

Human Anisakiasis in Italy: A Report of Eleven New Cases

Silvio Pampiglione¹, Francesco Rivasi², Mario Criscuolo³,
Anna De Benedittis³, Antonia Gentile⁴, Silvana Russo⁴,
Mario Testini⁵, Michele Villani⁶

¹Department of Veterinary Public Health and Animal Pathology, University of Bologna,

²Department of Morphological Sciences and Forensic Medicine, Section of Pathology,
University of Modena and Reggio Emilia, Modena,

³Service of Pathology, Hospital "A. Perrino", Brindisi,

⁴Department of Pathology and Genetics and

⁵Department of Surgery, University of Bari,

⁶Service of Pathology, Hospital "A. Cardarelli", Campobasso, Italy

Summary

The authors report on eleven new human cases of anisakiasis occurring in Italy, and emphasize the importance of the infection in clinical medicine, histopathology and public health. For ten of these cases, the diagnosis was based on histological findings: an eosinophilic granuloma associated with a larva of *Anisakis* sp. For one of them, the larva was removed from the stomach by endoscopy. Nine of the subjects were from Apulia and two from Molise (regions of southern and central Italy, respectively). Ten of them were surgically treated, and in one case the endoscopical extraction of the parasite resolved the situation. In two cases, the gastric wall was affected, in three the intestinal wall, in a further three the omentum, in one the spleen, and in the final two the mesentery and the epiploic appendix. In all the cases, the parasite was discovered unexpectedly during surgical treatment of the patients for supposed illnesses, which had originally been misdiagnosed. In three cases, the patients were also affected by cancer.

Human anisakiasis must be taken into account in the differential diagnosis of acute, abdominal syndromes in subjects who have ingested raw fish or squid a few hours to a few days before the onset of symptoms. Histopathologists should consider the possibility of this parasitic infection when confronted with an eosinophilic granuloma of the digestive tract, mesentery or peritoneum. The incidence of anisakiasis in Italy is probably

higher than reported, as some cases might not be diagnosed and others might heal spontaneously.

Key words: *Anisakis* – Human – Histopathology – Endoscopy – Italy

Introduction

In past decades, parasitologists and pathologists focused their attention on the apparently more frequent cases of helminthic infections in humans, which are caused by nematode parasites of other animal species. Among them, anisakiasis has become significantly important for several reasons: a) Its geographical distribution across all the continents. b) The public health issues it involves (it is inherent to the ingestion of marine animals that are potential carriers of the parasite). c) The diagnostic difficulties it causes both clinically and histopathologically. d) Last but not the least, the severity of the disease, which may often lead clinicians to resort to surgery.

Address for correspondence: Francesco Rivasi, Department of Morphological Sciences and Forensic Medicine, Section of Pathological Anatomy, University of Modena and Reggio Emilia, Via del Pozzo N. 71, 41100, Modena, Italy.
e-mail: rivasi@unimo.it

Anisakiasis is a parasitic zoonosis caused by larvae of nematodes of the family Anisakidae (Ascaridoidea), which are habitual parasites in adult marine mammals. The larvae utilize crustaceans, cephalopods and fish as intermediate and/or parathenic hosts. Man can accidentally be infected by eating such hosts, either raw or inadequately treated (pickled, smoked or salted). Once the infected food is ingested, the nematode enters the digestive tract by penetrating the gastric or intestinal wall. It sometimes reaches the peritoneum, the mesentery or the omentum, and in rare occasions other viscera, causing inflammatory reactions with acute to milder chronic forms of abdominal disorders. The pathogenesis seems to be caused not only by the physical action of perforation by the larvae (of a size ranging from 1.7–2.8 cm), but also by the associated allergic Arthus type and/or anaphylaxis reactions [12]. The infection is therefore linked to eating habits where raw fish salads are widely consumed, typical of some cultures such as in Japan [6]. These eating habits, spread among several populations inhabiting both ocean and sea coastal areas, also occur in Italy, where 14 cases of human anisakiasis have been recorded since 1996. The authors deem it useful to report eleven new cases observed recently in Italy.

