Infection, Genetics and Evolution 12 (2012) 1172–1177

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Pig *Ascaris:* An important source of human ascariasis in China

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ARTICLE INFO

Article history:
Received 23 February 2012
Received in revised form 13 April 2012
Accepted 17 April 2012
Available online 25 April 2012

Keywords:
Ascaris
Cross infection
Hybridization
Microsatellites
Control program
China

ABSTRACT

The aim of the present study is to detect the frequency and distribution of cross infection and hybridization of human and pig *Ascaris* in China. Twenty high polymorphic microsatellite loci were selected to screen 258 *Ascaris* worms from humans and pigs from six provinces in China. The software programs Structure, Baps and Newhybrids were used to determine the case of cross infection and hybridization of human and pig *Ascaris*. Results showed that cross infection was detected in all sampled locations and of the total 20 cross infection cases, 19 were indentified as human infections by pure-bred pig type *Ascaris* in contrast to only one case of pig infection by pure-bred human type *Ascaris*. Similar to the findings in cross infection, hybrid *Ascaris* was also detected in all locations and both host species and most of hybrids (95%) were detected from human host. The distribution of cross infection and hybrids showed significant difference between the two host species and among three categories of genotype in terms of G1, G2 and G3, and also between the south and north regions (for hybrids only). The results strongly suggest pig *Ascaris* as an important source of human ascariasis in endemic area where both human and pig *Ascaris* exist. In consideration of current control measures for human ascariasis targeting only infected people, it is urgently needed to revise current control measures by adding a simultaneous treatment to infected pigs in the sympatric endemics. The knowledge on cross transmission and hybridization between human and pig *Ascaris* is important not only for public health, but also for the understanding of genetic evolution, taxonomy and molecular epidemiology of *Ascaris*.

1. Introduction

Ascaris lumbricoides Linnaeus, 1758 and *Ascaris suum* Goeze, 1782, two of the most socioeconomically important parasitic nematode of humans and pigs, cause worldwide infection/disease ascariasis (Crompton, 2001; Dold and Holland, 2011; Peng et al., 1998a; Stephenson, 1987; Stephenson et al., 2000). Because of the difficulty in making morphological distinction between them and the reports that *Ascaris* of pig origin can infect humans and vice versa (Galvin, 1968; Takata, 1951; reviewed in Crompton, 1989), disputation about their taxonomic status has arisen for decades (Anderson, 2001; Macko and Dubinsky, 1997; Leles et al., 2012; Peng et al., 2007; Peng and Criscione, 2012). This problem is not only a taxonomic issue, but has important epidemiological concern with direct implications for the development and implementation of any control program for ascariasis. For example, in areas where human and pig *Ascaris* coexist, it is difficult to decide if control measures should be only applied to infected people (as it is usually carried out) or simultaneously to both infected humans and pigs.

During the past 20 years, molecular markers have been used in studies on *Ascaris* and ascariasis, and added valuable information to the understanding of two roundworms, especially in the aspects of molecular genetics and epidemiology (reviewed in Peng and Criscione, 2012). On the one side, in some areas of endemic human and pig transmission, there is strong neutral genetic differentiation between parasites from the two host species (Anderson et al., 1993; Peng et al., 1998a, 2003, 2005; Criscione et al., 2007a). On the other hand, the same *Ascaris* mtDNA haplotype and ITS genotype can be found in both humans and pigs (Anderson et al., 1993; Peng et al., 1998a, 2003, 2005). This could result from incomplete lineage sorting, current introgression, or cross-transmission but no interbreeding. Although there is good support for cross-transmission in locations of non-endemic human transmission (human ascariasis caused by pig Ascaris) (Anderson, 1995; Arizono et al., 2010; Maruyama et al., 1996; Nejsum et al., 2005), little is known about if cross infection occurs endemic areas and if such cross-transmission could lead to introgression between human and pig *Ascaris*? These questions have been recently addressed and results from the study showed that hybrid worms were detected in both sympatric

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http://dx.doi.org/10.1016/j.meegid.2012.04.016

samples from Guatemala and Hainan Province, China (Criscione et al., 2007a). These results indicate that there must have been contemporary interbreeding and thus, necessarily recent cross-transmission, between sympatric human and pig *Ascaris*, as the methods used by Criscione et al. (2007a) can only detect hybrids going back two generations (see Anderson and Thompson, 2002). This finding encourages further studies to detect the frequency of cross infection and hybridization in additional sympatric populations. These studies are important not only for understanding the transmission patterns of the two roundworms and the development and implementation of any control program, but also beneficial to studies exploring gene introgression between the host-associated populations, especially for genes related to host infectivity/affiliation or drug-resistance evolution. The present study, therefore, aimed to reveal the frequency of cross infection and hybridization between the human and pig *Ascaris* in China, and to explore if there would be any differences in the frequency distribution between host species, geographical regions and different genotypic categories in terms of G1–G3 marked by a part of the first internal transcribed spacer (pITS-1) (Peng et al., 2003).

