References

1. Mathai J, Raju B, Bavdekar A. Pediatric Gastroenterology Chapter, Indian Academy of Pediatrics. Chronic and persistent diarrhea in infants and young children: status statement. Indian Pediatr. 2011;48:37–42.
2. Lee KS, Kang DS, Yu J, Chang YP, Park WS. How to do in persistent diarrhea of children? Concepts and treatments of chronic diarrhea. Pediatr Gastroenterol Hepatol Nutr. 2012;15:229–36.
3. Holtman GA, Lisman-van Leeuwen Y, Reitsma JB, Berger MY. Noninvasive tests for inflammatory bowel disease: a meta-analysis. Pediatrics. 2016;137:1–11.
4. World Medical Association. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310:2191–4.
5. Day AS, Adamji M. Commentary: impact of fecal calprotectin measurement on decision-making in children with inflammatory bowel disease. Front Pediatr. 2017;5:133.
6. Holtman GA, Lisman-van Leeuwen Y, Kollen BJ, Norbruis OF, Escher JC, Kindermann A, et al. Diagnostic accuracy of fecal calprotectin for pediatric inflammatory bowel disease in primary care: a prospective cohort study. Ann Fam Med. 2016;14:437–45.
7. Salman YJ, Ali CA, Razaq A. Fecal calprotectin among patients infected with some protozoan infections. Int J Curr Microbiol Appl Sci. 2017;6:3258–74.

Esperanza Lepe-Balsalobre [a,*], Ricardo Rubio-Sánchez [a], Cristina Úbeda [b], José M. Úbeda Ontiveros [c]

[a] *Department of Clinical Biochemistry, Valme University Hospital, Seville, Spain*
[b] *Department of Nutrition and Food Science, Toxicology and Legal Medicine, Faculty of Pharmacy, University of Seville, Seville, Spain*
[c] *Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain*

* Corresponding author.
E-mail address: esplepbal@gmail.com
(E. Lepe-Balsalobre).

https://doi.org/10.1016/j.eimc.2020.07.014

Colonic anisakiasis, an infrequent case molecularly characterized by PCR-RFLP☆

Anisakiosis colónica, un caso infrecuente caracterizado molecularmente mediante PCR-RFLP

Anisakiasis is an emerging and underestimated zoonosis in Spain, where the incidence is the highest in Europe.[1] The main aetiological agents belong to the *Anisakis simplex* sensu lato (s.l.) complex, which is made up of three sibling species: *Anisakis simplex* sensu stricto (s.s.), *Anisakis pegreffii and Anisakis berlandi.* There are different clinical forms of anisakiasis: gastric, gastroallergic, intestinal and ectopic. In the intestinal form, the terminal ileum is usually the most affected segment[2] and, conversely, the colon and jejunum, the least involved.[3]

In this study, we present a case of colonic anisakiasis in a 69-year-old Spanish male with a history of colon polyps and endoscopic polypectomy of an adenomatous lesion 30 mm in size two years earlier, who was having a follow-up colonoscopy. The patient reported no gastrointestinal, or any other, symptoms.

In addition to 3 sessile polyps, which were removed, the colonoscopy detected a transparent whitish worm 6–8 mm in length penetrating into the mucosa in the descending colon (Fig. 1A). The worm was sent to the laboratory for molecular identification.

Initially, a morphological identification of the extracted larva was made under the microscope using the Petter and Maillard keys (1988).[4] The partially destroyed whitish, 19-mm-long larva was identified morphologically as compatible with *A. simplex* s.l. Automated extraction of genomic DNA was then performed using the commercial QIAamp® DNA FFPE kit (QIAGEN, Hilden, Germany) with prior overnight incubation at 56 °C with proteinase K and ATL buffer.

For molecular characterisation, the PCR products corresponding to the ITS1-5.8S-ITS2 region of ribosomal DNA were amplified and subsequently digested with HhaI and HinfI restriction enzymes (New England Biolabs, Massachusetts, USA) as per the protocol described by D'Amelio et al. in 2000.[5] The PCR results (Fig. 1B) established that the amplified DNA of the larva had a molecular weight (960 pb) compatible with that expected for the *A. simplex* s.l. complex. The larval transport fluid also showed an amplification at 960 pb, although with less intensity (amplification of free DNA).

In the analysis of the restriction fragment length polymorphisms (RFLP) of the amplified DNA, the larva had the characteristic profiles described for the species *A. simplex* s.s.[5] (Fig. 1C). Sequencing confirmed the identification of the *A. simplex* s.s. genotype and the sequence has been annotated in GenBank (NA: MT516319). This shows high homology with other isolates of the same genotype (99.9% NA: MN871437.1, NA: KY973681.1).

