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

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CHAPTER 3

MORPHOLOGICAL VARIABILITY WITHIN *OESOPHAGOSTOMUM BIFURCUM* (NEMATODA) AMONG DIFFERENT PRIMATE SPECIES FROM GHANA

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Abstract

In the present study, we compared morphologically adult *Oesophagostomum bifurcum* (order Strongylida: Oesophagostominae) from human and non-human primates from Ghana in order to investigate the extent of morphological variability within the species. Using analysis of variance (ANOVA) and principal component analysis we demonstrate that there are significant differences in morphological characters between *O. bifurcum* specimens from humans, the Mona, Patas or Green monkey, and/or Olive baboons. These findings suggest that *O. bifurcum* from different species of primate host represents distinct population variants, also supported by a recent epidemiological study of *O. bifurcum* from such hosts.

The nodular 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.[26,39] 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.[25,44] In the last two decades, human infection with *O. bifurcum* has been studied extensively in these countries,[20,22-24,26,46] but there are still serious gaps in our knowledge of various fundamental aspects, including host specificity and transmission of the parasite.

While it has been suggested that non-human primates may serve as a reservoir for human infection with *O. bifurcum*,[39] there is a significant difference in the distribution and prevalence of infection between human and non-human primates in Ghana (chapter 2). 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. While a large percentage (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 may comprise 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.[20] 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 (i.e., Patas monkey). Moreover, no statistical analysis of the 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 *Oesophagostomum bifurcum* (order Strongylida) from human and non-human primates from Ghana to investigate the extent of morphological variability within the species.

Adult specimens of *O. bifurcum* (n = 122) were obtained from humans (n = 6) and Patas monkeys (*Erythrocebus patas*) (n = 2) from the Garu area (10:85N; 0:18 W), from a Green monkey (*Cercopithecus sabeus*) (n = 1) and Olive baboons (*Papio anubis*) (n = 3) from Mole National Park (9:35N; 2:26W) and from a Mona monkey (*Cercopithecus Mona*) (n = 1) from Boabeng-Fiema (7:43N; 1:42W) (Table 1). Worms from humans, 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,[44] whereas worms from Olive baboons, 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. The worms were washed extensively in physiological saline. Worms from humans, Patas monkey, Green monkey and Olive baboon were stored in 70% ethanol whereas worms from Mona monkey were frozen until required for microscopy. Each specimen of *O. bifurcum* was identified morphologically using published keys and descriptions.[12,20,128]

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

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

The morphological comparison of adult *O. bifurcum* specimens from humans with those from species of non-human primates was based on measuring the overall length, the maximum width, the length of the oesophagus and the distance between the ventral groove and the anterior body end (vg-ae) of the worms. 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 the 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 included all *O. bifurcum* from Mona, Patas or Green monkey, and group C comprised all *O. bifurcum* from Olive baboon (Table 1).

To assess the presence of statistically significant differences in morphological characters between host groups analysis of variance (ANOVA) was performed. A two-way ANOVA factored by host-species and sex was utilized to analyze 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 utilized to analyze 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 the 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, Il).

The 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 of the morphological characters analysed, and as a result these data are not presented. One-way ANOVA analyses of the sex-specific morphological characters revealed that the length of the 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 within those of group B in any of the analyses performed. Furthermore it should be noted that worms from group A seemed to be darker in colour compared with those belonging to group B or group C.

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

(2a)

Group	Host	Length males	Width males	Oesophagus males	Vg-ae males	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]
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

(2b)

Group	Host	Length females	Width females	Oesophagus females	Vg-ae females	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

PCA showed that two principal components (PC) accounted for most (74.7%) of the total variance between *O. bifurcum* males from the 3 different groups of host species. PC 1 (41.6% of the total variance) was predominately influenced by the maximum width of the worm, the vg-ae and the length of the spicules, while PC 2 (33.1% of the total variance) was mainly influenced by the total length of the worm and the length of the oesophagus. For female worms, three principal components accounted for most (74%) of the total variance between groups of host species. PC 1 (30.8% of the total variance) was mainly controlled by the total length of the worm and the length of the oesophagus, PC 2 (24.2% of the total variance) was mainly influenced by the att and the 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*. In this scatterplot 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, figure 2A shows that females also cluster to host species. In figure 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. Overall, the ANOVAs and scatterplots of principal component analysis 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).

