time when it was not widely circulating prompted interstate notification for this out-of-state person.

This outbreak involving transmission in a bar between close social contacts and non–party-associated bar patrons demonstrates the high potential for Omicron transmission in indoor settings for which consistent mask use and distancing are challenging. Although no persons in this outbreak experienced severe disease, most were young and fully vaccinated. Local capacity for genomic sequencing, conducted across 7 laboratories in 5 states (Colorado, Illinois, Louisiana, Missouri, Michigan), enabled identification of linked case-patients beyond invited attendees who may have been excluded from traditional epidemiologic investigations.

Outbreak investigation limitations include incomplete identification of, or nonresponse from, dinner and party attendees; limited availability of clinical remnant specimens; and inability to estimate attack rates among persons in the bar. This outbreak highlights Omicron transmissibility; the value of local genomic surveillance capacity and interstate coordination; the value of vaccination for reducing the likelihood of severe disease; and the potential for rapid, widespread transmission of a novel variant across multiple states from 1 event over a holiday weekend.

Acknowledgements

We thank Sarah Patrick, Judy Kauerauf, Ann Wiringa, Ishani Doshi, Shirley Morales, Rachel Rubin, Matthew Sinn, Pauline Harrington, Heather Blankenship, Julie Hand, Christine Record, Alexis Burakoff, Christopher Grano, Carla Barrios, Christine Roloff, and Ashley Becht for their assistance with this study.

About the Author

Dr. Spencer is a pediatric infectious diseases physician and Epidemic Intelligence Service officer assigned to the Chicago Department of Public Health Bureau of Communicable Diseases and Bureau of Maternal, Infant, Child, and Adolescent Health. Her research interests include antimicrobial stewardship and infectious diseases in children.

References

1. Fisher KA, Tenforde MW, Feldstein LR, Lindsell CJ, Shapiro NI, Files DC, et al.; IVY Network Investigators; CDC COVID-19 Response Team. Community and close contact exposures associated with COVID-19 among symptomatic adults ≥18 years in 11 outpatient health care facilities—United States, July 2020. MMWR Morb Mortal Wkly Rep. 2020;69:1258–64. https://doi.org/10.15585/mmwr.mm6936a5
2. Sami S, Turbyfill CR, Daniel-Wayman S, Shonkwiler S, Fisher KA, Kuhring M, et al. Community transmission of SARS-CoV-2 associated with a local bar opening event—Illinois, February 2021. MMWR Morb Mortal Wkly Rep. 2021;70:528–32. https://doi.org/10.15585/mmwr.mm7014e3
3. Majra D, Benson J, Pitts J, Stebbing J. SARS-CoV-2 (COVID-19) superspreader events. J Infect. 2021;82:36–40. https://doi.org/10.1016/j.jinf.2020.11.021
4. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59:307–21. https://doi.org/10.1093/sysbio/syq010

Address for correspondence: Hillary Spencer, Chicago Department of Public Health, Rm 200, 333 S State St, Chicago, IL 60604, USA; email: rhq6@cdc.gov or hillary.spencer@cityofchicago.org

Molecular Diagnosis of *Pseudoterranova decipiens* Sensu Stricto Infections, South Korea, 2002–2020

Hyemi Song,[1] Seungwan Ryoo,[1] Bong-Kwang Jung, Jaeeun Cho, Taehee Chang, Sooji Hong, Hyejoo Shin, Woon-Mok Sohn, Jong-Yil Chai

Author affiliations: Korea Association of Health Promotion, Seoul, South Korea (H. Song, S. Ryoo, B.-K. Jung, J. Cho, T. Chang, S. Hong, H. Shin); Gyeongsang National University College of Medicine, Jinju, South Korea (W.-M. Sohn); Seoul National University College of Medicine, Seoul (J.-Y. Chai)

DOI: https://doi.org/10.3201/eid2806.212483

Human *Pseudoterranova decipiens* larval infections were diagnosed by molecular analysis of mitochondrial *cox*1 and *nd*1 genes in 12 health check-up patients in South Korea during 2002–2020. Based on high genetic identity (99.3%–100% for *cox*1 and 96.7%–98.0% for *nd*1), we identified all 12 larvae as *P. decipiens* sensu stricto.

