REVIEW ARTICLE

Insights into the epidemiology and genetic make-up of *Oesophagostomum bifurcum* from human and non-human primates using molecular tools

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SUMMARY

The nodule worm *Oesophagostomum bifurcum* (Nematoda: Strongylida) is a parasite of major human health importance predominantly in northern Togo and Ghana. Currently, it is estimated that 0·25 million people are infected with this nematode, and at least 1 million people are at risk of infection. Infection with this parasite causes 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 have been significant gaps in the knowledge of the biology, transmission and population genetics of the parasite. This review provides an account of some recent insights into the epidemiology and genetics of the parasite from human and non-human primate hosts in specific regions of Africa using molecular tools. Recent research findings are discussed mainly in relation to non-human primates being reservoirs of infection, and the consequences for the prevention and control of oesophagostomiasis in humans are briefly discussed.

Key words: *Oesophagostomum bifurcum*, oesophagostomiasis, human, diagnosis, molecular tools, genetics, epidemiology, control.

INTRODUCTION

Infection of humans with the nodule worm *Oesophagostomum bifurcum* (Nematoda: Strongylida: Oesophagostominae) is common in northern Togo and Ghana (Fig. 1) (reviewed by Polderman and Blotkamp, 1995; Polderman, Anemana and Asigri, 1999). While asymptomatic infection is common, the parasite can cause serious pathological effects and intestinal disease in humans (Elmes and McAdam, 1954; Haaf and van Soest, 1964; Gigase *et al*. 1987; Storey *et al*. 2000, 2001*a*, *b*; Ziem *et al*. 2005). Two distinct types of clinical disease can be distinguished. 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 (Polderman *et al*. 1999). Usually, patients 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 masses to avoid rupture and peritonitis. The less common, multi-nodular disease is characterized by hundreds of pea-sized, granulomatous nodules within the colonic wall and other intra-abdominal structures, together with gross thickening and oedema of the wall. This type of clinical presentation is often associated with abdominal pain, persistent diarrhoea and weight loss (Storey *et al*. 2000). Multinodular disease can lead to progressive destruction of the colonic wall, in which case, total or partial colonectomy is indicated (Gigase *et al*. 1987; Storey *et al*. 2000). In non-human primates, *O. bifurcum* infection can also be pathogenic, particularly in captive primates suffering from stresses of confinement and transportation (Stewart and Gasbarre, 1989).

In spite of the serious human health problems caused by *O. bifurcum*, there are significant gaps in the knowledge of the epidemiology of oesophagostomiasis (*cf*. Polderman and Blotkamp, 1995). It has been postulated that some species of non-human primates may act as reservoir hosts for human infection (Stewart and Gasbarre, 1989; Polderman and Blotkamp, 1995). Also, there has been an indication, based on morphological study, that size

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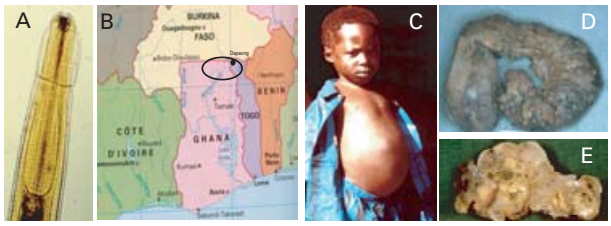

Fig. 1. *Oesophagostomum bifurcum* (A, anterior part of adult worm) is endemic in northern parts of Ghana and Togo in Africa (B) and causes clinical oesophagostomiasis (C, 'Dapaong tumour') as a consequence of granulomatous nodules in the wall of the large intestine (D and E, cross-section).

variation in the adults of *O. bifurcum* between human and non-human primates can occur (Blotkamp *et al.* 1993). The suggestion of population variation within *O. bifurcum* has stimulated recent investigations into the epidemiology and genetic make-up of *O. bifurcum* from human and non-human primates. In particular, molecular technologies, such as polymerase chain reaction (PCR)-coupled mutation scanning, sequencing and fingerprinting (reviewed by Gasser, 2005), have provided unique opportunities for the genetic characterization and/or specific identification of *O. bifurcum* to overcome limitations of traditional parasitological approaches. This review provides an account of new insights into the epidemiology and genetics of the parasite from different primate host species in parts of Africa employing molecular tools. The research findings are also discussed in relation to non-human primates being reservoirs of infection and the prevention and control of oesophagostomiasis in humans.