Materials and Methods

Of the eleven cases of human anisakiasis identified, six were from the files of the Department of Pathology of the Universi-

ty of Bari, three from the files of the Service of Pathology of the Hospital of Brindisi, and two from the files of the Service of Pathology of the Hospital of Campobasso, over the period 1996 to 2001.

The specimens consisted, in nine cases, of excisional, wedge biopsies, taken during surgical interventions and containing the nematodes. In the other two cases, the nematodes were picked up during surgery and endoscopy, respectively. Diagnosis was always based on histological sections. The excised nodules and the nematodes were fixed in buffered formalin (10%) and routinely processed. Three to six histological sections, stained with haematoxylin-eosin, PAS and Masson Goldner Trichrome, were examined in each case.

The histological slides were reviewed by two of the authors (S.P. and F.R.), one a parasitologist, the other a histopathologist, who paid particular attention to the parasitological and histological aspects.

Results

Clinical aspects

The clinical data are summarized in Table 1. Patient age ranges from 22 to 86 years. Eight patients were women and three were men. Nine of the subjects were from Apulia and two from Molise (southern and central regions of Italy, respectively). The clinical manifestations varied. Eight cases were acute: they were diagnosed as acute abdominal syndrome (three cases), acute appendicitis (two cases), and intestinal occlusion (three cases). Three cases were subacute: the clinical diagnoses were not attributed to the direct ac-

Table 1. Clinical and pathological data of the eleven cases of human anisakiasis

No.	Date	sex/age	Locality	Location	Raw fish ingested	Clinical diagnosis	Associated pathology	Therapy	Condition of the worm
1	1996	F/30 y.	Bari	ileum	now and then	acute abdominal syndrome	colon adeno-carcinoma, peritonitis	surgery	Necrotic
2	1996	F/22 y.	Campobasso	mesentery	few days before	acute appendicitis	stenosed ileal tract	surgery	well conserved
3	1997	F/54 y.	Bari	omentum	few days before	intestinal occlusion	sigmoid colon neoplasia	surgery	poorly conserved
4	1997	M/37 y.	Bari	omentum	now and then	gastric ulcer	diffuse intestinal metaplasia, cicatrized peptic ulcer	surgery	poorly conserved
5	1998	F/44 y.	Campobasso	omentum	4 days before	intestinal occlusion	stenosed ileal tract	surgery	well conserved
6	1998	F/60 y.	Bari	epiploic appendix	habitually	colon neoplasia	sigmoid flexure neoplasia	surgery	poorly conserved
7	2000	M/48 y.	Brindisi	ileum	8 hours before	intestinal occlusion	stenosed jejunal loop	surgery	well conserved
8	2000	F/41 y.	Brindisi	ileum	10 days before	acute appendicitis	ileitis and peritonitis	surgery	well conserved
9	2000	M/56 y.	Bari province	stomach	few hours before	acute abdominal syndrome	gastric adenocarcinoma with metastasis	surgery	necrotic
10	2000	F/86 y.	Bari	spleen	habitually	acute abdominal syndrome	small gastric perforation, spleen granuloma	surgery	poorly conserved
11	2001	F/56 y.	Brindisi	stomach	6 hours before	epigastralgia	hemorrhagic gastritis	endoscopy	well conserved

tion of the parasite, but to other concomitant pathology: gastric cancer, sigmoid colon cancer, and peptic ulcer, respectively. Treatment was always surgical, with the exception of the last case, where the parasite was detected and picked up by endoscopy. Some cases were completely dormant, the parasitic nodules coming to the attention of the surgeon only by chance. All the patients had eaten raw sardines or anchovies treated according to the customary recipe (i.e. filleted and left to soak overnight in lemon juice, oil, salt and parsley), from 6–8 hours to 7–8 days before the onset of the symptoms. Recurrent edemas in the form of transient swellings, erythema, rashes, widespread itching occurring before or during the development of the infection were rarely manifested in the patients.

