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ORIGINAL PAPER

Genetic identification of an oxyurid from a captive, black-handed spider monkey—implications for treatment and control

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Abstract Parasites are of major clinical significance in captive primates in zoos, particularly those with direct life cycles. Oxyurid nematodes can be a persistent problem, as infection intensity and environmental contamination with infective eggs are usually high. Observations at the Basel Zoo in Switzerland have revealed that particularly black-handed spider monkeys (*Ateles geoffroyi*) exhibit continuous oxyurid nematode infection(s), despite regular deworming with anthelmintics. In the present study, using a molecular approach, we were able to identify the nematode (*Trypanoxyuris atelis*) causing this ongoing problem, and we are now evaluating a practical treatment and control regimen to tackle this parasite problem.

Keywords Molecular tools · *Trypanoxyuris* · Black-handed spider monkey · Identification · Parasite control

Introduction

Parasites are of major animal health importance in experimental animal colonies and in zoological collections, particularly in primates. Parasites with direct life cycles and resilient infective stages can persist and accumulate over long periods of time, leading to sporadic or persistent clinical issues. Although not widely reported in the published literature, oxyurid nematodes of captive nonhuman primates are recognized as a continual problem when infection intensity and environmental contamination with infective eggs are continuously high. Although clinical signs are often mild or absent, anal pruritus, particularly in young primates, can be an ongoing issue and can lead to behavioural changes, such as irritability or aggression. Unpublished observations at Basel Zoo (www.zoobasel.ch) in Switzerland have shown that nonhuman primates, predominantly the black-handed spider monkey (*Ateles geoffroyi*), continuously excrete large numbers of oxyurid eggs in their faeces and exhibit intermittent diarrhoea for many years, despite regular deworming with anthelmintics. In the present study, the focus was on the specific identification of the likely agent and on establishing an effective treatment and control regimen to tackle this ongoing problem.

Materials and methods

A black-handed spider monkey (6-year-old male) from Basel Zoo, Switzerland, with an oxyurid infection, was euthanized due to a chronic and major orthopaedic disease. During the necropsy, one of us (SB) collected from the large intestine of this monkey 14 female oxyurids whose eggs were consistent in size and shape with those detected in faecal samples. We were also able to collect worms released in the faeces of two adult individuals of the same species of primate in the same captive troop. All specimens collected were preserved in ethanol (70 %) and initially examined morphologically using established criteria (Hasegawa et al. 2004; Hasegawa 2009). Although no male worms were detected, the morphological characteristics of the female worms (including cephalic features, lateral alae, position of vulva and direction of vagina and egg size and shape) were consistent with those of *Trypanoxyuris* species (Hasegawa et al. 2004; Hasegawa

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2009). In order to achieve an identification of the worms, we isolated genomic DNA from the mid-body section of individual females (n=4) using a sodium dodecyl-sulfate/proteinase K treatment and purification over a mini-column (Wizard DNA Clean-Up System, Promega, USA), amplified a region of the cytochrome *c* oxidase subunit I (*cox*1) gene using oligonucleotide primers JB3 (forward: 5′-TTTTTTGGGCATCCTGAG GTTTAT-3′; Bowles et al. 1992) and pr-b (reverse: 5′-AGAA AGAACCTAATGAAAATGAGCCA-3′; Nakano et al. 2006) and sequenced the amplicons. This *cox*1 region has already been demonstrated to be useful for the specific identification and classification of pinworms (Nakano et al. 2006). To do this, PCRs were carried out in a 50 μl volume containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl (Promega, USA), 3.0 mM of $MgCl_2$, 200 μM of each dNTP, 100 pmol of each primer and 1 U of *Go*Taq DNA polymerase (Promega, USA). The *cox*1 region was amplified using the following cycling protocol: 94 °C for 5 min (initial denaturation), followed by 35 cycles of 94 °C for 30 s (denaturation), 52 °C for 30 s (annealing) and 72 °C for 30 s (extension), with a final extension of 72 °C for 5 min. Following PCR, individual amplicons were treated with ExoSAP-IT (Affymetrix, USA), according to the manufacturer's instructions, and then subjected to direct, automated sequencing (BigDye Terminator v.3.1 chemistry, Applied Biosystems, USA) using the same primers employed in PCR. Sequence quality was verified by comparison with corresponding electropherograms using Geneious v.6.1.2 software (Biomatters, New Zealand). Subsequently, we aligned the sequence with publicly available sequences representing other primate oxyurids (Fig. 1) using the program MUSCLE (Edgar 2004); we then adjusted the alignment manually using the program Mesquite v.2.75 (Maddison and Maddison 2011) and subjected the sequence data to phylogenetic analysis by Bayesian inference (BI) employing Monte Carlo Markov Chain analysis in the program MrBayes v.3.2.2 (Huelsenbeck and Ronquist 2001). The likelihood parameters set for the BI analysis of sequence data were based on the Akaike Information Criteria test in jModeltest v.2.1.5 (Darriba et al. 2012); the number of substitutions was set at 6, with a gamma distribution and proportion on invariant sites. Posterior probability (pp) values were calculated by running 5,000,000 generations with four simultaneous tree-building chains. Trees were saved every 100th generation. At the end of each run, the SD of split frequencies was <0.01, and the potential scale reduction factor approached one. A 50 % majority rule consensus tree for each analysis was constructed based on the final 75 % of trees generated by BI. We conducted three independent analyses to ensure convergence and insensitivity to priors. *Syphacia obvelata* was selected as an out-group based on a tree constructed by Hasegawa et al. (2012).

