Transactions of the Royal Society of Tropical Medicine and Hygiene 106 (2012) 75–83

Contents lists available at SciVerse ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Genetic diversity of *Ascaris* in southwestern Uganda

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ARTICLE INFO

Article history:
Received 16 June 2011
Received in revised form 13 October 2011
Accepted 13 October 2011

Keywords:
Ascaris
Evolution
Cytochrome c oxidase subunit 1
Microsatellite
Population genetics
Uganda

ABSTRACT

Despite the common occurrence of ascariasis in southwestern Uganda, helminth control in the region has been limited. To gain further insights into the genetic diversity of *Ascaris* in this area, a parasitological survey in mothers (n = 41) and children (n = 74) living in two villages, Habutobere and Musezero, was carried out. Adult *Ascaris* worms were collected from infected individuals by chemo-expulsion using pyrantel pamoate treatment. Genetic diversity within these worms was assessed by inspection of DNA sequence variation in a mitochondrial marker and length polymorphism at microsatellite loci. Overall prevalence of ascariasis was 42.5% in mothers and 30.4% in their children and a total of 98 worms was examined from 18 hosts. Sequence analysis of a portion of the mitochondrial cytochrome c oxidase subunit 1 gene revealed 19 different haplotypes, 13 of which had not been previously encountered. Microsatellite analysis using eight loci provided evidence for high gene flow between worm populations from the two villages but comparing these worms with others obtained in a prior study on Unguja, Zanzibar, confirmed little genetic exchange and mixing of worm populations between the two areas. By adding to our understanding of the genetic diversity of *Ascaris* in Africa, this study provides useful information for monitoring changes in parasite population structure in the face of ongoing and future control.

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1. Introduction

It is estimated that around 1.2 billion people worldwide are infected with the giant roundworm *Ascaris lumbricoides*, a soil-transmitted helminth (STH).[1] Ascariasis is classified as a neglected tropical disease (NTD) by the WHO,[2] and is one of the targets of large-scale 'deworming' programmes based on regular distribution of anthelminthic drugs such as albendazole and/or mebendazole to school-aged children.[3] The main aim of these programmes is morbidity control associated with helminth infections rather than elimination of the infections themselves.

Across Uganda there is an extremely heterogeneous distribution of ascariasis with the majority of infections being found in the southwestern region bordering the Democratic Republic of Congo and Rwanda.[4,5] This is a highland area characterised by a predominantly rural population and a relatively cooler environment with higher annual rainfall.[6] Although Uganda was one of the first countries to launch a national school-based schistosomiasis

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doi:10.1016/j.trstmh.2011.10.011

and intestinal helminth control programme, this was initially focused in districts where school-aged children were considered at highest risk of intestinal schistosomiasis infection. Since schistosomiasis is largely absent in southwestern Uganda, this region was somewhat overlooked and did not feature strongly in the national helminth control strategy.[7,8] Although anthelminthic treatments (albendazole) were later incorporated into the package of health services through introduction of biannual community-based Child Health Days, established by the Ministry of Health, there is little formal recording of the anthelminthic coverage of this programme in southwestern Uganda.[9,10] Nevertheless analysis of recent disease surveillance data suggests that ascariasis remains a significant public health problem in southwestern Uganda.[11]

To enhance our understanding of ascariasis in Uganda, a detailed knowledge of the evolutionary origins and population genetics of worms is needed which can provide insights into local transmission dynamics; this is particularly useful in the context of disease control and especially relevant in an era where there are concerns that drug tolerance or resistance could arise due to the limited number of anthelminthics available for chemotherapy. Moreover, as ascariasis in domestic pigs is of public health concern, owing to zoonotic transmission potential, greater scrutiny of parasite populations is insightful.[12,13] A variety of molecular techniques have been applied to investigate the genetic diversity of *Ascaris* including DNA sequencing of mitochondrial and nuclear genes along with analysis of RFLPs and variation within microsatellites.[13–18]

In a previous study we investigated the genetic diversity of *Ascaris* worms from four villages in Zanzibar, where long-standing chemotherapy-based control has been ongoing, by sequencing a portion of the mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene and microsatellite fragment sizing analysis.[19,20] A large number of *cox*1 haplotypes in worms from Zanzibar was found which, in comparison with a limited number of samples from southwestern Uganda, was rather surprising as long-term drug selection could be assumed to reduce genetic diversity. Microsatellite analysis revealed some genetic differentiation between populations from Uganda and Zanzibar but little population structuring between villages on Zanzibar.

