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Morphological variability within *Oesophagostomum bifurcum* among different primate species from Ghana

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Abstract

Adult *Oesophagostomum bifurcum* (Nematoda: Strongylida) from human and non-human primates from Ghana were compared in order to investigate the extent of morphological variability within the species. Using analysis of variance and principal component analysis, significant differences in morphological characters (such as parasite length, width, length of the oesophagus and length of spicules) were demonstrated between *O. bifurcum* worms from humans, the Mona, Patas or Green monkey and/or Olive baboons. These findings suggest that *O. bifurcum* from different species of primate host represent distinct population variants, also supported by recent epidemiological and genetic studies of *O. bifurcum* from such hosts.

Introduction

The nodule worm *Oesophagostomum bifurcum* (Nematoda: Strongylida) infects both human and non-human primates and can cause significant disease as a consequence of encysted larvae in the wall of the large intestine (Stewart & Gasbarre, 1989; Storey *et al.*, 2000). Although infection in humans with this geo-helminth was originally thought to be rare, it is of major health importance in northern Togo and Ghana (Polderman *et al.*, 1991, 1999). In the last two decades, human infection with *O. bifurcum* has been studied extensively in these countries (Krepel *et al.*, 1992; Blotkamp *et al.*, 1993; Pit *et al.*, 1999; Polderman *et al.*, 1999; Storey *et al.*, 2000; Verweij *et al.* 2001; de Gruijter *et al.*, 2004, 2005) but there are still serious gaps in our knowledge of various fundamental aspects, including host specificity and parasite transmission.

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While it has been suggested that non-human primates may serve as a reservoir for human infection with *O. bifurcum* (Stewart & Gasbarre, 1989), there is a significant difference in the distribution and prevalence of infection between human and non-human primates in Ghana. For instance, in Mole National Park and Baobeng-Fiema, Central Ghana, there are villages where human and non-human primates live in close association and share the same habitat (van Lieshout *et al.*, 2005). While 70–99% of these non-human primates harbour *O. bifurcum*, no human infections with *O. bifurcum* have been detected in these areas. This observation has led to the hypothesis that *O. bifurcum* from human and non-human primates in Ghana comprises population variants or cryptic species.

Previously, in a preliminary morphological study of *O. bifurcum*, variation in parasite length between adult worms from humans and a Patas monkey was recorded (Blotkamp *et al.*, 1993). However, the number of adult *O. bifurcum* from non-human primates used in that study was limited and included only worms from one species of non-human primate, the Patas monkey. Moreover, no statistical analysis of morphometric data had been

performed to test the significance of the observed variation in parasite length. Since then, no detailed morphological study has been conducted to further investigate the existence of variation in parasite length and/or to define morphological characters which delineate adult *O. bifurcum* from human and non-human primates. Thus, in the present study, we compared morphologically a number of adult *O. bifurcum* from human and non-human primates from Ghana to investigate the extent of morphological variability within the species.

Materials and methods

Adult worms of *O. bifurcum* ($n = 122$) were obtained from humans ($n = 6$) and Patas monkeys (*Erythrocebus patas*) ($n = 2$) from the Garu area (10°85′ N; 0°18′ W), from a Green monkey (*Cercopithecus sabeus*) ($n = 1$) and Olive baboons (*Papio anubis*) ($n = 3$) from Mole National Park (9°35′ N; 2°26′ W) and from a Mona monkey (*Cercopithecus mona*) ($n = 1$) from Boabeng-Fiema (7°43′ N; 1°42′ W) (table 1). Worms from humans, the Green monkey and one of the Patas monkeys were obtained from the faeces of the infected hosts after treatment with pyrantel pamoate, as described previously (Polderman *et al.*, 1991), whereas worms from Olive baboons, the Mona monkey and the other Patas monkey were removed from the large intestine at necropsy. Informed consent for participation was obtained from all adult human participants and from parents of children of less than 15 years of age. Worms were washed extensively in physiological saline and stored in 70% ethanol until required for microscopy. Each specimen of *O. bifurcum* was identified morphologically using published keys and descriptions (Chabaud & Larivière, 1958; Popova, 1958; Blotkamp *et al.*, 1993).

Table 1. Numbers of male (N_M) and female (N_F) adult *Oesophagostomum bifurcum* used in this study per host group (A–C) and individual human (H1–H6), Mona monkey (M1), Patas monkey (P1 and P2), Green monkey (G1) and Olive baboon (B1–B3) hosts.

