Anisakiasis is a zoonotic nematode infection that causes acute and chronic gastrointestinal granulomatous disease in humans. For most patients, the causative agents are larvae of nematodes of the genera *Anisakis* and *Pseudoterranova*, and the source of infection is marine fish or squid harboring these larvae (1). Within 8–12 hours after infected fish are ingested, the larvae penetrate into the person’s stomach or intestinal wall, causing acute abdominal pain, indigestion, nausea, and vomiting; pathologic findings are edema, hyperemia, and bleeding in the surrounding mucosa (1,2). The diagnosis is usually based on morphologic identification of the larvae or on histopathologic identification of sectioned larvae (1). However, molecular techniques have recently been developed as effective tools not only for the diagnosis of individual cases but also for studies of taxonomy and evolution of anisakid nematodes (3,4).

Anisakiasis in humans was first reported in the Netherlands; since then, it has been reported extensively in Japan (~2,000 cases annually), South Korea (~200 cases annually), and some European countries (~500 cases annually) where people eat raw or undercooked fish (1,2). In the United States, up to 50 human cases are reported each year (1). Most infections in humans have been caused by *Anisakis simplex* sensu stricto and *Pseudoterranova decipiens* nematodes (1); however, since 1999, a few human infections with *Anisakis pegreffii* larvae (a sibling species of *A. simplex* s.s.), originally recovered from a Mediterranean monk seal (5), have been reported in Italy (6–9) and Japan (10,11). The larvae of *A. pegreffii* are morphologically distinguished, with difficulty, from those of *A. simplex* s.s. (both are *Anisakis* type I); however, molecular techniques can easily distinguish the 2 types of larvae (3,4).

In South Korea, *Anisakis* type I larvae recovered from humans and fish have been assigned to *A. simplex* s.s., on the basis of morphologic appearance (1,12). We performed molecular analyses of 26 *Anisakis* type I larvae recovered from 16 humans in South Korea by using DNA sequencing of the nuclear internal transcribed spacer (ITS) genes.

### The Study

A total of 30 *Anisakis* type I larvae were removed from the stomach of 16 patients referred to the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, Seoul, South Korea, from 2000 through 2013 (Table). Among them, 26 larvae were analyzed by DNA sequencing. All patients experienced acute gastric or abdominal discomfort, including epigastric pain and indigestion, and underwent gastroendoscopy. During the examinations, whitish nematode larvae were observed and extracted with biopsy forceps. Some larvae were preserved in 70% ethanol, and others were fixed in 10% formalin before being mounted on slides with glycerin jelly.

Total genomic DNA was extracted by using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany); nested PCR and nucleotide sequencing were performed on the ITS region (ITS1, 5.8S rRNA subunit, and ITS2) according to procedures reported previously (13). The PCR product was amplified by using the Cosmo Labopass X2 PCR Premix kit (Cosmo Genetech, Seoul, South Korea), and automated DNA sequencing was performed by Solgent Co., Ltd. (Daejeon, South Korea). Nucleotide sequences obtained were aligned by using the Geneious program, version 6.0.3 (Geneious Co., Wellington, New Zealand).

Of the 26 *Anisakis* larvae from 15 human patients, 25 showed 100% identity in the sequences of ITS region (244 bp, high-confidence variable positions) with those of the *A. pegreffii* sequence available in GenBank (accession no. AB277823), whereas their identity with *A. simplex* s.s. (accession no. AB277822) was 99.2% (Table). The remaining sample showed 100% identity with the sequences of *A. simplex* s.s. (accession no. AB277822) and 99.2% identity with those of *A. pegreffii* (accession no. AB277823). On the basis of these results, *A. pegreffii* nematode infection was diagnosed for 15 of the 16 patients, and *A. simplex* s.s. infection was diagnosed for only 1 patient.

### Conclusions

Our results confirm the presence of *A. pegreffii* nematode infection in humans in South Korea, making this the third...
Anisakiasis in Humans, South Korea