Human anisakiasis, which is caused by infection with larvae of the family Anisakidae after consuming infested marine fish or squids, is one of the most serious foodborne zoonotic diseases (*1*). Several species of *Anisakis* (*A. simplex* sensu stricto,

[1]These authors contributed equally to this article.

A. physeteris, and *A. pegreffii*) (*1*–*3*), *Pseudoterranova* (*P. decipiens* sensu stricto, *P. azarasi*, and *P. cattani*) (*4*–*6*), and *Contracecum* (*C. osculatum*) (*7*) nematodes have been reported to cause human infections.

Human anisakiasis was reported in the Netherlands during 1960 and has been found to occur in various parts of the world, including Japan and South Korea (*1*). Most human case-patients were infected with larvae of *A. simplex* s.s. (*1*). However, after 1999, a considerable number of cases infected with *A. pegreffii* nematodes (a sibling species of *A. simplex* s.s.) were diagnosed in Italy, Japan, and South Korea on the basis of molecular analysis of the larvae (*2*,*3*). Compared with *Anisakis* spp. nematodes, human infections with *Pseudoterranova* spp. nematodes have been relatively rare in Asia (*1*,*4*–*6*). In South Korea, among 645 anisakidosis cases recorded after 1971 until 2015, only ≈11.8% were infected with *Pseudoterranova* larvae (*8*). However, all of these *Pseudoterranova* infections were diagnosed on the basis of only the morphology of the larvae (*8*).

Within the genus *Pseudoterranova*, 8 species have been validated on the basis of molecular and morphologic/biologic characteristics: *P. decipiens* s.s., *P. kogiae*, *P. ceticola*, *P. azarasi*, *P. krabbei*, *P. bulbosa*, *P. decipiens* E, and *P. cattani* (*9*). Among those, 6 species (*P. decipiens* s.s., *P. krabbei*, *P. bulbosa*, *P. azarasi*, *P. decipiens* E, and *P. cattani*) are morphologically and biologically related to each another and designated as the *P. decipiens* species complex or *P. decipiens* sensu lato (*9*). These species can be discriminated by allozyme or molecular genetic analyses (*10*).

In our study, 12 human pseudoterranoviasis cases were found among patients who visited health check-up centers or hospitals in South Korea during 2002–2020 because of vague abdominal discomfort. Larvae were extracted by using gastrointestinal endoscopy (11 case-patients) or colonoscopy (1 case-patient). The larvae were confirmed to be *P. decipiens* s.s. by sequence analysis of the mitochondrial cytochrome *c* oxidase 1 (*cox*1) and NADH dehydrogenase subunit 1 (*nd*1) genes.

The patients consisted of 5 men (41–55 years of age) and 7 women (29–59 years of age). A total of 12 larvae (1 larva from each patient) were collected from the stomach (11 patients) or cecum (1 patient) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/6/21-2483-App1.pdf) and were processed for sequencing of 2 mitochondrial genes (Appendix).

Sequences of the *cox*1 (141 bp) (samples nos. OK539788–OK539799) and *nd*1 (153 bp) genes (OK539800–OK539807) showed high homologies with the sequences of *P. decipiens* s.s. (GenBank accession