Group	Host (individuals)	N_M	N_F
A	Human		
	H1	4	3
	H2	6	4
	H3	3	2
	H4	4	3
	H5	5	3
	H6	5	4
B	Mona monkey		
	M1	4	10
	Patas monkey		
	P1	3	2
	P2	9	7
	Green monkey		
	G1	–	4
C	Olive baboon		
	B1	9	8
	B2	4	6
	B3	5	5

The morphological comparison of adult *O. bifurcum* worms from humans with those from species of non-human primates was based on measuring the overall length, maximum width, length of the oesophagus and the distance between the ventral groove and the end of the anterior body (vg-ae). In addition, the distance between the vulva and the tip of the tail (vtt), and the anus and the tip of the tail (att) were assessed for all female worms, and the length of the spicules was determined for males. Worms were divided into groups A–C according to host species. As Mona, Patas and Green monkey all belong to the same tribe (Cercopithecini) within the subfamily Cercopithecinae of the family Cercopithecidae, *O. bifurcum* from these species of primate hosts were grouped together. Group A included all *O. bifurcum* from humans, group B all *O. bifurcum* from Mona, Patas or Green monkey, and group C comprised *O. bifurcum* from Olive baboon (table 1).

To assess the presence of statistically significant differences in morphological characters between host groups an analysis of variance (ANOVA) was performed. A two-way ANOVA factored by host species and sex was utilized to analyse those variables that were measured in both sexes, i.e. overall length, maximum width, length of oesophagus, and vg-ae, while a one-way ANOVA factored by host species was used to analyse those variables that were sex-specific (i.e. vtt, att and length of spicules). For all statistical analyses the significance level was set at $\alpha = 0.05$, and a Bonferroni correction for multiple comparisons was performed. Furthermore, a principal component analysis (PCA) with a varimax rotation was applied to reduce the dimensionality of data by examining the level of correlation between the morphological variables. All statistical analyses were performed using the software program SPSS 12.0 (SPSS Inc., Chicago, Illinois).

Results

Morphometric data for male and female worms are summarized in table 2a and 2b, respectively. Two-way ANOVA showed that there were significant differences in parasite length ($P = 2.3 \times 10^{-17}$), width ($P = 6.0 \times 10^{-19}$) and length of the oesophagus ($P = 2.7 \times 10^{-7}$) between male and female worms. Between host groups (A–C) highly significant differences were found in the total parasite length between groups A and B ($P = 6.3 \times 10^{-10}$), A and C ($P = 1.2 \times 10^{-5}$), and B and C ($P = 2.7 \times 10^{-16}$). Also, significant differences were found in the maximum width of worms between groups A and B ($P = 8.3 \times 10^{-29}$), and B and C ($P = 1.8 \times 10^{-15}$), and in the length of the oesophagus between groups A and C ($P = 5.6 \times 10^{-18}$), and groups B and C ($P = 2.6 \times 10^{-19}$). The interaction 'sex–host group' was not significant for any morphological characters analysed, and these data are not presented. One-way ANOVA analyses of sex-specific morphological characters revealed that the length of spicules of *O. bifurcum* from group A was significantly different from that of groups B ($P = 2.4 \times 10^{-5}$) and C ($P = 1.3 \times 10^{-4}$). No significant difference in morphological parameters was detected among individual specimens from Mona, Patas and Green monkey

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Table 2. Means ± standard deviations of measurements (in mm) of male (a) and female (b) adult *Oesophagostomum bifurcum* per host group (A–C) or per monkey species (Mona, Patas and Green monkey).

(a)

Group	Host	Length	Width	Oesophagus	Vg-ae	Length spicules
A	Human	11.6 ± 1.05^{B,C}	0.42 ± 0.03^{B}	0.62 ± 0.03^{C}	0.23 ± 0.02	1.12 ± 0.07^{B,C}
B	Monkey	12.5 ± 1.0^{C}	0.30 ± 0.02^{C}	0.63 ± 0.02^{C}	0.22 ± 0.02	0.98 ± 0.07
	Mona	13.2 ± 1.69	0.30 ± 0.01	0.62 ± 0.01	0.21 ± 0.01	0.96 ± 0.10
	Patas	12.3 ± 0.59	0.31 ± 0.03	0.63 ± 0.02	0.22 ± 0.02	1.0 ± 0.02
C	Baboon	10.3 ± 0.60	0.39 ± 0.02	0.50 ± 0.03	0.22 ± 0.01	0.99 ± 0.03

(b)