Anisakiasis is unusual in the more distal regions of the gastrointestinal tract; although cases have been reported in the ascending segment, it is rare to find it in the colon.[6,7] Until now Joo et al.[8] were the only authors to have described cases in the descending segment, and none have been reported in Spain.

The clinical manifestations of intestinal anisakiasis are quite diverse, from an asymptomatic condition, to diffuse abdominal pain with nausea, vomiting, etc., to acute abdomen due to intestinal perforation, obstruction, intussusception, etc.[8] An association between colonisation by *Anisakis* spp. and cancer has been suggested, due to the possible tumorigenic effect resulting from persistent local inflammation, as well as that induced by certain secretions of the parasite.[9]

The case we present here was an asymptomatic patient, in whom the anisakiasis was found incidentally. If it had been left to develop freely, there is no saying what the outcome might have been. The location of the larva in the descending colon makes our case more unique. One of the reasons for these larvae being more commonly found in the ascending colon is that intestinal peristalsis is slowed down in that area, and a larva therefore has the ideal conditions for penetration and is not forced to migrate to more distal regions of the colon.[8]

Molecular techniques are very useful in the diagnosis of intestinal anisakiasis, as they work even when the larva is in a poor state of preservation, which is common due to manipulation during the extraction process or the patient's immune response when attempting removal. These techniques also provide genotyping, with unequivocal characterisation at the species level, unlike the morphological study, in which not only must the integrity of the larva be taken into account, but also that it is impossible to distinguish sibling species. Also important is the high sensitivity of molecular techniques, enabling amplification and identification even with the DNA in the sample transport fluid, as demonstrated here.

☆ Please cite this article as: González-Bertolín B, Hernanz-Ruiz N, Pérez-Tanoira R, Perteguer-Prieto MJ. Anisakiosis colónica, un caso infrecuente caracterizado molecularmente mediante PCR-RFLP. Enferm Infecc Microbiol Clin. 2021;39:308–309.

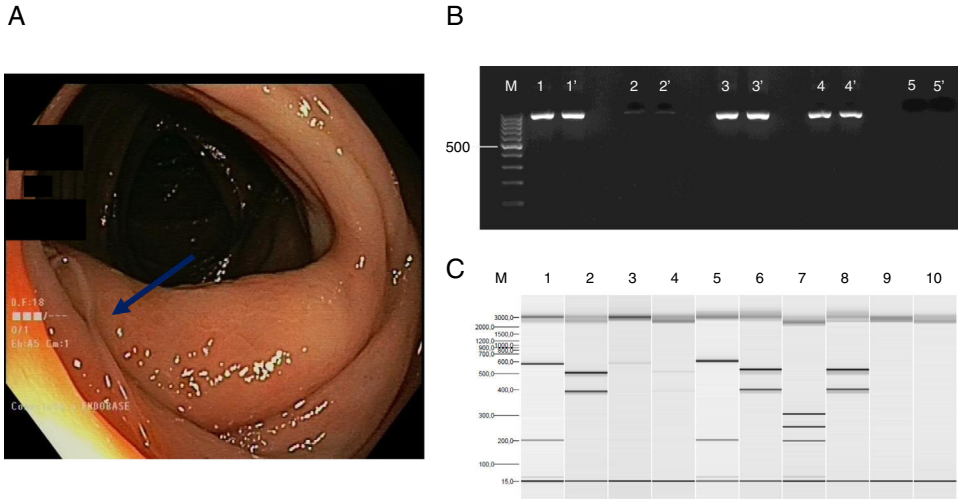

Fig. 1. Image of the parasite in the colon, PCR amplification products and RFLP pattern. (A) Photograph of the *A. simplex* s.s. larva in the descending colon. (B) Amplification products of the ITS1-5.8S-ITS2 region. M) Molecular weight marker. 1–1') Larva sample. 2–2') Larva sample transport fluid. 3–3') *A. simplex* s.s. positive control. 4–4') *A. pegreffii* positive control. 5–5') PCR negative control. (C) RFLP pattern after digestion with HhaI and HinfI restriction enzymes, visualised in QIAxcel® capillary electrophoresis system. M) Molecular weight marker. 1) Larva sample, HinfI. 2) Larva sample, HhaI. 3) Larva sample transport fluid, HinfI. 4) Larva sample transport fluid, HhaI. 5) *A. simplex* s.s positive control, HinfI. 6) *A. simplex* s.s positive control, HhaI. 7) *A. pegreffii* positive control, HinfI. 8) *A. pegreffii* positive control, HhaI. 9) PCR negative control, HinfI. 10) PCR negative control, HhaI.