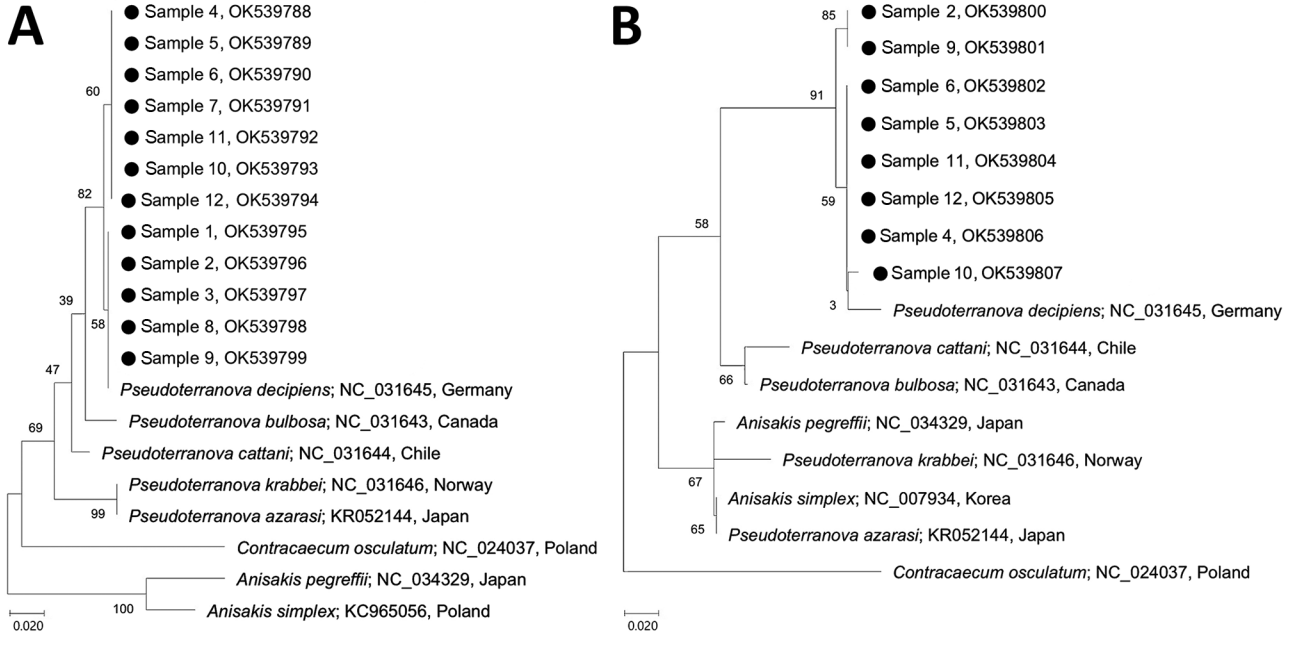

Figure. Phylogenetic analyses of *Pseudoterranova* nematode larvae extracted from 12 health check-up patients in South Korea, 2002–2020 (black dots), in comparison with other anisakid species. A) mitochondrial cytochrome oxidase c gene sequences; B) mitochondrial NADH dehydrogenase gene sequences. Trees were constructed by using the neighbor-joining method based on the Kimura 2-parameter model of nucleotide substitution with 1,000 bootstrap replications and viewed by using MEGA-X (https://www.megasoftware.net). GenBank accession numbers and country of origin are provided for reference sequences. Details of patient information for the 12 samples from this study are provided in Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/28/6/21-2483-App1.pdf). Numbers along branches are bootstrap values. Scale bars indicate nucleotide substitutions/site.

no. NC_031645 for *cox*1 and *nd*1). The homology between samples from this study and *P. decipiens* s.s. was 99.3%–100% for *cox*1 and 97.4%–98.0% for *nd*1 (Appendix Tables 1–3).

The phylogenetic tree for *cox*1 showed that the 12 study samples were tightly clustered with *P. decipiens* s.s. reported from Germany but separate from *P. bulbosa* from Canada, *P. cattani* from Chile, *P. krabbei* from Norway, and *P. azarasi* from Japan (Figure). The phylogenetic tree for *nd*1 showed that 8 study samples were closely aligned with *P. decipiens* s.s. reported from Germany but clearly separated from *P. cattani* from Chile, *P. bulbosa* from Canada, *P. krabbei* from Norway, and *P. azarasi* from Japan (Figure). We also determined genetic distances between the study specimens and *P. decipiens*, *P. azarasi*, *P. bulbosa*, *P. cattani*, and *P. krabbei* for *cox1* (Appendix Table 2) and *nd*1 (Appendix Table 3).

For the specific diagnosis of anisakid larvae, analysis of the larval morphology is highly useful. However, extracting a fully intact larva from human patients for high-quality morphologic analysis is usually difficult. In such instances, molecular analysis of the larvae is helpful and essential for obtaining a specific diagnosis. Analyses of the internal transcribed spacer region and partial 28S rDNA could discriminate *P. decipiens* s.s. from *P. bulbosa*, *P. krabbei*, *P. cattani*, and possibly *P. decipiens* E (*10*). However, great sequence similarity was observed between *P. decipiens* s.s. and *P. azarasi*. Thus, it was difficult to distinguish them by using nuclear genes (*10*). Some investigators used mitochondrial genes, including *cox*1, *cox*2, and *nd*1, to distinguish them (*4*,*5*).