Group	Host	Length	Width	Oesophagus	Vg-ae	Att	Vtt
A	Human	14.6 ± 1.86^{B,C}	0.50 ± 0.03^{B}	0.66 ± 0.04^{C}	0.24 ± 0.03	0.23 ± 0.02	0.47 ± 0.03
B	Monkey	16.4 ± 1.19^{C}	0.37 ± 0.03^{C}	0.65 ± 0.04^{C}	0.22 ± 0.03	0.21 ± 0.03	0.45 ± 0.05
	Mona	16.9 ± 1.29	0.38 ± 0.03	0.64 ± 0.05	0.21 ± 0.03	0.21 ± 0.04	0.45 ± 0.02
	Patas	16.0 ± 0.97	0.36 ± 0.03	0.66 ± 0.01	0.24 ± 0.02	0.20 ± 0.01	0.44 ± 0.07
	Green	16.9 ± 1.29	0.38 ± 0.03	0.64 ± 0.05	0.21 ± 0.03	0.21 ± 0.04	0.45 ± 0.02
C	Baboon	12.0 ± 1.32	0.48 ± 0.03	0.55 ± 0.03	0.22 ± 0.02	0.21 ± 0.03	0.44 ± 0.02

Vg-ae = length between ventral groove and anterior body end; vtt = length between vulva and tip of the tail; att = length between anus and tip of the tail.^{B,C} indicates a significant difference compared to worms of the same sex of group B or C, respectively.

within those of group B in any of the analyses performed. Furthermore, worms from group A appeared to be darker in colour compared with those belonging to groups B or C.

PCA showed that two principal components (PC) accounted for most (74.7%) of the total variance between *O. bifurcum* males from the three different host groups. PC 1 (41.6% of the total variance) was predominantly influenced by the maximum worm width, vg-ae and length of spicules, while PC 2 (33.1% of the total variance) was mainly influenced by the total worm length and length of the oesophagus. For female worms, three principal components accounted for 74% of the total variance between the host groups. PC 1 (30.8% of the total variance) was mainly controlled by the total worm length and length of the oesophagus, PC 2 (24.2% of the total variance) was mainly influenced by the att and vtt, and PC3 (19% of the total variance) was largely a function of the vg-ae. Figure 1 shows a scatterplot of PC 1 versus PC 2 for male *O. bifurcum*, where a clear separation of the three groups of host species can be seen. Figure 2A and 2B show the scatterplot of PC 1 versus PC 2, and PC 1 versus PC 3, respectively, for female *O. bifurcum*. Although less prominent compared to males, fig. 2A shows that females also cluster to host species. In fig. 2B there is more overlap of worms from different host species. However, this is not unexpected as PC 3 explains less of the total variance compared with PC 2.

Discussion

The ANOVAs and scatterplots of the PCA show that there are significant differences in morphology between adult *O. bifurcum* from human (group A), Mona monkey, Patas monkey or Green monkey (Group B), and Olive baboon (group C). Also, in this study a profound difference in colour was detected between worms from humans and non-human primates. Similar variations in parasite length and colour between human and non-human primate hosts have been reported for adult worms of *Ternidens deminutus* (Nematoda: Strongylida) (Goldsmid, 1991), which belongs to the same subfamily (Oesophagostominae) as *O. bifurcum*. Goldsmid & Lyons (1973) reported that adult *T. deminutus* from humans were larger and darker compared with those obtained from baboons. While this size difference may be due to population variation, these authors suggested that 'stunting' of worms from baboons may be due to a higher intensity of infection in this host compared with that in humans. For *O. bifurcum*, higher numbers of third

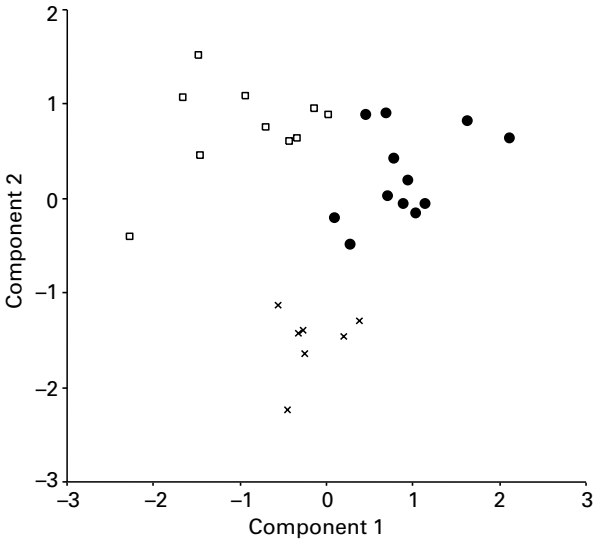

Fig. 1. Scatterplot of principal component (PC) 1 versus 2 for male *Oesophagostomum bifurcum* from human (●, group A), Mona monkey, Patas monkey or Green monkey (□, group B) and Olive baboon (×, group C).