In order to gain further insights into the population genetics of *Ascaris* in southwestern Uganda, where there has been little anthelminthic chemotherapy to date,[21] a cross-sectional survey including collection of adult *Ascaris* was carried out in two Ugandan villages, one in Kabale District and one in Kisoro District. The results of *cox*1 haplotype analysis and microsatellite profiling of the *Ascaris* samples are presented here.

2. Materials and methods

2.1. Study sites and participants

The study was carried out in June 2010 in the villages of Habutobere, Kabale District (lat 1°13'S, long 29°50'E) and Musezero, Kisoro District (lat 1°13'S, long 29°43'E), both located in the southwestern region of Uganda. The villages are separated by a large hill and the journey between them on a newly created road is around 45 km. Before the survey, the community in each village was sensitised by the local Vector Control Division Officer, community leaders and the survey team as to the nature and purpose of the study. Mothers with children aged between 3 and 12 years old were then invited to participate. In Habutobere, 22 mothers and 38 children were recruited into the study (1 or 2 children per mother), and in Musezero 19 mothers and 36 children took part (1 or 2 children per mother).

2.2. Epidemiological background of study participants

The mean age of the children was 6.5 years (range 3–12 years) and of the mothers was 30.8 years (range 17–47 years). In the children the female to male ratio was 1:1.55. The prevalence levels of STH infections, malaria and anaemia categorised by village and age group are summarised in Table 1. No hookworm infections were identified. Interestingly, two mothers from Musezero were infected with *Schistosoma mansoni* based on the presence of eggs in stool.

2.3. Detection of parasites and morbidity markers

Each mother and child provided a single stool and urine sample upon registration. Parasitological diagnosis of *A. lumbricoides*, *Trichuris trichiura*, hookworms and *S. mansoni* was carried out in the field by microscopy on a double thick Kato–Katz smear (41.7 mg).[22] Initially, slides were examined for the presence or absence of eggs to enable rapid treatment decisions to be made on site. Subsequently, more detailed egg counts were carried out on the thick smears from each sample and results were expressed as eggs per gram (epg) of faeces.

Fingerprick blood was taken from each mother and child and haemoglobin (Hb) levels were tested using a HemoCue spectrophotometer (HemoCue AB, Angelholm, Sweden). In addition, all study participants were tested for malaria using the OptiMAL-IT LDH rapid diagnostic test (Diamed GmBH, Cressier, Switzerland) according to the manufacturer's instructions.

2.4. Treatment and Ascaris worm collection

Mothers and children who had an egg-patent *A. lumbricoides* infection, or who had a family member with an *A. lumbricoides* infection, received a single treatment of pyrantel pamoate (Combatrin®, Pfizer, UK) at 10 mg/kg for chemo-expulsion. Each participant was provided with a plastic bag for collection of total stool and any worms which were expelled over the subsequent 24 h. The following day, the expelled worms were collected, washed thoroughly in water to remove faecal material and placed in 96% ethanol for long-term storage. Worms from different hosts were stored separately. In total 99 worms were collected.

All study participants were also offered treatment with albendazole and the two mothers who were positive for *S. mansoni* infection were offered praziquantel (40 mg/kg). Three of the individuals who were

Table 1
Prevalence levels of soil-transmitted helminth infections, malaria and anaemia in study participants

Infection/morbidity	Intensity	Habutobere		Musezero	
		Children (n = 38) % (95% CI)[a]	Mothers (n = 22) % (95% CI)[a]	Children (n = 36) % (95% CI)[a]	Mothers (n = 19) % (95% CI)[a]
Ascaris lumbricoides	Any	33.3 (18.6–51.0)	47.6 (25.7–70.2)	27.3 (13.3–45.5)	36.8 (16.3–61.6)
	Light[b]	16.7 (6.4–32.8)	28.6 (11.3–52.2)	9.1 (1.9–24.3)	10.5 (1.3–33.1)
	Medium/heavy[b]	16.7 (6.4–32.8)	19.1 (5.4–41.9)	18.2 (7.0–35.4)	26.3 (9.1–51.2)
Trichuris trichiura	Any	52.8 (35.5–69.6)	33.3 (14.6–57.0)	54.6 (36.3–71.9)	47.4 (24.4–71.1)
	Light[b]	50.0 (32.9–67.0)	28.9 (11.3–52.2)	45.5 (28.1–63.6)	42.1 (20.3–66.5)
	Medium/heavy[b]	2.8 (0.07–14.5)	4.8 (0.1–23.8)	9.1 (1.9–24.3)	5.3 (0.1–26.0)
Malaria	Any	16.7 (6.4–32.8)	9.5 (1.2–30.4)	3.1 (0.08–16.2)	5.6 (1.4–27.3)
Anaemia	<110 mg/l	2.7 (0.07–14.1)	4.6 (0.1–22.8)	20.6 (8.7–37.9)	0

[a] 95% confidence intervals determined using the exact method.
[b] Intestinal helminth infections were classified according to intensity based on WHO recommendations.[23]

infected with *T. trichiura* were treated with ivermectin (0.2 mg/kg). In addition, artemether–lumefantrine treatment was provided for those who had a positive malaria test.