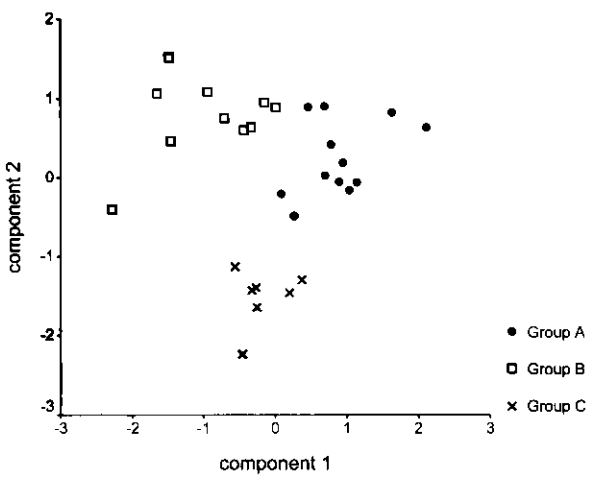

Figure 1 Scatterplot of principal component (PC) 1 versus 2 for male *O. bifurcum* from human (group A), Mona monkey, Patas monkey or Green monkey (group B) and Olive baboon (group C).

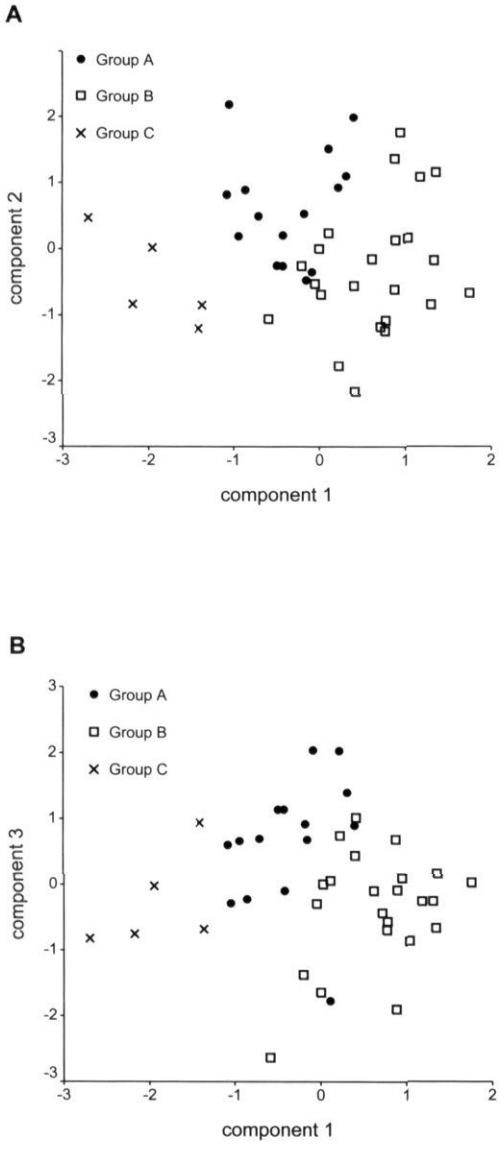

Figure 2 Scatterplot of principal component (PC) 1 versus 2 (**2A**) and 1 versus 3 (**2B**) for female *O. bifurcum* from human (group A), Mona monkey, Patas monkey or Green monkey (group B) and Olive baboon (group C).

Variation in morphology (i.e., parasite length) and colour between human and non-human primate hosts has also been reported for adults of *Ternidens deminutus* (Nematoda: Strongylida),[130] which belongs to the same subfamily (Oesophagostominae) as *O. bifurcum*. Goldsmid and Lyons (1973) described that adult *T. deminutus* from humans were larger and darker compared with those obtained from baboons. While the size difference may have been due to population variation, the authors suggested that it may also have been due to 'stunting' of the worms from baboons caused by a higher intensity of infection in this host compared with that of humans. It is unclear whether there are differences in the intensity of infection with *O. bifurcum* between different host species. However, similar number of adult worms (n = ~20) were obtained from the different hosts included in this study, which may indicate that there is not a profound difference in the intensity of the infection. Therefore 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 (see chapter 2). 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 this endemic area for human oesophagostomiasis. To obtain additional support for the existence of population variation within *O. bifurcum* genetic studies could be conducted. For instance, the cytochrome *c* oxidase subunit I (*cox*1) gene of mitochondrial DNA (mtDNA) has been shown to provide valuable genetic markers to study population variation within a range of parasitic nematodes, and therefore might also be useful to investigate population substructuring within *O. bifurcum*.[62,64,65,131]