In our study, we used 2 mitochondrial genes, *cox*1 and *nd*1, to distinguish the species of *Pseudoterranova*. Our results showed that the nematode specimens from these patients nested within *P. decipiens* s.s. but were clearly separated from *P. azarasi*, *P. bulbosa*, *P. cattani*, and *P. krabbei* samples available in GenBank. Molecular analysis of larvae will be useful for obtaining specific diagnoses of infection.

Acknowledgments

We thank the endoscopy doctors and staff in the branch offices of the Korea Association of Health Promotion for assistance in collecting *P. decipiens* sensu stricto larval specimens from 12 health check-up patients, and members of the Institute of Parasitic Diseases, Korea Association of Health Promotion for assistance in processing the specimens for morphologic and molecular studies.

About the Author

Dr. Song is a research scientist at the Institute of Parasitic Diseases at Korea Association of Health Promotion, Seoul, South Korea. Her primary research interests are molecular aspects of parasites and parasitic diseases.

References

1. Sohn WM, Chai JY. Anisakiosis (Anisakidosis). In: Palmer SR, Soulsby L, Torgerson PR, Brown DW, editors. Oxford textbook of zoonoses. Oxford (UK): Oxford University Press; 2011. p. 774–6.
2. Lim H, Jung BK, Cho J, Yooyen T, Shin EH, Chai JY. Molecular diagnosis of cause of anisakiasis in humans, South Korea. Emerg Infect Dis. 2015;21:342–4. https://doi.org/10.3201/eid2102.140798
3. Mattiucci S, Fazii P, De Rosa A, Paoletti M, Megna AS, Glielmo A, et al. Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. Emerg Infect Dis. 2013;19:496–9. https://doi.org/10.3201/eid1903.121017
4. Nordholm A, Kurtzhals JA, Karami AM, Kania PW, Buchmann K. Nasal localization of a *Pseudoterranova decipiens* larva in a Danish patient with suspected allergic rhinitis. J Helminthol. 2020;94(E187):e187. https://doi.org/10.1017/S0022149X20000681
5. Arizono N, Miura T, Yamada M, Tegoshi T, Onishi K. Human infection with *Pseudoterranova azarasi* roundworm. Emerg Infect Dis. 2011;17:555–6. https://doi.org/10.3201/eid1703.101350
6. Weitzel T, Sugiyama H, Yamasaki H, Ramirez C, Rosas R, Mercado R. Human infections with *Pseudoterranova cattani* nematodes, Chile. Emerg Infect Dis. 2015;21:1874–5. https://doi.org/10.3201/eid2110.141848
7. Schaum E, Müller W. Heterocheilidiasis: human infection with fish ascarides [in German]. Dtsch Med Wochenschr. 1967;92:2230–3. https://doi.org/10.1055/s-0028-1106122
8. Sohn WM, Na BK, Kim TH, Park TJ. Anisakiasis: report of 15 gastric cases caused by *Anisakis* type I larvae and a brief review of Korean anisakiasis cases. Korean J Parasitol. 2015;53:465–70. https://doi.org/10.3347/kjp.2015.53.4.465
9. 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. https://doi.org/10.1016/S0065-308X(08)00202-9
10. Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA. Molecular phylogenetics and diagnosis of *Anisakis, Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. J Parasitol. 2005;91:1413–29. https://doi.org/10.1645/GE-522R.1

Address for correspondence: Jong-Yil Chai, Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul 03080, South Korea; email: cjy@snu.ac.kr

Article DOI: https://doi.org/10.3201/eid2806.212483

Molecular Diagnosis of *Pseudoterranova decipiens* Sensu Stricto Infections, South Korea, 2002–2020

Appendix

Methods

Larvae preserved in 70% ethanol (3 cases), 10% formalin (1 case), or mounted in glycerin jelly after clearing with glycerin–alcohol (8 cases) were processed for molecular analysis of the mitochondrial genes, in particular, cytochrome *c* oxidase 1 (*cox*1) and NADH dehydrogenase subunit 1 (*nd*1). Genomic DNA was isolated by using the DNeasy Blood and Tissue Kit (QIAGEN, https://www.qiagen.com) according to the manufacturer's instructions.

