular biology have led to major developments in the genetic typing of various causative agents of infectious diseases (i.e., bacteria, viruses and parasites). Sequence data have been used to investigate ancestral relationships of pathogens and to assist in addressing relevant questions as to their genetic make up. Furthermore, DNA fingerprinting has been applied to identify and differentiate pathogenic organisms, and to detect sources of infection. The results of the present study reinforce the usefulness of molecular tools for the identification and/or differentiation of parasitic nematodes (chapters 2 and 7) and studying population genetic substructures (chapters 4 to 6). In particular, the method of AFLP (which previously had not been applied to parasitic nematodes of human health importance) proved to be a valuable tool in establishing genetic variation within *O. bifurcum*. Hence, this technique could be important in addressing key questions regarding the population genetics and systematics of other nematodes (chapter 6). For example, it could be used to genotype *Ascaris* from humans and pigs. Currently, there is an ongoing debate as to whether *Ascaris* infecting humans is the same species as the one infecting pigs. Genetic studies (based on ribosomal and mitochondrial genes) have been performed to resolve this taxonomic problem, but have been inconclusive.\(^{96,201-205}\) High throughput AFLP analysis of *Ascaris* from human and pigs from different geographical regions might give more insights into the transmission cycle(s) and elucidate the species status of this strongylid nematode that currently infects a quarter of the world's human population.\(^{206,207}\) Also, the present AFLP might be a valuable method to genotype *Ternidens deminutus* from humans and non-human primates. A recent study, based on the second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) of *T. deminutus* from Mona monkey and Olive baboon from Ghana (Schindler et al., unpublished) suggests the existence of at least two cryptic species within *T. deminutus*. It would be interesting to further study the systematics of this strongylid nematode by AFLP analysis of a large number of adult worms from different primate host species and from different geographical locations. Finally, the AFLP approach developed herein may have important implications to study the geographical spread of helminthiasis and/or to investigate possible sources and routes of infection.

In conclusion, the present thesis has filled some gaps in the knowledge of *O. bifurcum* in northern Ghana. In particular, it has advanced our understanding of the epidemiology and population biology of the parasite. It suggests that *O. bifurcum* from humans and non-human primates have distinct transmission cycles and that non-human primates are not a threat to the public health in Ghana. This knowledge should, in the longer term, contribute to reducing the
health impact of *O. bifurcum* and hookworm in Ghana and is likely to prove useful in the present efforts to monitor and control human oesophagostomiasis and hookworm disease in this country. Overall, the results of this study emphasized the importance of the accurate identification and differentiation of parasites and demonstrated that DNA-based techniques provide powerful complementary tools to traditional methods for parasite identification. While this thesis focused on key strongylid helminths in Ghana, the molecular approaches described and used herein have broad applicability to other parasites of socio-economic and public health importance globally.