Histopathological and parasitological findings

Histopathological features of the lesions in cases 1 to 6 and 9 consisted of a granuloma surrounding the larva of

the nematode (Fig. 1a, b). Necrotic tissue was located on the inner layer of the granuloma, with an intense infiltration of eosinophils and neutrophils. The surrounding granulomatous tissue was infiltrated by eosinophils, histiocytes, plasmocytes and lymphocytes with edema and fibrin exudation or fibrinoid degeneration. More peripheral tissues were also infiltrated by scattered, inflammatory and mononuclear cells.

In cases 1 and 9, the parasite was located in the thickened sub-serosa, without evident ulceration of the surface mucosa.

In the seventh case, the ileal wall was completely affected: it was thicker than normal due to intensive edema. Massive cellular infiltration (with eosinophils, neutrophils, monocytes and lymphocytes) was present, particularly in the sub-mucosal and serosal layers. The most intensive histological changes were seen around the *Anisakis* larva. Ulceration and necrosis were observed in the mucosal layer; the muscular layer present-

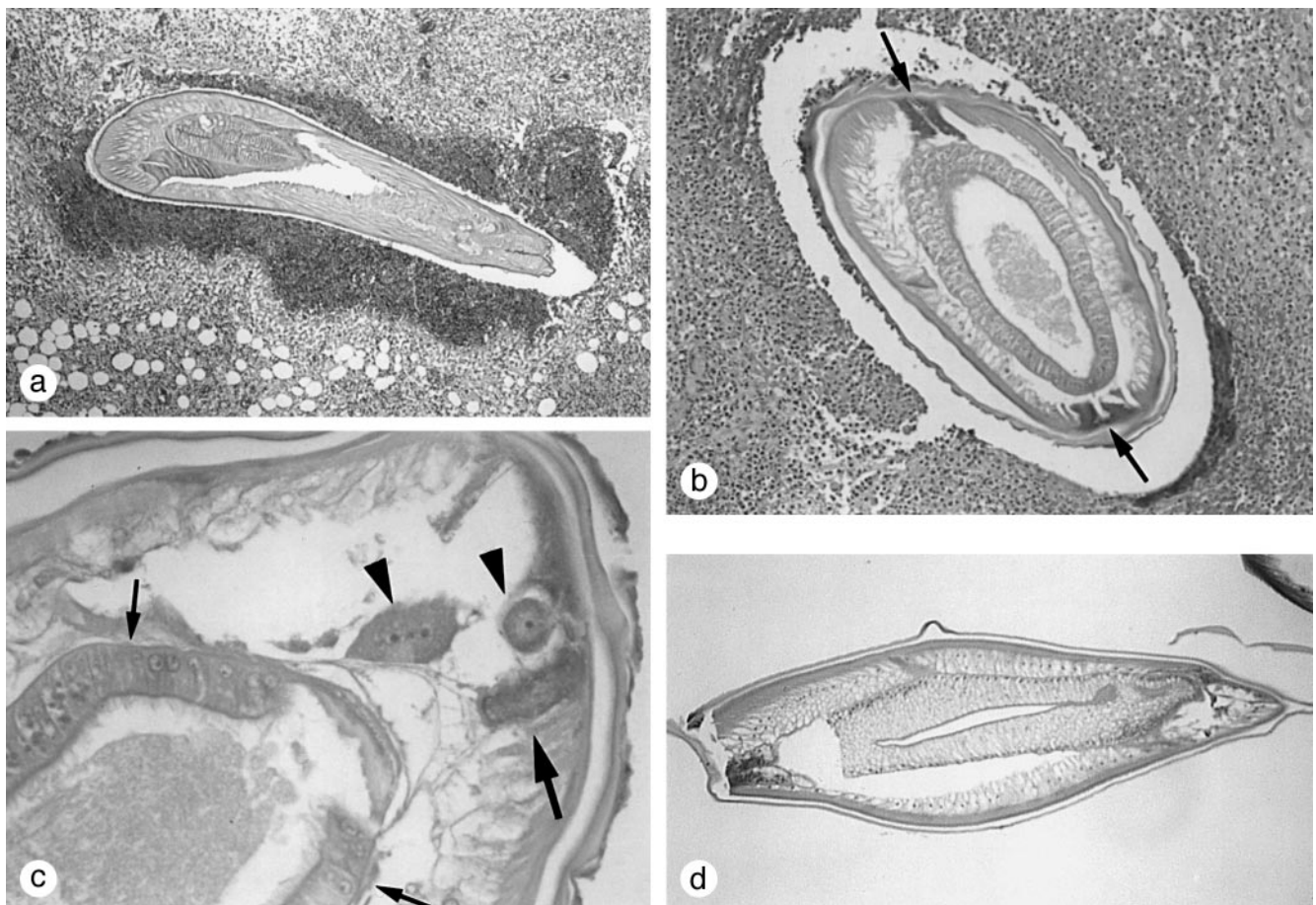


Fig. 1. **a:** Oblique section of larva of *Anisakis* sp. in an eosinophilic granuloma of ileal mesentery (hematoxylin and eosin; original magnification $\times 60$). **b:** Para-transverse section of a larva surrounded by abscess granulomatous type tissue. Lateral chords are well visible (arrows), (hematoxylin and eosin; original magnification $\times 150$). **c:** Higher magnification through the anterior end of *Anisakis* sp. larva showing the excretory gland cell (arrow heads), a lateral chord (thick arrow) and the intestinal ciliated epithelium (thin arrows), (hematoxylin and eosin; original magnification $\times 250$). **d:** A longitudinal section of the larva extracted by endoscopy from the stomach (hematoxylin and eosin; original magnification $\times 150$).

ed less inflammatory changes, neither edema nor fibrin exudation and only mild cell infiltration. The sub-serosa was swollen and showed fibrosis, especially in perivascular areas.

In case 8, the nematode adhered to the sub-serosa, with signs of peritonitis.