2. Material and methods

2.1. Sample information

The samples used in the present study (totally 258 pig and human *Ascaris*, see Table 1 for details) were from the collection made during 1998–2001 from Jiangxi, Xinjiang, Liaoning, Qinghai, Yunnan and Hainan province (region) in China. Human-derived worms came from the collection after treatment with pyrantel pamoate (Hangzhou Minsheng Pharmaceutical Group Co., Ltd., 10 mg/kg, 1 dosage), majority of pig-derived worms came from the local pig slaughter house, a few after anthelmintic treatment. Individual adult worms were washed extensively in physiological saline and stored at −80 °C for samples collected in 2001 from Jiangxi province, or in Carnoy's solution (60% ethanol, 30% chloroform, 10% acetate v/v/v) at 4 °C for samples collected during 1998–1999 from other provinces until required for DNA isolation (see Peng et al. (2003) for details).

2.2. Isolation of genomic DNA

Isolation of genomic DNA from each worm was conducted using a small section of body wall (5–10 mm) and the method by SDS/proteinase K treatment, column purified (Wizard® SV Genomic DNA Purification System, Promega, Madison, WI, USA) and eluted into 60 μl H_2O according to the manufacturer's recommendation (Zhou et al., 2011a).

2.3. Amplification of pITS-1

Most of worms used in this study have records of identification of genotypes of G1–G5 by PCR based single strand conformation polymorphism combined with direct sequencing from our previous researches (Peng et al., 2003). For worms not having the identification, amplification of pITS-1 was performed using the primers XZ5 forward 5′-TGATGTAATAGCAGTCGGCG-3′ and NC13 reverse 5′-GGCTGCGTTCTTCATCAT-3′ to genotype in terms of G1–G6 (Peng et al., 2003; Leles et al., 2010). Direct sequencing of the product of PCR of pITS-1 was conducted first. In the case of the sequencing data not clear, these samples were cloned into the pMD18-T Easy Vector System (TaKaRa), and sequenced by Sangon Biotech (Shanghai) Co., Ltd.). We picked 1–3 clones for each sample, depending on if the sequencing data is clear.

2.4. Amplification of multiple microsatellite loci

For Microsatellite amplification and alleles genotyping, we selected 20 microsatellite loci with higher polymorphism from the 23 autosomal microsatellite loci employed by Criscione et al., 2007a,b) (GenBank ID: DQ988845, DQ988847-DQ988849, DQ988853, DQ988855, DQ988857, DQ988859, DQ988860, DQ988862–DQ988864, DQ988866, DQ988867, DQ988869–DQ988872, CB101754 and BQ835581). To all worms, PCR reactions (25 μl) were performed in Premix Taq Version2.0 (Takara, Dalian, China), 0.2 μm each primer, 2 μl template DNA, with an initial denaturing step (96 °C for 5 min), followed by 5 cycles of high-temperature PCR (96 °C for 45 s, 55 °C or 53 °C for 30 s, 72 °C for 1 min), then 35 cycles of low-temperature PCR (96 °C for 45 s, 47 °C or 45 °C for 30 s, 72 °C for 1 min), and a final step of 72 °C for 7 min. The PCR products of 1 μl were detected on an ABI3730xl capillary DNA sequencer (Invitrogen™, Shanghai, China). Fragment sizes of alleles were calculated against the size standard GS-500-ROX using GeneMapper® software, version 3.7 (Invitrogen™).

2.5. Data analysis

For the sequences of pITS-1, we downloaded sequences of G1–G5 (Peng et al., 2003) and G6 (Leles et al., 2010) (GenBank ID: AJ554036–AJ554043; EF150649–EF150653, EU073131–EU073134, EF1536919–EF15361923). Then we aligned the measured and downloaded sequences by clustal X procedure (Thompson et al., 1997) and identified the genotypes of the measured samples. For microsatellite data, we determined the size of alleles based on capillary electrophoresis and converted raw data into the required format using the microsatellite format conversion software CREATE v 1.1 (Coombs et al., 2008). To search for cross infection and hybrids within sympatric populations of human and pig *Ascaris*, the software programs, the program settings and the criterion for classifying individuals as cross infection or hybridization used in the present study are the same as Criscione et al. (2007a), except for a minor changes that following recommendations in the BAPS v. 4.14 manual, we used 300 iterations to estimate the admixture coefficients and simulated 300 reference individuals with 300 iterations each for significance testing.

Table 1
Ascaris sample information of location, host origin and genotype from China.

Province	Worm no. from			Genotypes of worms			No. of host	
	Human	Pig	Total	G1	G2	G3	Human	Pig
Liaoning	25	20	45	12	16	17	21	11
Xinjiang	21	21	42	9	16	17	17	15
Qinghai	22	21	43	12	10	21	18	14
Jiangxi	26	15	41	22	1	18	(No records)	
Yunnan	21	23	44	13	13	18	21	22
Hainan	22	21	43	15	13	15	20	20
Total	137	121	258	83	69	106	97	82