BRIEF ACCOUNT OF THE LIFE-CYCLE AND EPIDEMIOLOGY OF HUMAN OESOPHAGOSTOMIASIS IN NORTHERN TOGO AND GHANA

Some aspects of the life-cycle and transmission of *O. bifurcum* in primates have been elucidated, but details are still lacking. *O. bifurcum* is a dioecious nematode. Each adult female produces ~5000 eggs per day (Krepel and Polderman, 1992) which are excreted in the faeces and subsequently develop into the first-, second- and third-stage larvae (L1, L2, L3, respectively) outside of the host. The development from egg to L3 takes 4–7 days, depending on environmental conditions. The infection of humans with *O. bifurcum* is considered to occur *via* the ingestion of the L3 in contaminated water, food, soil or dust (Krepel, 1994), rather than percutaneous transmission (Krepel, Baeta and Polderman, 1992; Polderman and Blotkamp, 1995). After ingestion, the L3 enter the intestinal mucosa and form tiny nodules, in which they develop into the fourth-stage larvae (L4). These larvae can remain in the nodules or return to the intestinal lumen where they develop to adults. After copulation of the male and female adults, the latter start to produce eggs. Importantly, the L3 of *Oesophagostomum* species are resistant to environmental stresses. Even after long periods of desiccation (months) or freezing (−15 °C), some of them (~20%) can remain viable for extended periods of time (Spindler, 1936; Rose and Small, 1980; Barger, Lewis and Brown, 1984; Polderman and Blotkamp, 1995; Pit *et al.* 2000). During periods of desiccation, the L3s shrink within their sheath. 'Revival' of the dormant L3 occurs after rehydration (*cf.* Polderman and Blotkamp, 1995).

Oesophagostomum bifurcum infection in humans in northern Togo and Ghana occurs in almost every village, with the highest prevalence (⩽90%) in the rural areas (Krepel *et al.* 1992; Pit *et al.* 1999*a*; Storey *et al.* 2000; Yelifari *et al.* 2005). Poor hygiene and lack of medical assistance in these villages may be explanations for this observation. Krepel *et al.* (1992) reported that the prevalence in northern Togo and Ghana is usually low in children of <5 years of age, although heavy infection in children of 3–4 years of age has been reported in a more recent survey (Pit *et al.* 1999*a*). There is a significant increase in the prevalence in children between the ages of 2 and 10 years, which indicates an increased rate of transmission of the parasite in this age group. Females of >5 years of age are usually more frequently 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. It is possible that the difference is caused by the daily activities of females, such as cooking, washing and the fetching of water, which may imply a higher frequency of contact with L3 in contaminated water, soil or dust. It is also possible that the higher prevalence relates to differences in the immune response(s) or susceptibility in females.

In almost all villages endemic for *O. bifurcum*, co-infection with the hookworm *Necator americanus* occurs, whereas 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 persons were either infected with both parasites, or none at all, and suggested explanations for this observation. Firstly, similar factors could be associated with the risk of infection with both parasites, such as poor hygiene, agricultural practices and/or the relative lack of potable water. Secondly, there could be a similarity in transmission, although this seems to be unlikely, as the transmission of *N. americanus* occurs percutaneously (Hotez *et al.* 2005) and *O. bifurcum* transmission is considered to occur *via* the oral route (Polderman and Blotkamp, 1995). In contrast, the transmission of the human hookworm *Ancylostoma duodenale* can occur both percutaneously and orally (Hotez *et al.* 2005). Until

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recently, it was unclear whether *N. americanus* is the only species of hookworm infecting humans in Togo and Ghana but, recently, *A. duodenale* has been demonstrated (de Gruijter *et al.* 2005*b*).