Despite the high number of anisakiasis cases diagnosed in Spain, the study by Roca-Geronès et al.[10] is the only one published in which molecular identification of the larvae was performed, and the species identified is the same as in our case. Knowledge at the species level adds the benefit of providing information about their invasive and pathogenic potential; in this case, the ability of *A. simplex* s.s. to survive gastric juice and its penetration potential in the colon, previously reported by other authors.[11]

Funding

Study funded by the projects PI17CIII/00019 — Intramural Health Strategic Action (Acción Estratégica de Salud Intramural, ISCIII-AESI) and RD16/0027/0000 — Cooperative Research Network on Tropical Diseases (Red de Investigación Cooperativa en Enfermedades Tropicales, RICET). Belén González-Bertolín has a research assistant contract, PEJ-2017-AI/BMD- 6632, - POEJ-CAM.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Bao M, Pierce GJ, Pascual S, González-Muñoz M, Mattiucci S, Mladineo I, et al. Assessing the risk of an emerging zoonosis of worldwide concern: anisakiasis. Sci Rep. 2017;7:1–17, http://dx.doi.org/10.1038/srep43699.
2. Repiso Ortega A, Alcántara Torres M, González de Frutos C, de Artaza Varasa T, Rodríguez Merlo R, Valle Muñoz J, et al. Anisakiasis gastrointestinal. Estudio de una serie de 25 pacientes. Gastroenterol Hepatol. 2003;26:341–6, http://dx.doi.org/10.1016/s0210-5705(03)70370-7.
3. Shirahama M, Koga T, Ishibashi H, Uchida S, Ohta Y, Shimoda Y. Intestinal anisakiasis: US in diagnosis. Radiology. 1992;185:789–93, http://dx.doi.org/10.1148/radiology.185.3.1438764.
4. Petter A, Maillard C. Larves d' Ascarides parasites de Poissons en Méditerranée occidentale. Bull Mus natn Hist nat. 1988;2:347–69, section A.
5. D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanço M, et al. Genetic markers in ribosomal DNA for the identification of members of the genus Anisakis (Nematoda: Ascaridoidea) defined by polymerase-chain-reaction-based restriction fragment length polymorphism. Int J Parasitol. 2000;30:223–6, http://dx.doi.org/10.1016/S0020-7519(99)00178-2.
6. Tsukui M, Morimoto N, Kurata H, Sunada F. Asymptomatic anisakiasis of the colon incidentally diagnosed and treated during colonoscopy by retroflexion in the ascending colon. J Rural Med. 2016;11:73–5, http://dx.doi.org/10.2185/jrm.2914.
7. Amir A, Ngui R, Wan Ismail WH, Wong KT, Ong JSK, Lim YAL, et al. Case report: anisakiasis causing acute dysentery in Malaysia. Am J Trop Med Hyg. 2016;95:410–2, http://dx.doi.org/10.4269/ajtmh.16-0007.
8. Joo SK, Kim JW, Kim BG, Kim W, Lee JK, Lee KL. Clinical and endoscopic features of colonic anisakiasis in Korea. Korean J Parasitol. 2019;57:411–6, http://dx.doi.org/10.3347/kjp.2019.57.4.411.
9. Garcia-Perez JC, Rodriguez-Perez R, Ballestero A, Zuloaga J, Fernandez-Puntero B, Arias-Diaz J, et al. Previous exposure to the fish parasite anisakis as a potential risk factor for gastric or colon adenocarcinoma. Medicine (Baltimore). 2015;94, http://dx.doi.org/10.1097/MD.0000000000001699.
10. Roca-Geronès X, Alcover MM, Godínez-González C, González-Moreno O, Masachs M, Fisa R, et al. First molecular diagnosis of clinical cases of gastric anisakiosis in Spain. Genes. 2020;11:452, http://dx.doi.org/10.3390/genes11040452.
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.

Belén González-Bertolín[a,*], Nerea Hernanz-Ruiz[b], Ramón Pérez-Tanoira[c], María Jesús Perteguer-Prieto[a]

[a] *Laboratorio de Helmintos, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain*
[b] *Servicio de Aparato Digestivo, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain*
[c] *Servicio de Microbiología, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain*

* Corresponding author.
E-mail addresses: belen.gonzalez@isciii.es, belengbertolin@gmail.com (B. González-Bertolín).

https://doi.org/10.1016/j.eimce.2021.04.001