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The zoonotic potential of *Oesophagostomum bifurcum* in Ghana. Epidemiological, morphological and genetic studies

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SUMMARY

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The nodule worm *Oesophagostomum bifurcum* (Nematoda: Strongylida) is a parasite that is commonly found in humans in northern Togo and Ghana. Currently, it is estimated that 250 000 people are infected with this nematode and at least one million people are at risk of infection. Infection can cause significant disease as a consequence of encysted larvae in the wall of the large intestine. In spite of the health problems caused by *O. bifurcum*, there are serious gaps in the knowledge of the biology and transmission of the parasite. For instance, it has been proposed that non-human primates can act as a reservoir for human infection with *O. bifurcum*, but it has been unclear to what extent non-human primates in Ghana are infected with *O. bifurcum* and whether the parasite infecting humans represents the same species as is found in non-human primates.

The overall objective of the present study was to assess the zoonotic potential of *O. bifurcum*, in other words to assess the risk of transmission of the simian parasite to humans. Knowledge of the zoonotic potential of *O. bifurcum* is central to controlling the infection in humans. The specific research aims were (1) to establish the presence of *O. bifurcum* in different species of non-human primates in two geographical areas outside of the endemic region in Ghana, (2) to determine whether there is any significant morphological variation between *O. bifurcum* from human and from different species of non-human primates, (3) to test the hypothesis that *O. bifurcum* from humans is genetically distinct from the parasite from non-human primates, and (4) to establish whether the hookworm *Ancylostoma duodenale* occurs in sympatry with *O. bifurcum* and impacts on human health in northern Ghana.

In chapter 2, the prevalence of infection with *O. bifurcum* in non-human primates in Mole National Park (MNP) and Baobeng-Fiema monkey sanctuary (BFMS) (central Ghana) was established using classical, parasitological and molecular methods. The localities are situated outside of the endemic area of human oesophagostomiasis, and, in both areas, there is a close contact between humans and non-human primates. Stool samples from different species of non-human primates were collected and examined for the presence of *O. bifurcum* by larval culture and microscopy as well as by species-specific polymerase chain reaction (PCR). The results showed that a high percentage (75-99%) of the Olive baboon (*Papio anubis*) and Mona monkey (*Cercopithecus Mona*) samples contained *O. bifurcum*. No *O. bifurcum* was detected in the faeces from the Black-and-white colobus monkeys (*Colobus vellerosus*). Studies of the behaviour of the non-human primates, focusing on food consumption, defecation and physical contact with humans, indicated favourable conditions for zoonotic transmission.

In chapter 3, a study of the morphology of adult *O. bifurcum* from humans and different species of non-human primates from Ghana was undertaken to assess whether there were any morphological differences between adult *O. bifurcum* from human and non-human primates. The results showed that both male and female *O. bifurcum* specimens from human

were significantly smaller compared with those from the Mona, Patas (*Cercopithecus Patas*) and Green monkeys (*Cercopithecus sabeus*), and larger than those from Olive baboons. Furthermore, differences in length of the oesophagus and the length of the spicules were detected between different host species. Also, worms obtained from humans were found to be darker in the colour than those derived from monkeys and baboons. Together with the detection of a difference in distribution between *O. bifurcum* from human and non-human primates (chapter 2), the results of chapter 3 stimulated investigations into the genetic make-up of the parasite.

In chapters 4 to 6, several molecular approaches were used to study genetic variation within *O. bifurcum* from different species of primate host. Part of the cytochrome *c* oxidase subunit I (*pcox1*) gene was screened for sequence variation between *O. bifurcum* from humans and the Mona monkey from Ghana using PCR-coupled single-strand conformation polymorphism (SSCP) analysis (chapter 4). The results of the study showed that there was no fixed sequence difference in the *pcox1* between *O. bifurcum* from these two species of primates. Cluster analysis of the *pcox1* data showed that there was no apparent relationship between *O. bifurcum* haplotype and the specific primate host infected. However, it was still possible that genetic sub-structuring did indeed exist, but that this was not adequately reflected in the *pcox1*. Therefore, two DNA fingerprinting methods, namely random amplified polymorphic DNA (RAPD) (chapter 5) and AFLP™ (chapter 6) were employed to further investigate genetic variation within the parasite. The results obtained using these methods demonstrated clearly the existence of population genetic sub-structuring according to host species, and that *O. bifurcum* from humans, the Mona monkey, the Patas monkey and the Olive baboon represented genetically distinct groups.

Taken together, the epidemiological, morphological and genetic data obtained in chapters 2 to 6 provided support for the proposal that the genetic variant of *O. bifurcum* infecting humans in northern Ghana has a distinct transmission cycle from *O. bifurcum* of Mona monkeys, Patas monkeys and Olive baboons. This information suggests that these non-human primates are not a reservoir for *O. bifurcum* infection to humans in Ghana and thus are not a threat to the health of either local people or and international travellers.

In chapter 8, an investigation was undertaken to establish whether (in addition to *N. americanus*) the human hookworm *A. duodenale* was present in northern Ghana and to estimate its prevalence. A two-step, semi-nested PCR approach was established and utilized for the differential diagnosis of *N. americanus* and *A. duodenale*. Surprisingly, *A. duodenale* DNA was specifically detected in 74 (19.6%) of the 378 faecal samples tested. This finding confirmed that this species of hookworm co-exists with *N. americanus* in northern Ghana and has a human health impact in this part of the country. This study also demonstrated that the present PCR approach is a useful complementary tool for the diagnosis of *A. duodenale* infection. Consequently, it provides a valuable additional method for other epidemiological studies and in

monitoring the success of control programs of *A. duodenale*, in Ghana and other parts of Africa.

Overall, this thesis has contributed to a better understanding of the epidemiology and/or genetic make-up of *O. bifurcum* and 'hookworm' in Ghana. The results and knowledge gained are of importance for the present efforts to prevent and control human infection with *O. bifurcum* and hookworm in northern Ghana by mass treatment with albendazole. As it is unlikely that non-human primates in this region represent a reservoir for human infection with *O. bifurcum*, control can now focus predominantly on the human population. Given that the efficiency of anthelmintic treatment can vary considerably between infections with *N. americanus* and *A. duodenale*, the finding that human infection with *A. duodenale* occurs in northern Ghana is of importance for monitoring the effect of mass treatment. Finally, the DNA-based methods established in the present thesis have important implications for epidemiological and genetic studies of a wide range of (other) infectious agents and therefore contribute more broadly to the control of infectious diseases and the improvement of public health world-wide.