Interestingly, human oesophagostomiasis appears to be localized to a well-defined region in northern Togo and Ghana, concentrated in several foci and with a decreasing prevalence toward the south of these countries (Krepel *et al.* 1992; Pit *et al.* 1999*a*; Yelifari *et al.* 2005). In the north, the number of non-human primates has decreased significantly in the last decades which may suggest that humans have become a preferred host for *O. bifurcum* (Polderman, unpublished observations). In the south, there are locations (e.g. Baobeng-Fiema and 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 important questions regarding the epidemiology of human oesophagostomiasis, such as which factors prevent the parasite from infecting humans in the South of Togo and Ghana and whether the parasite causing human oesophagostomiasis in northern Togo and Ghana represents a different species or variant compared with that found in monkeys, thereby having a different host preference and/or geographical distribution.

IDENTIFICATION OF *OESOPHAGOSTOMUM* SPECIES AND DIAGNOSIS OF INFECTION

The classification of members of the genus *Oesophagostomum* is primarily based on morphological features of adult worms (Skrjabin *et al.* 1991). However, the relatively simple body plan and an overlap in some morphological characters between members of this genus have caused some taxonomic controversies. Between 1905 and the early 1980s, there was confusion as to the the taxonomic status of *Oesophagostomum* species infecting humans. For instance, specimens referred to as *O. apiostomum* by Leiper (1911) are now classified as either *O. bifurcum* or *O. aculeatum* (Chabaud and Larivière, 1958), and *O. bifurcum* and *O. brumpti* considered to be synonymous by Chabaud and Larivière (1958) were considered as two different species by Glen and Brooks (1985). There has been controversy about the species status of *O. bifurcum* infecting primates in northern Togo and Ghana. It has been 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 *et al.* 1993) and genetic (Gasser *et al.* 1999) studies were conducted but were inconclusive.

Traditionally, the specific diagnosis of gastrointestinal nematode infections in primates is based on the detection of eggs in the faeces. However, the eggs of many species of strongylids are morphologically indistinguishable, representing a serious diagnostic limitation. For instance, the eggs of *Oesophagostomum* spp., hookworms, *Trichostrongylus* spp. and *Ternidens deminutus* are morphologically identical or very similar (*cf.* Goldsmid, 1991; Polderman and Blotkamp, 1995). To overcome this limitation, coproculture is used to allow eggs to develop into the L3s, which can then be identified microscopically to the genus level (Polderman *et al.* 1991; Blotkamp *et al.* 1993). However, coprological diagnosis using this approach is laborious, time consuming, unreliable and requires relatively skilled personnel to identify and differentiate larvae.

MOLECULAR TOOLS FOR THE IDENTIFICATION OF *O. BIFURCUM* AND DIAGNOSIS OF INFECTION – IMPLICATIONS FOR EPIDEMIOLOGY AND ECOLOGY

Central to the PCR-based identification of parasites to species is the choice of an appropriate DNA target region (genetic marker or locus) (Gasser, 2001). As different genes evolve at different rates, the DNA region chosen should contain a sufficient magnitude of sequence variability to allow the identification of parasites to the taxonomic level required. For specific identification, the target DNA should differ enough in sequence to allow the delineation of species, but display no or minor variation within a species. In contrast, for the purpose of identifying population variants or 'strains', a considerable degree of variation in the sequence should exist within a species. Various target regions, including nuclear ribosomal DNA (rDNA), mitochondrial DNA (mtDNA) or repetitive DNA elements have been employed to achieve the identification of parasites to species or strains (reviewed by Gasser, 2001, 2005).