Based on the complete mitochondrial genome of *Pseudoterranova decipiens* (GenBank accession no. NC_031645), specific pairs of primers were designed by using the tool of the National Library of Medicine (https://www.nlm.nih.gov). The *cox*1 primers used were Pse_*cox*1_F (5′-TGCTGGTTTACACGGTTTTCC-3′) and Pse_*cox*1_R (5′-CGATGA CCCACAAAAGACTCC-3′). The *nd*1 primers were Pse_*nd*1_F (5′-TATTAGGTGGCA GTCAGCAGC-3′) and Pse_*nd*1_R (5′-AAAAGACCCCCGGAACCAAAA-3′). The thermal cycling profile was denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 20 s, annealing at 61°C (*cox*1) or 62°C (*nd*1) for 20 s, and extension at 72°C for 1 min with a final extension at 72°C for 5 min.

DNA sequencing was performed at Macrogen Co. Ltd. (https://www.macrogen.com). Phylogenetic trees were constructed by using the neighbor-joining method and viewed by using the MEGA-X program (https://www.megasoftware.net).

Appendix Table 1. Characteristics of 12 case-patients who had *Pseudoterranova decipiens* larvae infection diagnosed*

Case-patient	Year detected	Age, y/sex	Infection site	Sample storage	Morphologic features of larva, IC/BT/M	% Identity for *cox*1/*nd*1†
1	2002	45/F	Stomach	GJ mount	+/NO/+	100/–
2	2002	45/F	Stomach	GJ mount	+/+/+	100/96.7
3	2003	55/M	Stomach	GJ mount	NO/NO/NO	100/–
4	2004	54/F	Stomach	GJ mount	+/+/+	99.3/98.0
5	2005	39/F	Stomach	GJ mount	+/+/+	99.3/98.0
6	2005	59/F	Stomach	GJ mount	+/+/+	99.3/98.0
7	2008	46/M	Stomach	GJ mount	+/+/NO	99.3/–
8	2010	48/F	Cecum	GJ mount	NO/NO/NO	100/–
9	2015	29/F	Stomach	70% ethanol	NO/+/+	100/96.7
10	2016	48/M	Stomach	10% formalin	+/+/+	99.3/97.4
11	2018	54/M	Stomach	70% ethanol	NO/NO/+	99.3/98.0
12	2020	41/M	Stomach	70% ethanol	+/+/+	99.3/98.0

*BT, boring tooth; GJ, glycerin jelly; IC, intestinal cecum; M, mucron; NO, Not observable because of partial destruction of larva. –, negative; +, positive.
†For *cox1*, GenBank accession no. is NC_031645; for *nd1*, GenBank accession no. is NC_031645.

Appendix Table 2. Genetic distances shown as % differences of *cox*1 sequences (141 bp) among specimens of *Pseudoterranova* larvae

Sample no. and name	1	2	3	4	5	6	7
1 OK539788~OK539794 (this study)							
2 OK539795~OK539799 (this study)	0.7						
3 *P. decipiens* s.s. NC_031645 (Germany)	0.7	0.0					
4 *P. azarasi* KR052144 (Japan)	6.3	7.0	7.0				
5 *P. bulbosa* NC_031643 (Canada)	3.5	2.8	2.8	6.3			
6 *P. cattani* NC_031644 (Chile)	2.8	3.5	3.5	4.9	4.9		
7 *P. krabbei* NC_031646 (Norway)	6.3	7.0	7.0	0.0	6.3	4.9	

Appendix Table 3. Genetic distances shown as % differences of *nd1* sequences (153 bp) among specimens of *Pseudoterranova* larvae

Sample no. and name	1	2	3	4	5	6	7	8
1 OK539800~OK539801 (this study)								
2 OK539802~OK539806 (this study)	1.3							
3 OK539807 (this study)	2.0	0.7						
4 *P. decipiens* s.s. NC_031645 (Germany)	3.3	2.0	2.6					
5 *P. azarasi* KR052144 (Japan)	13.7	13.7	14.4	15.7				
6 *P. bulbosa* NC_031643 (Canada)	8.5	8.5	9.2	9.2	7.8			
7 *P. cattani* NC_031644 (Chile)	10.5	10.5	11.1	11.1	10.5	2.6		
8 *P. krabbei* NC_031646 (Norway)	16.3	16.3	17.0	18.3	3.3	9.8	7.2	