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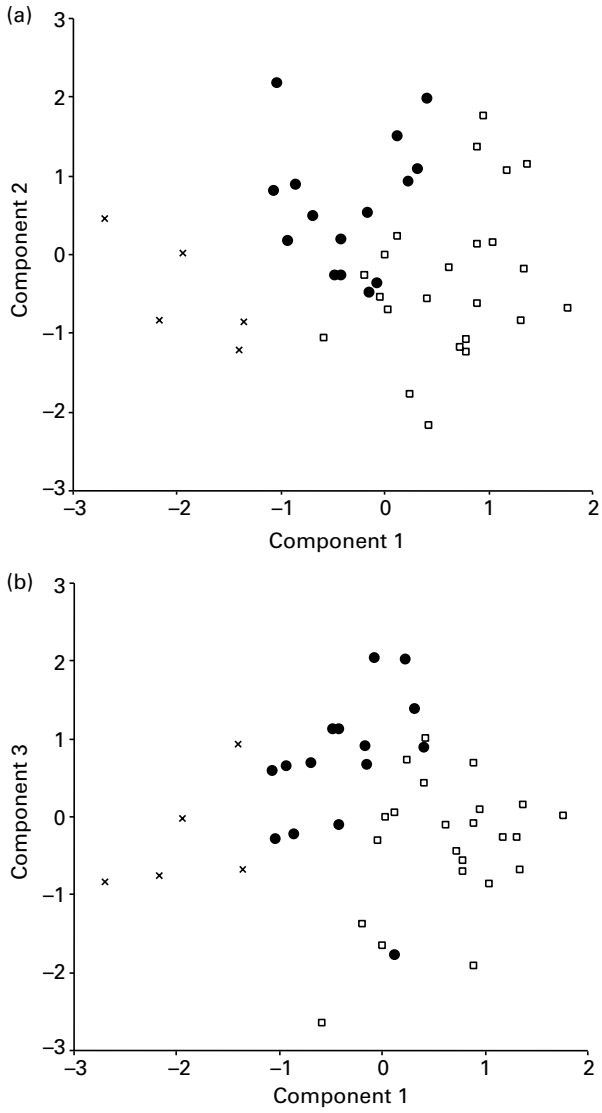

Fig. 2. Scatterplot of principal component (PC) 1 versus 2 (2a) and 1 versus 3 (2b) for female *Oesophagostomum bifurcum* from human (●, group A), Mona monkey, Patas monkey or Green monkey (□, group B) and Olive baboon (×, group C).

stage larvae (L3) have been found in the faeces from Mona monkey and Olive baboon (van Lieshout *et al.*, 2005) than those reported in studies for human faeces (Pit *et al.*, 1999, Yelifari *et al.*, 2005), indicating a higher intensity of infection in these non-human primate species. However, the present results show that, although adult *O. bifurcum* from the Olive baboon are smaller in size compared with those of humans, worms from the Mona monkey are larger (fig. 2a). This suggests that for *O. bifurcum* it is unlikely that size differences are related to stunting.

We suggest that the morphological differences between *O. bifurcum* from human and non-human primates detected in this study relate to population variation within the species. This suggestion is supported by epidemiological data. For instance, while *O. bifurcum* from humans is limited to the north of Togo and Ghana, *O. bifurcum* from non-human primates can also be found outside of this area endemic for human oesophagostomiasis (van Lieshout *et al.*, 2005). Furthermore, molecular studies have indicated the existence of population variation within the species (Gasser *et al.*, 1999; de Gruijter *et al.*, 2002) and showed that *O. bifurcum* from humans, Patas or Mona monkey, and Olive baboon are genetically distinct (de Gruijter *et al.*, 2004, 2005). Given that there are no unequivocal sequence differences in the first and second internal transcribed spacers (ITS-1 and ITS-2) of ribosomal DNA (rDNA) between *O. bifurcum* from human and non-human primate hosts (Gasser *et al.*, 1999; de Gruijter *et al.*, 2002, 2004, 2005), morphological data presented herein together with previously obtained epidemiological and molecular data sets provide strong support for population variation (or substructuring) within *O. bifurcum* according to primate host (group). These data suggest that *O. bifurcum* from humans and some species of non-human primates have distinct transmission patterns and that these non-human primates are therefore not a threat to public health in Ghana. Although further work is needed to provide further proof that cross-infection does not take place, i.e. reciprocal cross-infection studies, this knowledge should, in the long term, contribute to reducing the health impact of *O. bifurcum* in Ghana and is likely to prove useful in monitoring and controlling human oesophagostomiasis in this country.

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