In case 10, the nematode caused an eosinophilic granuloma in the spleen. The spleen was surrounded by gastric juice and showed a 3 cm solid, not well circumscribed nodular area at the superior pole. In this area, some necrotic foci were located around the degenerated worm. Histological examination of the excised stomach tissue revealed the presence of an ulcerous-type gastritis with acute, inflammatory cells and a few multinucleated, foreign-body giant cells.

In case 11, the larva was detected in the gastric mucosa during endoscopy (Fig. 2). No severe tissue lesions were observed.

In all cases, the morphological characteristics of the nematode definitely pointed to a stage III *Anisakis* larva, probably *A. simplex*. Its diameter ranged between 265–470 µm, and its cuticle thickness was 5–6 µm; its lateral chords were typically shaped, with two butterfly-like lobes. The larva also had polymiary-shaped myoid tissue, a thick, muscular esophagus and an intestinal tubule occupying most of the pseudocelomatic cavity, with a cylindrical and slightly ciliated epithelium (Figs. 1c, d).

Discussion

The first published cases of anisakiasis came from the Netherlands [19] in 1960 and concerned patients who had eaten raw herrings a few hours or a few days before

admission to hospital. Since then a progressive increase in cases has been recorded, more attention being paid to the subject by doctors and histopathologists. Japan and other Far-Eastern countries account for thousands of cases: almost 13,000 were diagnosed between 1968 and 1989 in Japan, where anisakiasis is considered a “leading problem” [6]. Just over 100 cases have been detected in Europe, distributed across countries with a coastline (the Netherlands, the United Kingdom, France, Spain, Italy) [4, 9, 14, 15, 17]. Many more cases are suspected where subjects manifest serological positivity, without detection of parasites. The relatively small number of cases recorded in Europe may be explained as follows: a) The eating habits favoring the infection are more limited than in Far-Eastern countries. b) European clinicians have little knowledge of the subject. c) The histological diagnosis can often be difficult due to a poor conservation state of the larva. Some cases considered as eosinophilic granuloma of the digestive tract with unknown etiology might also have been caused by *Anisakis*.

The presence of *Anisakis* larvae has been reported several times in many species of fish from Italian seas, such as sardines, anchovies, mackerel, whiting and horse mackerel. The larvae were found both in the lumen of the digestive tract and in the musculature of the fish (Fig. 3) [15].