2.5. Statistical analysis

Data were entered using Microsoft Excel 2004 for Mac v11.6.5 (Microsoft Corp., Redmond, WA, USA) and were analysed using Stata v9.0 (StataCorp LP, College Station, TX, USA) and R v2.8.1 (R Foundation for Statistical Computing, Vienna, Austria; http://cran.r-project.org/bin/windows/base/). Intestinal helminth infections were classified according to intensity based on WHO recommendations.[23]

2.6. Genomic DNA extraction

A 0.4 cm segment was cut from each *Ascaris* worm, the gut and outer cuticle were removed and genomic DNA was extracted from the remaining material using the Genomic DNA Isolation Kit for Tissue and Cells (nexttec GmbH, Leverkusen, Germany) according to the manufacturer's instructions.

2.7. cox1 sequencing and microsatellite amplification

A 450 bp fragment of the *Ascaris cox*1 gene was amplified using the primers As-Co1F and As-Co1R.[16] Cycle sequencing of the PCR fragments from both strands was carried out as described previously.[19]

Eight microsatellite loci (ALAC07, ALAC09, ALGA48, ALTN04, ALGA31, ALGA15, ALAC32 and ALAC08) were PCR-amplified for 96 *Ascaris* samples using published primer sets and cycling conditions and fluorescently-labelled forward primers.[24] PCR fragments were multiplexed and their size was determined as described previously.[19]

2.8. cox1 haplotype analysis

The *cox*1 PCR fragment was successfully sequenced for all worm samples but one. Assembly and manual editing of the sequences was carried out in Sequencher v4.8 (Gene Codes Corp., Ann Arbor, MI, USA; http://www.genecodes.com). Sequence alignment and construction of the TCS network was conducted as described. Unique haplotypes identified previously were included in the analysis for comparison.[16,19]

To investigate population structure using the *cox*1 haplotypes, pairwise estimators of F_{ST} (Φ_{ST}, which takes account of the relationships between haplotypes) were determined for each population pair using Arlequin v3.11.[25] Haplotype data from villages in Zanzibar (Kandwi, Ghana, Kizimbani and Tumbatu-Jongowe; n = 57) and Uganda (Hamukaaka; n = 19) were included for comparison.[19]

2.9. Microsatellite fragment analysis

Microsatellite allele sizes were read using PeakScanner v1.0 (Applied Biosystems, Carlsbad, CA, USA). Good reads were obtained from 89 worm samples. Population genetic analysis was carried out using data from eight microstatellite loci (ALAC07, ALAC09, ALGA48, ALTN04, ALGA31, ALGA15, ALAC32 and ALAC08). The number of alleles per locus (N_A), number of private alleles (N_P), the observed heterozygosity (H_O), gene diversity (expected heterozygosity, H_E) and estimate of inbreeding (F_{IS}) were determined for worms from Habutobere and Musezero using Genepop on the Web.[26] In cases of six or more worms per host, observed and expected heterozygosity and F_{IS} were determined for all the worms from each host using FSTAT v2.9.3.2 (http://www.unil.ch/izea/softwares/fstat.html) and the Microsatellite Toolkit (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/).[27] Estimators of F_{ST} (θ) were calculated in FSTAT v2.9.3.2[27] using the

method of Weir and Cockerham[28] and were adjusted to take account of population size in RecodeDate v0.1 using the method of Meirmans.[29] The significance of differentiation was evaluated in FSTAT by random permutation of genotypes between samples and using the overall *G*-statistic to classify the table. Standard Bonferroni corrections were then applied to adjust reported probabilities.[27] When worms from Zanzibar and Hamukaaka village, Uganda, were included for comparison, the analysis was based on data from just four microsatellite loci (ALAC07, ALAC09, ALGA48 and ALTN04).[19]

The program STRUCTURE v2.3 was used to infer potential structure in the Habutobere and Musezero populations (eight loci).[30] We used an admixture model assuming independent allele frequencies. Values of *K* from 1 to 7 were tested after a burn-in length of 10 000 steps followed by a run length of 100 000. In order to test for consistency of estimates across runs, 10 independent runs were performed for each value of *K*. For the data set including the seven villages in Uganda and Zanzibar the structure analysis was performed as described above but including sampling location as prior information to assist the clustering which is recommended in case of weak structure (e.g. low F_{ST}) and few markers (four loci).[31] The average of the 10 iterations for each *K* was calculated and the highest value used to infer the most likely number of populations. All worms in a village were considered as one population.