Studies of a range of nematodes, including those of the order Strongylida (bursate nematodes, to which nodule worms belong) have consistently shown that the internal transcribed spacers (ITS) of nuclear rDNA yield genetic markers for the identification and characterization of species (Gasser, 1999, 2001). The definition of specific markers in the second ITS (ITS-2) of *O. bifurcum* has had important implications for diagnosis and for studying the epidemiology of oesophagostomiasis. Based on the research of *O. bifurcum* in humans in Togo (Romstad *et al.* 1997*a*, *b*), Verweij *et al.* (2000) established a PCR for the specific amplification of the ITS-2 from *O. bifurcum* DNA (femtograms) from human faeces. Using a panel of 155 well-defined faecal and DNA samples, the assay achieved a diagnostic sensitivity of ~95% and a specificity of 100%. Verweij *et al.* (2001) extended this work to determine by PCR the prevalence of both *O. bifurcum* and *N. americanus* infections in humans in northern Ghana based on

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the specific amplification of DNA from these parasites from faecal samples. The *Oesophagostomum bifurcum*-PCR was positive for 57 of 61 (93%) faecal samples known to contain *O. bifurcum* L3s after coproculture. The *Necator americanus*-PCR was positive for 137 of 146 (94%) faecal samples known to contain *N. americanus* L3s in coproculture. PCR also detected 26 additional *O. bifurcum* cases in 72 samples from *O. bifurcum*-endemic villages for which no *O. bifurcum* L3s were found and 45 *N. americanus* cases in 78 samples from which no *N. americanus* L3 were obtained from culture. No *O. bifurcum* DNA was detected in 91 faecal samples from individuals from 2 non-endemic villages. Clearly, these results demonstrated the usefulness and advantages of the PCR assays (Verweij *et al*. 2000, 2001) as epidemiological tools to estimate the prevalence and distribution of *O. bifurcum* in humans and for the differentiation of *O. bifurcum* from *N. americanus* infection. The development of conventional PCR-based copro-diagnostic methods represents a significant improvement and overcomes the limitations of traditional coproscopic methods, and recent results (Verweij *et al*. unpublished) indicate the prospect for developing real-time PCR assays for the quantitation or semi-quantitation of DNA in the faeces from infected hosts as tools for investigating the epidemiology and ecology of *O. bifurcum*.

RECENT OBSERVATIONAL STUDY OF NON-HUMAN PRIMATES AS POTENTIAL RESERVOIRS OF *O. BIFURCUM* INFECTION IN HUMANS

As it has been proposed that non-human primates may represent a zoonotic reservoir for human oesophagostomiasis (Polderman *et al*. 1999), van Lieshout *et al*. (2005) recently undertook an epidemiological investigation of *O. bifurcum* in different primate host species. These authors examined faecal samples ($n = 349$) from Olive baboon, Mona monkey and Black-and-white Colobus monkey from 2 distinct geographical areas external to the area in Ghana endemic for human *O. bifurcum* using both microscopic and species-specific PCR methods (Verweij *et al*. 2000, 2001). The results showed that a high percentage (75–99%) of samples from the Olive baboon and Mona monkeys harboured *O. bifurcum*. The majority of the test-positive samples contained a large number of L3 (>100) after coproculture, whereas no *O. bifurcum* was detected in the faeces from the Colobus monkeys. Studying the behaviour of the non-human primates, focusing on defaecation, food consumption and physical contact with humans, indicated favourable conditions for zoonotic transmission.