Table. Anisakiasis characteristics among 16 human patients, South Korea, 2000–2013*

| Patient no. | Patient sex | Year of larva recovery | No. larvae recovered | Clinical signs and symptoms | Anisakis larvae sequencing results (% identical sites) |
|-------------|-------------|------------------------|----------------------|----------------------------|--------------------------------------------------|
|             |             |                        |                      |                            | A. simplex s.s.    | A. pegreffii |
| 1           | M           | 2000                   | 1                    | Abdominal pain, nausea     | 99.2               | 100          |
| 2           | M           | 2000                   | 1                    | Indigestion, vague gastric pain | 99.2               | 100          |
| 3           | M           | 2002                   | 1                    | Abdominal pain, nausea, vomiting | 99.2               | 100          |
| 4           | F           | 2002                   | 1                    | Abdominal pain and tenderness, anorexia | 99.2               | 100          |
| 5           | F           | 2003                   | 1                    | Epigastric pain, nausea, vomiting | 99.2               | 100          |
| 6           | M           | 2003                   | 1                    | Abdominal pain, indigestion | 99.2               | 100          |
| 7           | F           | 2003                   | 1                    | Abdominal pain, nausea, vomiting | 99.2               | 100          |
| 8           | F           | 2003                   | 1                    | Abdominal pain, nausea, anorexia | 99.2               | 100          |
| 9           | F           | 2004                   | 1                    | Epigastric pain, abdominal fullness | 99.2               | 100          |
| 10          | M           | 2005                   | 1                    | Abdominal pain, nausea, vomiting | 99.2               | 100          |
| 11          | F           | 2005                   | 1                    | Abdominal pain, indigestion, nausea, vomiting | 99.2               | 100          |
| 12          | M           | 2005                   | 1                    | Abdominal pain, nausea, vomiting | 99.2               | 100          |
| 13          | M           | 2005                   | 1                    | Epigastric pain, indigestion, nausea | 99.2               | 100          |
| 14          | M           | 2006                   | 1                    | Abdominal discomfort, nausea | 99.2               | 100          |
| 15          | M           | 2012                   | 1                    | Abdominal pain and tenderness | 100                | 99.2         |
| 16          | M           | 2013                   | 15†                  | Abdominal pain and tenderness, indigestion, nausea, vomiting, anorexia | 99.2               | 100          |

*All patients underwent gastroduodenoscopy, during which Anisakis larvae were removed with biopsy forceps.
†Of these 15 larvae, 11 were analyzed by use of molecular techniques.

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country (after Italy and Japan) in which this infection in humans has been identified. This high proportion of A. pegreffii nematode infections in humans is surprising and suggests that most cases of anisakiasis in humans in South Korea may be caused by A. pegreffii rather than A. simplex s.s. larvae. To confirm the source of infection, molecular analyses of Anisakis larvae extracted from human patients are required in South Korea.

Human infection with A. pegreffii nematodes was first documented in Italy by use of PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis (6). The second case was reported from Japan (10), in which 1 of 100 anisakid larvae extracted from patients in Kyushu and Hokkaido was identified by PCR-RFLP analysis as A. pegreffii. Then, in 2009, A. pegreffii nematode infection was diagnosed for 2 women in Italy by PCR-RFLP and sequencing of the 28S gene (7). In 2011, A. pegreffii DNA was extracted from a paraffin-embedded granuloma from a man in Italy (8). Also in Italy, 8 more A. pegreffii nematode infections in humans were reported in 2013 (9). Thus, to date, including the 15 cases reported here, a total of 28 cases of A. pegreffii nematode infections in humans have been documented in the literature.

The markedly high proportion of A. pegreffii nematode infections identified among patients in South Korea (25/26 larvae from 15/16 patients) was not expected because in Japan (Kyushu and Hokkaido), which are geographically close to South Korea, Anisakis larvae from humans are mostly A. simplex s.s. (99/100 larvae from 84/85 patients); only 1 larva was identified as A. pegreffii (10). This remarkable discrepancy between South Korea and Japan remains to be further investigated. However, it is of note that the species of Anisakis larvae detected in fish varied according to the 2 large localities of Japan; from northern Japan to the Pacific sides and from the Sea of Japan to the eastern China Sea sides (14). The former locality, such as Hokkaido and eastern Japan, showed more A. simplex s.s. than A. pegreffii larvae, whereas the latter locality (southwestern Japan), including Kyushu and Fukuoka (close to South Korea), showed more A. pegreffii than A. simplex s.s larvae (14). This finding might partly explain the discrepancy between the Anisakis larvae species that infect humans in South Korea and Japan.

When the pathogenic potential of A. simplex s.s. larvae for human patients was compared with that of A. pegreffii (11), it was found that A. simplex s.s. larvae had greater potential than A. pegreffii larvae to survive acidic gastric juice and to penetrate the human stomach, small intestine, and colon. However, further studies are needed to elucidate this finding.