3. Results

3.1. Worm chemo-expulsion

Overall, 30.4% of children and 42.5% of mothers were infected with *A. lumbricoides* (Table 1). In total 47 study participants (40.9%) were treated with pyrantel pamoate and chemo-expelled worms were collected from 12 individuals in Habutobere and six individuals in Musezero (Table 2). The number of worms collected from each individual ranged from 1 to 39 (for one child in Musezero). In Habutobere, hosts 6 and 10 were from the same family. In Musezero, hosts 3 and 4 were from the same family as were hosts 2, 5 and 6.

3.2. Haplotype and gene network analysis

Haplotypes were obtained for 98 worms, 37 from Habutobere and 61 from Musezero. Nineteen different haplotypes were identified, six of which have previously been found in *Ascaris* from Zanzibar and/or China (Table 3). Nine different haplotypes were found in Habutobere and 12 were found in Musezero. Haplotypes not identified in our previous study were numbered from 17 to 30. As observed previously, haplotype 1 was the most common (found in 64 worms), followed by haplotype 3 (found in 14 worms). These two haplotypes were found in worms from both villages (Table 2). In contrast, 14 haplotypes were found only in single worms.

Pairwise F_{ST} analysis comparing haplotypes between three Ugandan villages (Habutobere, Musezero and Hamukaaka) and four Zanzibari villages (Kandwi, Ghana, Kizimbani and Tumbatu Jongowe) did not indicate significant genetic differentiation between the Ugandan villages but there was evidence for differences between the three Ugandan villages and Kizimbani (Table 4). When haplotype data for all the Ugandan villages and all the Zanzibari villages was pooled, the F_{ST} was 0.009 and there was no evidence of genetic differentiation.

Network analysis revealed two main groups of haplotypes, one centred around haplotype 1 and one centred around haplotypes 3 and 7 (Figure 1). Many of the rare haplotypes clustered around haplotype 1 and a couple of 'bridging' haplotypes were identified, e.g. H24 and H28.

Table 2
Summary of worms and haplotypes from different study participants

Host			Total no. of worms collected	Haplotypes					
ID	Age (years)	Sex		H01	H03	H11	H21	H29	Rare[a]
HAB1	40	F	3	2	1	0	0	0	0
HAB2	10	M	7	7	0	0	0	0	0
HAB3	10	F	4	2	0	0	0	0	2
HAB4	25	F	1	1	0	0	0	0	0
HAB5	26	F	2	0	0	0	0	0	2
HAB6	30	F	1	1	0	0	0	0	0
HAB7	32	F	1	1	0	0	0	0	0
HAB8	34	F	3	1	1	0	0	0	1
HAB9	4	F	1	0	1	0	0	0	0
HAB10	10	F	3	3	0	0	0	0	0
HAB11	38	F	1	1	0	0	0	0	0
HAB12	30	F	10	6	1	0	0	2	1
MUS1	7	F	3	2	0	0	0	0	1
MUS2	7	F	4	3	0	0	0	0	1
MUS3	28	F	1	0	1	0	0	0	0
MUS4	3	F	2	2	0	0	0	0	0
MUS5	20	F	13	6	2	1	2	0	2
MUS6	8	M	39[b]	26	7	1	0	0	4

[a] Only found in one worm.
[b] 39 worms in total but haplotypes obtained from 38 worms.

Table 3
A comparison of 19 DNA haplotypes identified in 98 worms from Habutobere and Musezero indicating numbers of worms for each haplotype, variant positions and nucleotide base differences

Haplotype	No. of worms	15	18	21	25	49	52	60	61	69	81	90	102	114	132	168	186	234	261	273	285	330	339	358	360	366	371
01[a]	64	G	T	T	A	C	A	A	A	C	G	T	G	A	A	C	C	T	G	A	A	C	G	G	T	C	A
02	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
03	14	A	.	.	.	T	.	.	.	T	.	.	A	G	.	T	T	.	A	G	.	.	A	.	C	.	.
07[a]	1	A	.	.	.	T	.	.	.	T	.	.	A	G	.	T	T	.	A	G	.	.	A	.	.	.	.
11	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
17	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
18	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
19	1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
20	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
21	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
22	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	1	.	C	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
24	1	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
25	1	.	.	.	.	.	G	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
26	1	.	.	.	.	.	G	G	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
27	1	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28[a]	1	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
29	2	A	.	.	G	T	.	.	.	T	.	.	.	G	.	T	T	.	A	G	.	.	A	.	C	.	.
30	1	A	.	.	.	T	.	.	.	T	A	.	A	G	.	T	T	.	A	G	.	.	A	.	C	.	.