Interestingly, greater numbers of L3 have been found in the faeces from Mona monkey and Olive baboon (van Lieshout *et al*. 2005) than samples from humans (Pit *et al*. 1999*b*; Yelifari *et al*. 2005), suggesting a higher intensity of infection and effective transmission for non-human primates. In contrast, there appeared to be no risk of infection in the aboreal (i.e. tree-living) Colobus monkeys. Interestingly, to date, no cases of human oesophagostomiasis have been reported in the two study areas. Also, the majority of inhabitants ($n = \sim 700$) living in the Mole National Park (Northern region of Ghana) and a selection of individuals ($n = \sim 100$) from the Baobeng Fiema Monkey Sanctuary (Brong-Ahafo region) were tested on multiple occasions by copro-culture and/or PCR for the presence of *O. bifurcum*. None of the human stool samples were shown to be test-positive for *O. bifurcum*, whereas *N. americanus* infection was common (prevalence of ~30%). The apparent absence of *Oesophagostomum* from humans in both regions is remarkable for multiple reasons. Firstly, the ecological conditions for transmission seem to be favourable for *O. bifurcum*; the estimates of the prevalence and the intensities of infection in non-arboreal, non-human primates are high, with no major seasonal fluctuations in prevalence and intensity of infection. This indicates that the free-living, potentially infective, larval stages develop successfully throughout the year. Secondly, the behavioural study indicated a close relationship and interaction between human and non-human primates in the same habitat. Thirdly, humans are suitable hosts for *O. bifurcum*, as demonstrated by the high prevalence of human infection several hundred kilometres to the north of the study areas (*cf*. Polderman *et al*. 1991). Thus, it is still unclear why the parasite does not infect humans in the two study areas. Given that *O. bifurcum* infection was not detected in humans in either study area, the findings from this study (van Lieshout *et al*. 2005) supported the proposal that *O. bifurcum* from humans in the north of Ghana is biologically distinct from *O. bifurcum* from Mona monkey and/or Olive baboon hosts.

ASSESSMENT OF GENETIC VARIABILITY IN NUCLEAR RIBOSOMAL AND MITOCHONDRIAL DNA REGIONS WITHIN *O. BIFURCUM* FROM HUMAN AND MONA MONKEY FROM GHANA BY MUTATION SCANNING

In order to examine whether *O. bifurcum* from human and the Mona monkey from Ghana represented genetic variants or 'cryptic' (i.e. 'hidden'; morphologically similar or identical but genetically distinct) species, Gasser *et al*. (1999) used a PCR-based mutation scanning approach, the single-strand conformation polymorphism (SSCP), to scan for nucleotide alterations in the ITS-2 region among individual *O. bifurcum*. Although some sequence micro-heterogeneity was detectable among individuals, no unequivocal sequence difference was detected in the ITS-2 of *O. bifurcum* between the host species. Since mitochondrial DNA mutates at a

higher rate than the nuclear DNA (see Blouin, 2002; Hu, Chilton and Gasser, 2004), the former was considered more appropriate for the detection of genetic variation within the species. Therefore, de Gruijter *et al*. (2002) employed the SSCP in combination with DNA sequencing to estimate haplotypic variability in a portion of the *cox*1 gene (p*cox*1; 393 bp) within *O. bifurcum* from the same host species. SSCP analysis of 24 p*cox*1 amplicons representing *O. bifurcum* from humans displayed 10 distinct haplotypes. Amplicons representing these 10 profiles were subjected to sequencing. Pairwise comparison between the *O. bifurcum* samples showed sequence differences ranging from 0·3–8·4%. In spite of the variation in the nucleotide and amino acid sequences within *O. bifurcum*, there was no unequivocal (i.e. fixed) nucleotide difference between *O. bifurcum* individuals from humans and those from Mona monkeys. Compared with the magnitude of sequence differences in the p*cox*1 among representatives of 5 other species of *Oesophagostomum*, the absence of an unequivocal nucleotide difference provided support that *O. bifurcum* from the two host species represented a single species, and that the haplotypic variability detected represented population variation, which was consistent with the previous results for the ITS-2 nuclear rDNA region (Gasser *et al*. 1999). Nonetheless, based on the biological evidence, it was still considered possible that *O. bifurcum* from human and non-human primates were genetically distinct, but that this was not adequately reflected in both the ITS-2 and the p*cox*1.

