

Short Communication

Ascariasis in Japan: Is Pig-Derived *Ascaris* Infecting Humans?

Naoki Arizono*, Yuta Yoshimura, Naoki Tohzaka, Minoru Yamada, Tatsuya Tegoshi, Kotaro Onishi, and Ryuichi Uchikawa¹

Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto 602-8566; and ¹Medicine Chemical Research, Chiba Institute of Science, Choshi 288-0025, Japan

(Received August 27, 2010. Accepted October 14, 2010)

SUMMARY: Human ascariasis is caused by infection with the common roundworm *Ascaris lumbricoides*, although the pig roundworm *Ascaris suum* has also been reported to infect humans and develop into the adult stage. To elucidate whether pig-derived *Ascaris* infects humans in Japan, 9 *Ascaris* isolates obtained from Japanese patients and further 9 *Ascaris* isolates of pig origin were analyzed to determine their internal transcribed spacer-1 sequences. Six of the 9 clinical isolates showed the *Ascaris* genotype which predominantly infects humans in endemic countries, while the other 3 clinical isolates and 9 pig-derived isolates showed the genotype predominant in pigs worldwide. These results suggest that at least some cases of human ascariasis in Japan are a result of infection with pig-derived *Ascaris*.

Ascaris lumbricoides is one of the most common intestinal parasites in humans. However, it has long been debated whether *Ascaris suum*, the pig roundworm which is morphologically indistinguishable from *A. lumbricoides*, also parasitizes humans. Recent studies have shown that some genetic markers, especially the rRNA internal transcribed spacer (ITS) region, provide clear evidence for genetic differentiation between *Ascaris* populations from humans and pigs (1–5). Indeed, these genetic markers have been used to show that *Ascaris* parasites in humans and pigs are reproductively isolated in endemic regions such as Central America and China (2–4), whereas most cases of human ascariasis in non-endemic regions, such as North America and Denmark, have been suggested to be a cross-infection with pig *Ascaris* (1,5). In Japan, however, the source (or species) of *Ascaris* parasites which infect humans in recent years remains unknown. To gain an insight into the epidemiological status of ascariasis in Japan, where the incidence of human ascariasis has declined markedly, we analyzed *Ascaris* worms obtained from Japanese patients to determine their ITS sequences and compare them with those of *Ascaris* isolated from pigs.

Nine adult *Ascaris* worms, which were either passed in the feces, expelled through the mouth, or extracted from the stomach by endoscopy, were obtained from 9 patients aged 23–84 years in the period 1991–2010. All patients were residents of either Kyoto, Nara, or Shiga Prefecture, Japan. Two of the 9 patients had travelled to Bolivia or India within the year prior to recovery of the worms, whereas the others had no history of recent travel to endemic regions. Nine adult worms of pig *Ascaris* were collected from pigs in a slaughterhouse in Gifu Prefecture in 2002 and 2006, or in Kyoto in 2006. All the worms had been kept frozen, or fixed in forma-

lin and then preserved in 70% alcohol, until their DNA was extracted.

The primers 5'-CCGGGCAAAGTCGTAACAA-3' (AscITF) and 5'-TAGTGCTCAATGTGTCTGCA-3', which amplify the ITS1 region, or AscITF and 5'-ATATGCTTAAATTCAGCGGGT-3', which span ITS1, 5.8S rRNA, and ITS2, were used for PCR. Amplification products were directly sequenced on both strands, and the nucleotide sequences determined were deposited in DNA databases (accession nos. AB571295–AB571302, AB571305–AB571307, AB576588–AB576594).

The sequences of the ITS1 regions of all 18 isolates were found to be very similar to each other, with overall average divergence of 0.003 (Jukes-Cantor model). Previous studies have shown that ITS1 sequences exhibit two polymorphic sites (positions 133 and 246) that differ between *Ascaris* of human and pig origin (4). As expected, the nucleotides at these positions for the 9 pig isolates were the same as those reported previously for pig isolates (Table 1). In contrast, 6 of the 9 isolates from clinical samples had G and T at these two positions, respectively, as would be expected for human *Ascaris* isolates, whereas the other 3 isolates had C and A, thus suggesting that the parasite was of pig origin. Nucleotide position 133 is at 1 of 2 restriction recognition sites for *HaeIII*, thereby allowing human and pig

Table 1. Summary of the results for two polymorphic sites in the ITS1 region of *Ascaris* spp. of 9 pig- and 9 human-derived isolates

Source of <i>Ascaris</i>	No. of isolates	Position ¹⁾		Reference
		133	246	
Pig		C	A	4
Pig	9	C	A	present study
Human		G	T	4
Human	6	G	T	present study
Human	3	C	A	present study

¹⁾ Nucleotide position according to reference no. 4.

*Corresponding author: Mailing address: Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kyoto 602-8566, Japan. Tel: +81-75-251-5325, Fax: +81-75-251-5328, E-mail: arizonon@koto.kpu-m.ac.jp

samples to be differentiated by PCR-RFLP (1–4). Although previous population genetics studies using PCR-RFLP showed the occurrence of intermediates/hybrids of the two taxa in some *Ascaris* samples (1–4), none of the *Ascaris* specimens analyzed in the present study showed either heterozygote C/G at position 133 or A/T at position 246 in their electropherograms.