A dot indicates an identical base to that in haplotype 1.
[a] Haplotype matches one identified by Peng et al.[16] Haplotypes 1–11 were previously identified in *Ascaris* worms from Zanzibar.

3.3. Microsatellite allelic diversity and population structuring

All eight microsatellite loci were polymorphic in both populations and the number of alleles per locus ranged from 13 to 27 (Table 5). The size ranges of the loci ALTN04, ALGA48, ALAC09 and ALAC07 were similar to those observed previously.[19] In practically all cases, the observed heterozygosity was lower than the expected heterozygosity and there was evidence for departure from Hardy–Weinberg equilibrium for three loci in worms from Habutobere and seven loci in worms from Musezero. Taking all loci into account, there was evidence for departure from Hardy–Weinberg equilibrium for both worm populations (Habutobere: $p = 0.002$; Musezero: $p < 0.0001$).

Genetic structuring within hosts was investigated for worm populations from the four individuals who had expelled six or more worms. In all cases the observed heterozygosity was lower than the expected heterozygosity and the F_{IS} was positive. There was evidence for departure from Hardy–Weinberg for both populations from hosts in Musezero (see Table 6). When pairwise F_{ST} values were calculated between populations from each host, there was no evidence of genetic differentiation between any population pair (data not shown).

When population structuring at the village level was investigated, there was little evidence for genetic differentiation between worm populations from Habutobere and Musezero ($F_{ST} = 0.002$; $p > 0.05$, 1000 permutations). However, when populations from Ugandan villages were compared with populations from Zanzibari villages, genetic differentiation was apparent between most of the Ugandan-Zanzibari population pairs (Table 7). A similar result was found when all worms collected from Uganda were compared with all worms collected from Zanzibar ($F_{ST} = 0.054$; $p < 0.05$, 1000 permutations).

The STRUCTURE analysis supported the F_{ST} results above suggesting that *Ascaris* from Habutobere and Musezero belonged to one population ($K = 1$) (Supplementary Figure 1). Likewise, when including worms from the seven villages an optimal $K = 2$ was identified where the populations of worms in each country were assigned to the same cluster, i.e. worms on Uganda and Zanzibar were identified as two different populations.

4. Discussion

In this study of the epidemiology and genetic diversity of *Ascaris* in two villages in southwestern Uganda we found 30% of the children and 43% of the mothers with egg-patent ascariasis, suggesting that this remains a substantial health burden in southwestern Uganda. Sequencing of a portion of the *cox*1 gene from 98 worms obtained by chemo-expulsion revealed 19 different DNA haplotypes. Based on the haplotype data and microsatellite analysis, there was evidence for panmixia or high gene flow between the worms obtained from the two villages despite their physical separation. There was, however,

Table 4
Population pairwise F_{ST} values based on DNA haplotypes

	Population	Zanzibar				Uganda	
		Kandwi	Ghana	Kizimbani	Tumbatu Jongowe	Hamukaaka	Habutobere
Zanzibar	Ghana	0.00	–	–	–	–	–
	Kizimbani	0.00	0.22[a]	–	–	–	–
	Tumbatu Jongowe	0.04	0.00	0.28[a]	–	–	–
Uganda	Hamukaaka	0.00	0.00	0.24[a]	0.00	–	–
	Habutobere	0.04	0.00	0.29[a]	0.00	0.00	–
	Musezero	0.05	0.00	0.31[a]	0.00	0.00	0.00

[a] Significant genetic differentiation ($p < 0.05$) based on 1023 permutations.

genetic differentiation between the Ugandan worms and *Ascaris* obtained previously from individuals living on Unguja, Zanzibar suggestive of limited present or past gene exchange between worm populations in these locations.

4.1. *cox1* haplotypes

The number of different *cox*1 haplotypes identified in this study (19 different haplotypes in 98 worm samples) is similar to that found in our previous study on worms