The first correctly diagnosed human case in Italy dates back to 1996 [18] and referred to a 40-year-old woman from Bari. The parasite was located in the gastric wall. Eight other cases were subsequently detected [1, 2, 3, 10, 16, 18]: two in Sicily, five in Apulia and one in Rome. Five more cases were diagnosed as “probable” (i.e. only positive serologically); three of these occurred in



Fig. 2. Gastrosopic direct visualization of *Anisakis* sp. larva adherent to the gastric mucosa (courtesy of Doctor G. Chiriatti).



Fig. 3. Larva of *Anisakis* sp. coming out from anchovy (*Engraulis encrasicolus*) (courtesy of Professor M.L. Fioravanti).

Pescara, one in Bari and one in Sicily, respectively [5, 16]. Of these fourteen cases, eight underwent surgery, i.e. resection of the ileal loop, partial colectomy, colectomy, explorative laparotomy and splenectomy.

In this study, the larva was located in the gastric mucosa in one case, in the wall of the stomach in another and in the wall of the small bowel in three cases; in the six other cases, it had an extra-intestinal location (three in the omentum, one in the spleen and two in the mesentery and the epiploic appendix, respectively). The location in the spleen (case no. 10) seems to be unusual. No such cases, in fact, have been described in the literature. Three of our cases were associated with malignant tumors of the intestinal tract, confirming the possible relationship between cancer and anisakiasis: a certain fraction of the antigens of *Anisakis* larvae demonstrated mutagenic factors and tumor-promoting activity [6].

From a histopathological point of view, according to Kojima et al. (1966) [8], our cases can be classified as follows: seven abscess-granulomatous type, one granulomatous, one phlegmon-type and one abscess type. In one case, no severe tissue lesions were observed.

From a clinical point of view, eight cases were considered acute, and three sub-acute. Clinical diagnosis was wrong in every case.

Despite the small number of cases examined, it seems that *Anisakis* parasite prefers human adults to children; also, women seem to be infected more frequently than men.

Conclusions

Human anisakiasis must be considered in diagnoses of acute abdominal pains, in subjects who have ingested raw or insufficiently processed fish a few hours or a few days before the onset of the pathological symptoms. Clinicians must be aware of this, especially in seaside localities where eating habits involve raw fish and whenever the patient's history enhances infection. Peripheral eosinophilia, as well as the increase in leukocytes and in the ESR, is not constant, especially at the beginning of the clinical manifestations. Positive serological tests can be a useful indicator, but clinicians can only be certain of the diagnosis when finding the nematode larva. Histopathologists should also consider the possibility of *Anisakis* infection when facing an eosinophilic granuloma of the digestive tract, the mesentery, or the peritoneum. Several sections of the eosinophilic granuloma must be performed in seeking sections of the nematode. Before surgery is decided upon, however, it is necessary to examine endoscopically the stomach or the intestinal tract. It is also useful to carry out serological testing (i.e. complement fixation test, indirect hemoagglutination test, Ouchterlony immunoelectrophoresis, ELISA) or to test for PCR [3]. The diagnosis, however, may be difficult as the symptoms are not specific; the histological sections may be difficult to read and,

lastly, serological reagents are not completely reliable or difficult to obtain. With regard to diagnosis, the importance of the patient's history (whether they ingested raw fish and cephalopods) should be taken into account. In cases of no fish consumption, it is possible to eliminate the presence of *Anisakis*. However, it is worthwhile bearing in mind that patients may be bad at remembering what they have eaten.

Specific therapy with ascaricide drugs may be attempted [7, 14]. Experiments on rats treated with pyrantel pamoate seem to have shown favorable results [7]. It must, however, be remembered that once the *Anisakis* larvae have penetrated the thickness of the intestinal wall, though subsequently killed by the drug, they will still cause their hosts to experience a sustained, antigenic reaction, contributing to the development of eosinophilic granuloma [13]. Anti-inflammatory therapy and antibiotics may be advantageous if there is suspicion of an overlapping bacterial infection.

Prophylaxis is based on health education principles aiming at informing communities of the risks of the infection associated with the ingestion of raw fish or cephalopods.

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