EVIDENCE OF GENETIC SUBSTRUCTURING WITHIN *O. BIFURCUM* FROM HUMAN AND NON-HUMAN PRIMATES USING DNA FINGERPRINTING APPROACHES

Since investigations using nuclear ribosomal and mitochondrial DNA loci did not detect genetic substructuring within *O. bifurcum* according to primate host species (Gasser *et al*. 1999; de Gruijter *et al*. 2002), further studies (de Gruijter *et al*. 2004, 2005*a*) were undertaken using DNA fingerprinting data sets. In brief, DNA fingerprinting methods rely on the screening of the genome(s) for variation in sequence and/or organization. The data generated for populations can be used to investigate genetic diversity and relationships of organisms without knowledge of the genome sequence. The random amplification of polymorphic DNA (RAPD) or arbitrarily primed-polymerase chain reaction (AP-PCR) (Welsh and McClelland, 1990; Williams *et al*. 1990) is such a technique which relies on the amplification of genomic DNA fragments using (usually) single primers (~10-mers) of arbitrary sequence, and subsequent electrophoretic separation of the amplicons.

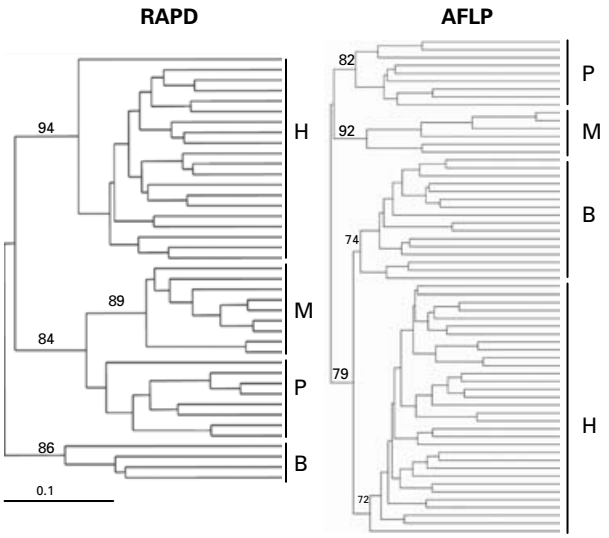

Fig. 2. Simplified dendrograms based on separate cluster analyses of random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) data sets, displaying the genetic variation among adult *Oesophagostomum bifurcum* individuals from humans (H), Mona monkey (M), Patas monkey (P) and Olive baboon (B) (de Gruijter *et al*. 2004, 2005*a*). Totals of 41 and 63 adult worms of *O. bifurcum* were used in the RAPD and AFLP analyses, respectively. The numbers on branches represent bootstrap (RAPD) or co-phenetic (AFLP) values.

'High stringency' RAPD analyses using selected primers (designated OPA-10, OPB-1, OPB-6 and OPB-8, Operon) revealed a high degree of polymorphism (320 polymorphic bands) among individuals of *O. bifurcum* ($n=41$) from different species of primate from different regions in Ghana (de Gruijter *et al*. 2004). Cluster analysis of the profile data (including a total of 326 RAPD bands) showed that *O. bifurcum* represented three distinct groups, namely those from humans, those from the Patas or the Mona monkey, and those from the Olive baboon (Fig. 2). This result demonstrated clearly the existence of population genetic substructuring within *O. bifurcum* according to host species. The fact that *O. bifurcum* from humans and from the Patas monkey (from the Bolgatanga-Bawku region) grouped into different clusters (i.e. cluster I and cluster II, respectively) showed that there was no association between *O. bifurcum* genotype and the geographical origin of the host species based on the RAPD data set. This was also indicated for *O. bifurcum* from the Patas monkey and from the Olive baboon (from the Tamale region) which were divided into distinct clusters.