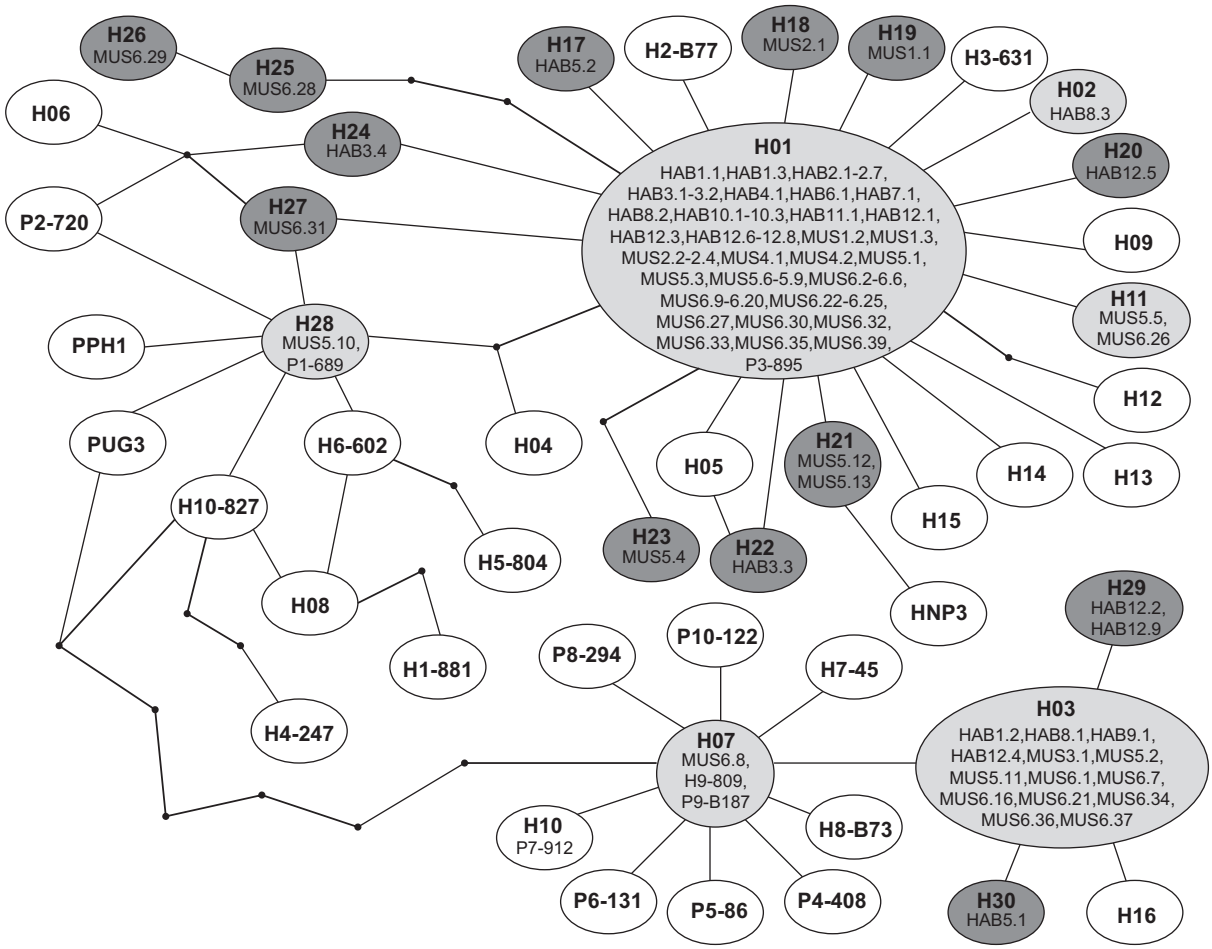

Figure 1. Minimum spanning TCS network of all haplotypes identified together with 20 sequences from China, 16 haplotypes previously identified in Zanzibar and Uganda and 3 other sequences.[16,19] A line indicates one base change. A black dot indicates a non-sampled or extinct haplotype. The size of the ovals is representative of the number of samples with this haplotype. Dark grey indicates novel haplotypes identified in this study, light grey indicates haplotypes found in this and previous studies and white indicates haplotypes found in previous studies which are included for comparison. Haplotypes 1–16 were identified in Betson et al.[19] and haplotypes 17–30 were identified in this study. Haplotype 28 was also identified by Peng et al.[16] HAB1.1-HAB12.10: *Ascaris* sequences from Habutobere; MUS1.1-MUS6.39: *Ascaris* sequences from Musezero; H1-881, H2-B77, H3-631, H4-247, H5-804, H6-602, H7-45, H8-B73, H9-809 and H10-827: human *Ascaris* sequences from China; P1-689, P2-720, P3-895, P4-408, P5-86, P6-131, P7-912, P8-294, P9-B187 and P10-122: pig *Ascaris* sequences from China; HNP: human *Ascaris* sequence from Nepal; PUG: pig *Ascaris* sequence from Uganda; PPH: pig *Ascaris* sequence from the Philippines; PGT: pig *Ascaris* sequence from Guatemala.