Although polymorphism at the *Hae*III restriction site (position 133), and at position 246 in ITS1, is not an absolute diagnostic marker for differentiating *A. lumbricoides* and *A. suum* because of the presence of a heterozygous genotype in many regions of the world, it is still informative and better than any other single marker for estimating the origin of individual *Ascaris* worms obtained from humans. Previous studies have shown that 84.2–98.5% of *Ascaris* worms obtained from people in endemic regions in Central and South America and Asia had the genotype with G at position 133 (tentatively designated as genotype “H” hereafter), and that 79.8–98.2% of worms isolated from pigs had C at position 133 (designated as genotype “P”) (1,3,5). The frequency distribution of the genotypes of *Ascaris* obtained from Japanese patients (6 H and 3 P genotype isolates) differed significantly ($P = 0.010$, Fisher’s exact test) from those in Chinese patients (48, 1, and 8 for H, P, and the hybrid genotype, respectively) (3). Three of the 9 clinical isolates analyzed in this study were obtained in 1991, 2001, and 2002, respectively, and were found to have the H genotype. The other 6 isolates were obtained between 2006 and 2010. Two of these patients, which were found to be genotype H, were obtained from patients who had travelled to an endemic region, whereas the remaining 4 were obtained from patients with no travel history. One of these 4 isolates was found to be genotype H and 3 genotype P; the possibility of contact with pig manure in the latter cases was unknown. Although the numbers analyzed here are small, the results suggest that the epidemiological status of human ascariasis in Japan has been changing from that of endemic countries, where human-to-human transmission is the norm, toward that of non-endemic countries, where cross-infection with pig-derived parasites occurs more than would otherwise be expected.

We also analyzed the sequence divergence of the cytochrome oxidase subunit 1 (CO1) gene of 7 clinical and 4 pig isolates (accession nos. AB591795–AB591805) and found a total of 8 distinct haplotypes, with sequence divergences of up to 0.026. Neighbor-joining tree analyses, however, showed no association between the mtDNA haplotype lineages and host species of *As-*

caris worms (detailed data not shown), which is consistent with previous reports whereby each of the mtDNA haplotype lineages included worms collected from pigs and humans (2,3).

The possibility that *A. suum* reaches maturity in humans has been suggested as a result of experimental or accidental laboratory infections of humans with pig *Ascaris* (6). On the other hand, there have been a number of clinical cases of larva migrans supposedly caused by *A. suum* larvae, which invade the liver and lungs but do not necessarily develop to maturity (7). However, the pathogenetic (or physiogenetic) factors that determine the course of human infection with pig-derived *Ascaris* remain to be elucidated.

Although a continuous person-to-person cycle of ascariasis transmission is rarely, if ever, likely to occur in the present agricultural environment in Japan, ascariasis could still continue to occur at a low incidence, at least in part through cross-infection with *Ascaris* eggs of pig origin or through imported fresh or processed food. Indeed, a few *Ascaris* eggs of genotype H have been found in imported kimchi made from vegetables with various seasonings (8).

Conflict of interest None to declare.

REFERENCES

1. Anderson, T.J. (1995): *Ascaris* infections in humans from North America: molecular evidence for cross-infection. *Parasitology*, 110, 215–219.
2. Anderson, T.J. and Jaenike, J. (1997): Host specificity, evolutionary relationships and macrogeographic differentiation among *Ascaris* populations from humans and pigs. *Parasitology*, 115, 325–342.
3. Peng, W., Anderson, T.J., Zhou, X., et al. (1998): Genetic variation in sympatric *Ascaris* populations from humans and pigs in China. *Parasitology*, 117, 355–361.
4. Zhu, X., Chilton, N.B., Jacobs, D.E., et al. (1999): Characterisation of *Ascaris* from human and pig hosts by nuclear ribosomal DNA sequences. *Int. J. Parasitol.*, 29, 469–478.
5. Nejsum, P., Parker, E.D., Jr., Frydenberg, J., et al. (2005): Ascariasis is a zoonosis in Denmark. *J. Clin. Microbiol.*, 43, 1142–1148.
6. Morishita, K. (1972): Studies on epidemiological aspects of ascariasis in Japan and basic knowledge concerning its control. p. 3–153. In K. Morishita, Y. Komiya and H. Matsubayashi (ed.), *Progress of Medical Parasitology in Japan*. vol. 4. Meguro Parasitological Museum, Tokyo.
7. Maruyama, H., Nawa, Y., Noda, S., et al. (1996): An outbreak of visceral larva migrans due to *Ascaris suum* in Kyushu, Japan. *Lancet*, 347, 1766–1767.
8. Sugiyama, H., Morishima, Y., Kawanaka, M., et al. (2006): Molecular identification of eggs of roundworm-like parasite found in imported kimchi. *Clin. Parasitol.*, 17, 153–155 (in Japanese).