Extending from the RAPD analyses (de Gruijter *et al*. 2004), the fingerprinting method of amplified fragment length polymorphism (AFLP) (Vos *et al*. 1995) was employed as a comparative tool (*cf*. de Gruijter *et al*. 2005*a*). In brief, the AFLP method is a 'high stringency' DNA fingerprinting method which

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relies on the selective amplification of restriction fragments produced from genomic DNA and their subsequent display by denaturing gel electrophoretic analysis (Vos *et al.* 1995). de Gruijter *et al.* 2005*a*) subjected 63 *O. bifurcum* adults from human, Patas monkey, Mona monkey and Olive baboon hosts from Ghana to AFLP analysis. The cluster analysis of the data set revealed 4 genetically distinct groups, namely *O. bifurcum* from the Patas monkey, from the Mona monkey, from humans and from the Olive baboon (Fig. 2). These findings were in accordance with those achieved using the RAPD analysis (Fig. 2). Hence, in contrast to the studies conducted employing nuclear and mitochondrial loci (Gasser *et al.* 1999; de Gruijter *et al.* 2002), the DNA fingerprinting data revealed genetically distinct, host species-affiliated variants of *O. bifurcum* in Ghana.

CONCLUSIONS

Interestingly, the infection of humans with *O. bifurcum* appears to be restricted to the very north of Togo and Ghana, where at least 0·25 million people are infected (Polderman *et al.* 1991, 1999). The non-human primates in this geographical area are also infected, but their numbers have decreased substantially in the last decades (Polderman, unpublished data). In other locations, further to the south of Ghana (e.g. Mole National Park and Baobeng-Fiema Monkey Sanctuary), non-human primates remain numerous and in close contact with human settlements. There, the prevalence of infection in various non-human primates is high and, surprisingly, there is no evidence of infection in humans. The recent evidence of genetically distinct, host-affiliated groups within *O. bifurcum* (see de Gruijter *et al.* 2004, 2005*a*) and findings and interpretations from epidemiological and experimental studies (Polderman *et al.* 1999; Eberhard *et al.* 2001; van Lieshout *et al.* 2005) provide support for the proposal that cross-infection between non-human primates and humans in Togo and Ghana is limited or absent. Nonetheless, further work is required to provide proof.

Recent work has demonstrated the usefulness of molecular tools for the specific diagnosis and for studying the genetic make-up of *O. bifurcum* populations, which has given new insights into the epidemiology of this parasite(s) from human and non-human primates. Since PCR has proven to be a valuable diagnostic tool for *O. bifurcum* and hookworm infections (Romstad *et al.* 1997*a*, *b*; Verweij *et al.* 2000, 2001), future studies should focus on developing an advanced, multiplex real-time PCR for assessing the intensity of these infections. Such a quantitative PCR should have important implications, for example, for monitoring the effectiveness of mass treatment of humans with albendazole (*cf.* Krepel *et al.* 1993; Ziem *et al.* 2004). Also, the AFLP fingerprinting method (not previously applied to parasitic nematodes of human health importance) has proven to be a powerful tool for establishing genetic variation within *O. bifurcum*. This high-stringency and high-resolution technique should be useful for addressing key questions regarding the population genetics and systematics of other parasites of primates. For instance, the present AFLP might be a useful method to investigate the population genetics of *Ternidens deminutus* from humans and non-human primates (*cf.* Goldsmid, 1991). A recent study using the ITS-2 of rDNA of *T. deminutus* from Mona monkey and Olive baboon from Ghana (Schindler *et al.* 2005) has revealed at least 2 genetic variants within *T. deminutus*. It would be interesting to investigate the genetic variability within this bursate nematode by AFLP analysis of a large number of adult specimens from different primate host species and from different geographical locations in Africa. Finally, the AFLP approach may have implications for studying the geographical spread of parasites and/or for investigating possible sources and routes of infection.

In conclusion, recent studies have filled some gaps in the knowledge of *O. bifurcum*. In particular, they have elucidated some aspects of the epidemiology and population genetics of the parasite. Epidemiological and molecular investigations have provided evidence to suggest that *O. bifurcum* from humans and some species of non-human primates have distinct transmission patterns and that non-human primates are not a source of human oesophagostomiasis in Ghana, having important implications for the control of oesophagostomiasis in this country.

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