Results and discussion

The four *cox*1 sequences derived from oxyurid nematodes from multiple black-handed spider monkeys of the same captive troop were identical. Upon pairwise comparison, there was 95.4–95.7 % similarity over the consensus alignment length (328 nt) with the three reference sequences representing *Trypanoxyuris atelis* (GenBank accession nos.

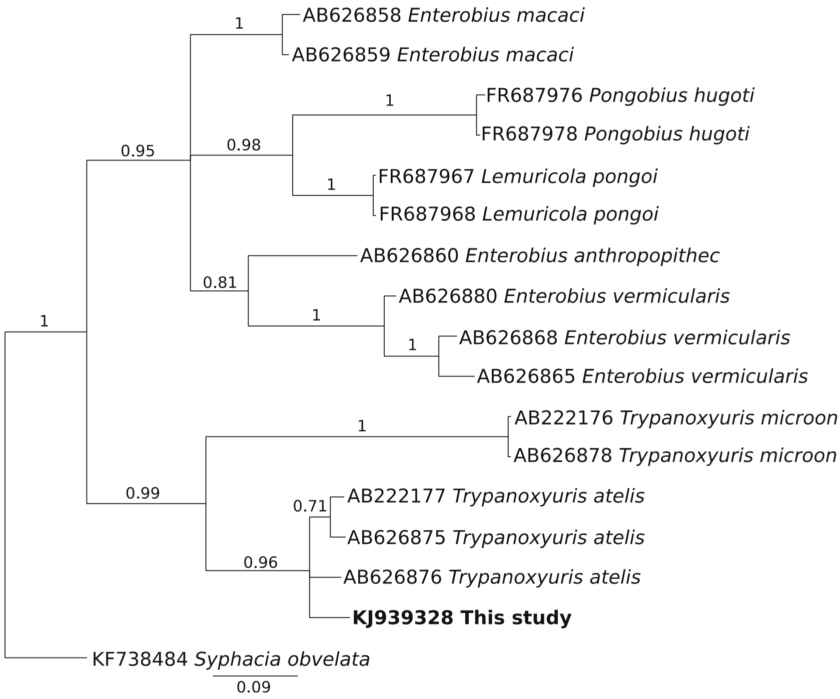

Fig. 1 Phylogenetic relationship of an oxyurid nematode (*Trypanoxyuris atelis*) collected at necropsy from a black-handed spider monkey (*Ateles geoffroyi*) at Basel Zoo, Switzerland. An analysis of the 328 bp partial sequence of *cox*1 in the present study (*bold-type*) was performed by Bayesian inference; the tree is rooted to *Syphacia obvelata*. Posterior probabilities are indicated adjacent to nodes. Accession number KJ939328 represents four identical sequences of *T. atelis* specimens from multiple monkeys in the same captive troop

AB222177, AB626875 and AB626876; Hasegawa et al. 2012) and 86.7 % similarity to the related species, *Trypanoxyuris micron* (accession nos. AB222176 and AB626878; Hasegawa et al. 2012). Additionally, phylogenetic analysis of the data by BI showed that the four identical sequences determined here grouped, with strong support (pp=0.96) with the three reference sequences from *T. atelis* (Fig. 1), suggesting that the oxyurids studied herein are *T. atelis*. Furthermore, the sequence variation (4.6 %) in *cox*1 within *T. atelis* is similar to that recorded (6.4 %) among individuals of a related oxyurid, *Enterobius vermicularis*, for this region of *cox*1 (cf. Fig. 1).