Table 5
Microsatellite allelic diversity by marker and village

Locus		Habutobere	Musezero	Both villages
ALTN04	N_A (N_P)	6 (0)	13 (7)	13
(148–216 bp)	R_A	5.70	9.90	12.80
	H_O	0.42	0.56[a]	0.45[a]
	H_E	0.40	0.52	0.47
	F_{IS}	−0.07	0.10	0.05
ALGA31	N_A (N_P)	12 (1)	16 (5)	17
(291–318 bp)	R_A	12.00	13.96	17.00
	H_O	0.72[a]	0.70[a]	0.71[a]
	H_E	0.87	0.87	0.82
	F_{IS}	0.18	0.19	0.19
ALGA48	N_A (N_P)	9 (2)	13 (6)	15
(191–227 bp)	R_A	8.91	10.50	14.89
	H_O	0.58[a]	0.60[a]	0.59[a]
	H_E	0.72	0.77	0.75
	F_{IS}	0.21	0.22	0.22
ALAC08	N_A (N_P)	22 (5)	22 (5)	27
(300–345 bp)	R_A	21.61	17.93	26.73
	H_O	0.85	0.75[a]	0.79[a]
	H_E	0.90	0.87	0.88
	F_{IS}	0.06	0.14	0.11
ALAC09	N_A (N_P)	11 (2)	14 (5)	16
(182–210 bp)	R_A	11.00	12.19	15.97
	H_O	0.79	0.73[a]	0.76[a]
	H_E	0.85	0.86	0.88
	F_{IS}	0.04	0.16	0.12
ALGA15	N_A (N_P)	17 (5)	23 (9)	27
(286–345 bp)	R_A	16.82	18.61	26.73
	H_O	0.76[a]	0.71[a]	0.73[a]
	H_E	0.89	0.90	0.81
	F_{IS}	0.15	0.21	0.19
ALAC32	N_A (N_P)	11 (2)	13 (4)	15
(99–145 bp)	R_A	10.91	12.10	14.98
	H_O	0.73	0.80	0.77
	H_E	0.82	0.85	0.84
	F_{IS}	0.12	0.07	0.08
ALAC07	N_A (N_P)	8 (1)	15 (8)	16
(221–251 bp)	R_A	7.94	12.48	15.83
	H_O	0.85	0.75[a]	0.79[a]
	H_E	0.81	0.81	0.81
	F_{IS}	−0.05	0.07	0.03

N_A: number of different alleles found at each locus; N_P: number of private alleles; R_A: allelic richness; H_O: observed heterozygosity; H_E: gene diversity (expected heterozygosity); F_{IS}: estimate of inbreeding. The size ranges observed for each locus are in parentheses below the locus name.
[a] Evidence for departure from Hardy–Weinberg equilibrium ($p < 0.05$).

from Zanzibar and Uganda and is high compared with that in China, suggesting that there is raised *cox*1 diversity in *Ascaris* across East Africa.[16,19] The number of haplotypes identified per village is similar to that found in the village of Tumbatu Jongwe in Zanzibar. Although 13 novel haplotypes were discovered, gene network analysis revealed that all new haplotypes could be easily fitted into a network created previously based on data from Zanzibar, Uganda and China, either as branches from central haplotypes 1 and 7 or 'bridging' haplotypes.[19] The two groupings of haplotypes clustered around haplotype 1 and haplotypes 3 and 7 are still very obvious (Figure 1). A similar pattern is seen in a recently published network analysis of haplotypes from Chinese *Ascaris*.[18] It will be interesting to see whether haplotypes from *Ascaris* worms from other parts of the world also fit into this network or form new more disconnected groups.

As in worms from Zanzibar, 'pig-like' haplotypes (genotypes originally identified in *Ascaris* from pigs in China) were found in the *Ascaris* from southwestern Uganda. These

Table 6
Summary of genetic variation at eight microsatellite loci for worms from six different hosts

Village	ID	N	H_O	H_E	F_{IS}
Habutobere	HAB2	6	0.73	0.80	0.10
	HAB12	9	0.77	0.79	0.03
Musezero	MUS5	12	0.70[a]	0.79	0.12
	MUS6	34	0.67[a]	0.80	0.16

N: number of worms analysed per host; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: estimate of inbreeding.
[a] Evidence for departure from Hardy–Weinberg equilibrium ($p < 0.05$).