Another clinicopathologic concern associated with anisakiasis in humans is the potential for A. simplex s.s.
and A. pegreffii larvae to elicit gastroallergic reactions. These reactions are characterized by urticaria on the arms and abdomen and by angioedema or anaphylaxis when the live parasite attempts to penetrate the gastric mucosa (9). We did not notice such allergic reactions in the patients reported here. However, because of an increasing tendency toward Anisakis nematode allergy among patients in South Korea (15), attention should be paid to this clinical feature.

Our study demonstrates the predominance of A. pegreffii over A. simplex s.s. nematode infection among humans with anisakiasis in South Korea. The study highlights the need to perform molecular analysis for each larva extracted from human patients in this country.

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References

1. Sohn WM, Choi JY. Anisakiosis (anisakidosis). In: Palmer SR, Soulsby L, Torgerson PR, Brown WG, editors. Oxford textbook of zoonoses. 2nd ed. Oxford (UK): Oxford University Press; 2011. p. 774–86.

2. Choi JY, Murrell KD, Lymbery AJ. Fish-borne parasitic zoonoses: status and issues. Int J Parasitol. 2005;35:1233–54. http://dx.doi.org/10.1016/j.ijpara.2005.07.013

3. Mattiucci S, Nascetti G. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Adv Parasitol. 2008;66:47–148. http://dx.doi.org/10.1016/S0065-308X(08)00202-9

4. Mattiucci S, Cipriani P, Webb SC, Paolelli M, Marcier F, Bellissaro B, et al. Genetic and morphological approaches distinguish the three sibling species of the Anisakis simplex species complex, with a species designation as Anisakis berlandi n. sp. for A. simplex sp. C (Nematoda: Anisakidae). J Parasitol. 2014;100:199–214. http://dx.doi.org/10.1645/12-120.1

5. Campana-Rouget PY, Biocca E. Une nouvelle espèce d’Anisakis chez un poquhe Méditerranée Annales de Parasitologie. 1955;30:477–80.

6. D’Amelio S, Mathiopoulos KD, Brandonisio O, Lucarelli G, Doronzo F, Paggi L. Diagnosis of a case of gastric anisakidosis by PCR-based restriction fragment length polymorphism analysis. Parasitologia. 1999;41:591–3.

7. Fumarola L, Monno R, Ierardi E, Rizzo G, Giannelli G, Lalle M, et al. Anisakis pegreffii etiological agent of gastric infections in two Italian women. Foodborne Pathog Dis. 2009;6:1157–9. http://dx.doi.org/10.1089/fpd.2009.0325.

8. Mattiucci S, Paolelli M, Borrini F, Palumbo M, Palmieri RM, Gomes V, et al. First molecular identification of the zoontic parasite Anisakis pegreffii (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. BMC Infect Dis. 2011;11:1–6.

9. Mattiucci S, Fazii P, Rosa AD, Paolelli M, Megna AS, Gielmo A, et al. Anisakiasis and gastroallergic reactions associated with Anisakis pegreffii infection, Italy. Emerg Infect Dis. 2013;19:496–9. http://dx.doi.org/10.3201/eid1903.121017

10. Umehara A, Nakawaki Y, Araki J, Uchida A. Molecular identification of the etiological agent of the human anisakiasis in Japan. Parasitol Int. 2007;56:211–5. http://dx.doi.org/10.1016/j.parsint.2007.02.005

11. Arizono N, Yamada M, Tegoshi T, Yoshikawa M. Anisakis simplex sensu stricto and Anisakis pegreffii: biological characteristics and pathogenetic potential in human anisakiasis. Foodborne Pathog Dis. 2012;9:517–21. http://dx.doi.org/10.1089/fpd.2011.1076

12. Chai JY, Cho SR, Kook J, Lee SH. Infection status of the sea eel (Astroconger myriaster) purchased from the Noryangjin fish market with anisakid larvae [in Korean]. Kisaengchunghak Chapchi. 1992;30:157–62. http://dx.doi.org/10.3347/kjp.1992.30.3.157

13. Suzuki J, Murata R, Hosaka M, Araki J. Risk factors for human Anisakis infection and association between the geographic origins of Scomber japonicus and anisakid nematodes. Int J Food Microbiol. 2010;137:88–93. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.10.001

14. Quiaizon KMA, Yoshinaga T, Ogawa K. Distribution of Anisakis species larvae from fishes of the Japanese waters. Parasitol Int. 2011;60:223–6. http://dx.doi.org/10.1016/j.parint.2011.03.002

15. Choi SJ, Lee JC, Kim MJ, Hur GY, Shin SY, Park HS. The clinical characteristics of Anisakis allergy in Korea. Korean J Intern Med. 2009;24:160–3. http://dx.doi.org/10.3201/kjim.2009.24.2.160

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