Although oxyurids may not cause clinical signs as a consequence of the worms themselves, high intensity infections can cause diarrhoea and also indirect problems associated with pruritus ani, and might lead to behavioral issues in captive primates, such that it becomes important to control the worm problem. The identification of the causative agent can assist in selecting a treatment option. Here, it was important to identify the parasite, gain insight into its biology and to explore treatment and control options. The molecular findings indicated that the parasite was *T. atelis*. Like other nematode congeners, this parasite likely has a direct life cycle: the ovigerous females migrate to the perianal region, where eggs are deposited; following a rapid phase of embryonation (days), eggs are infective. The primary route of transmission is via the ingestion of eggs by the same (autoinfection) or another host individual (heteroinfection); however, it is also possible that larvae can hatch from eggs in the anus of the infected animal and then migrate back into the intestine (retroinfection) (Felt and White 2005). According to Pinto et al. (2013), 18 species of *Trypanoxyuris* are recognized and have been ascribed to four subgenera: (i) *Trypanoxyuris* (*Trypanoxyuris*) Vevers 1923, with eight species recorded mainly in the subfamily Cebinae, and some in the Aotidae, Atelidae, and Pitheciidae; (ii) *Trypanoxyuris* (*Hapaloxyuris*) Inglis and Cosgrove 1965, represented by four species reported from the Callitrichinae; (iii) *Trypanoxyuris* (*Paraoxyuronema*) Artigas 1936 with four species from the Atelidae; and (iv) *Trypanoxyuris* (*Rodentoxyuris*) Quentin and Tenora 1974 represented by two species found in rodents (family Sciuridae) (Hugot et al. 1996; Hugot 1999).

Although there is almost no detailed information on effective control of oxyurids in captive primates, there is one study (Bentzel and Bacon 2007) that compares various anthelmintic treatments of *Trypanoxyuris micron* infection in the owl monkey (*Aotus nancymae*). In this study, the authors were focused on establishing an effective anthelmintic therapy against *T. microon* infection in *A. nancymae*. Monkeys shown to be infected by perianal sticky tape testing were treated twice 14 days apart with pyrantel pamoate, ivermectin or thiabendazole; an untreated group was included. Using the same test, monkeys were examined for eggs for a period of 28 days. If no eggs were detected at five consecutive negative test, treatment was regarded as successful. Pyrantel pamoate and ivermectin were each significantly more effective at achieving “egg clearance” than thiabendazole and no treatment. Overall, all monkey groups treated with pyrantel pamoate and ivermectin cleared eggs, while 60 % of the thiabendazole-treatment group became test-negative for eggs. Moreover, the time after treatment until clearance was 1 to 2 days for pyrantel pamoate, 2 to 4 days for thiabendazole and 4-6.5 days for ivermectin. These findings suggested that pyrantel pamoate was the most effective and rapidly acting drug against adult worms of *T. microon*. However, because of likely reinfections, it was recommended that affected monkey colonies should be treated every 1 to 2 weeks, in combination with environmental sanitation. Although the pharmacokinetics of these drugs are not yet known in non-human primates and might differ between spider monkeys and owl monkeys, the efficacy of pyrantel pamoate (an insoluble form) against pinworms in humans and other animals (Pitts and Migliardi 1974) suggests that this treatment-sanitation approach could have been an option to reduce the infection intensity of the nematode congener, *T. atelis*, in the spider monkey colony in the Basel Zoo. However, repeated administrations of this and, subsequently, benzimidazole compounds did not eliminate the problem. One reason might be, from the practical perspective, an inability to treat newborn monkeys. Thus, we selected an alternative macrocyclic lactone (moxidectin), which is commercially available in formulation that is palatable to primates (paste with apple-flavour) and known to be transmitted lactogenically. In this way, we were not only able to target adult and juvenile individuals in captive troops, but also offspring via milk from the dam. A long-term study is now underway to assess a pinworm control strategy similar to that described by Bentzel and Bacon (2007), also including an environmental sanitation approach.

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