Table 7
Population pairwise F_{ST} values based on microsatellite data

	Population	Zanzibar				Uganda		
		Kandwi	Ghana	Kizimbani	Tumbatu Jongowe	Hamukaaka	Habutobere	Musezero
Zanzibar	Kandwi	–	0.00	0.00	0.00	0.46	0.33	0.16
	Ghana	0.00	–	0.10	0.06	0.54	0.40	0.29
	Kizimbani	0.00	0.02	–	0.06	0.48	0.31	0.13
	Tumbatu Jongowe	0.00	0.01	0.02	–	0.33	0.23	0.12
Uganda	Hamukaaka	0.13	0.16[a]	0.14[a]	0.10[a]	–	0.15	0.16
	Habutobere	0.09	0.11[a]	0.08[a]	0.06[a]	0.05[a]	–	0.02
	Musezero	0.04	0.07[a]	0.03	0.03[a]	0.05	0.01	–

Unadjusted F_{ST} values are below the diagonal; F_{ST} estimators calculated according to the method of Weir and Cockerham.[28]
Adjusted F_{ST} values are above the diagonal; F_{ST} estimators standardized to take account of differences in population size using the method of Meirmans.[29]
[a] Significant differentiation at the 1% nominal level after Bonferroni corrections, based on 21 tests each consisting of 1000 permutations.[27]

could possibly represent cases of humans cross-infected with pig worms which might not be surprising as pig farming is common in Uganda and the prevalence of *A. suum* infections in pigs from Kabale is close to 40%.[32] The pigs are housed in close proximity to human dwellings so it is likely that humans are sporadically infected with *Ascaris* eggs from pig faeces and vice versa, potentially also allowing gene flow between populations of pig and human *Ascaris*.[33] Alternatively, shared haplotypes between human and pig *Ascaris* can also be explained by evolutionary processes such as introgression and retention of ancestral polymorphisms[14] and seems to be the reason for such findings in *Ascaris* populations in Guatemala.[34] Nevertheless human and zoonotic transmission might exist concurrently in this part of Uganda.

4.2. Microsatellite analysis of Ascaris

Microsatellite analysis revealed departures from Hardy–Weinberg equilibrium for a number of loci in worm populations in both villages. As there was no evidence of genetic structuring between hosts, departures from Hardy–Weinberg are likely caused by inbreeding within hosts (F_{IS}). This could be due to non-random mating within hosts, individual hosts collecting parasites from different locations or null alleles.

The villages of Habutobere and Musezero are separated by a large hill and the journey between them by the main road is arduous, around 45 km and takes 1.5–2 h. This physical separation seems not to be a substantial barrier to gene flow as, based on both the sequence and microsatellite data, there was no evidence of genetic differentiation between worm populations from the two villages. By contrast there are significant differences in population structure between worms from the Ugandan villages and three villages on Zanzibar. Interestingly, even though STRUCTURE assigned worms from the three villages to the same population, the F_{ST} data suggest some differentiation between Hamukaaka and the two other Ugandan villages. Although Hamukaaka and Habutobere are only 7 km apart as the crow flies, the road between these two villages is not direct and consists of narrow winding dirt tracks whereas the route from Habutobere and Musezero is predominantly along a main road. Although our data contrast with work from Nepal suggesting local foci of transmission, it must be remembered that the number of worms and hosts sampled in our study and the number of markers analysed was far smaller than in the Nepalese study and so we are unlikely to find fine-scale genetic structure.[15,19] More detailed epidemiological mapping in Kabale and Kisoro is needed to investigate fine-scale microepidemiology.

5. Conclusion

Ascariasis remains a public health problem in south-western Uganda and further control interventions are needed which should include treatment of both adults and children. From a parasite perspective, substantial genetic diversity exists within the *Ascaris* populations from this region based on *cox*1 sequence analysis and microsatellite profiling. By adding to our understanding of the population genetics of *Ascaris* in Uganda and Zanzibar, this study has provided further useful information for monitoring parasite transmission dynamics in Africa before and during the implementation of control programmes.

Authors' contributions: JRS, MB and NBK were responsible for the overall conception and design of the study with the assistance of AA, MAr, MAd, AR and GT; MB, AA, MAr, MAd, AR, GT and JRS carried out the field surveys; MB carried out laboratory DNA work; MB analysed and interpreted the data; PN, JLH and CG undertook DNA sequencing, fragment size determination and analysis; MB and JRS drafted the manuscript. All authors revised the manuscript and read and approved the final version. JRS is guarantor of the paper.

Acknowledgements: The authors are extremely grateful to all the study participants, the local Vector Control Division Officers and community mobilisers.

Funding: This study was funded by the Natural History Museum, London, UK and the Wellcome Trust.

Competing interests: None declared.

Ethical approval: Ethical approval was granted by the London School of Hygiene & Tropical Medicine, UK (application no. LSHTM 5538·09, amended) and the Ugandan Council of Science and Technology. All participating mothers gave informed consent in writing or by fingerprint

(in cases of illiteracy) on behalf of themselves and their children.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2011.10.011.

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