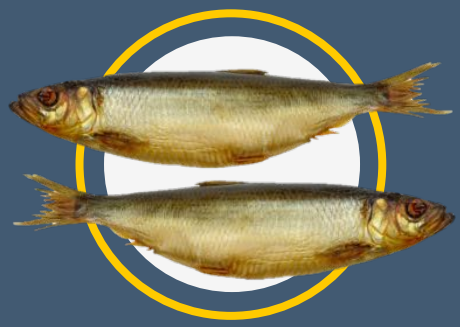
Anisakis spp. larvae in marketed products made of herring (*Clupea harengus*)



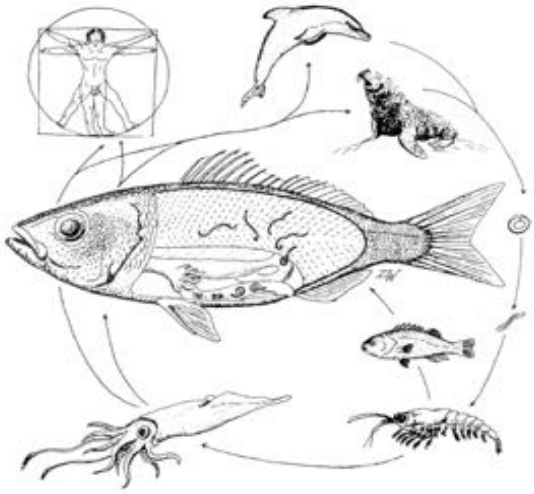
Guardone L 1, Bordino M 1, Rosellini N 1, Nucera D 2, Tinacci L 1, Guidi A 1, Armani A 1
1 Department of Veterinary Sciences, University of Pisa, viale delle Piagge 2, 56124 Pisa; 2 Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Braccini 2, 10095, Grugliasco - Turin (Italy)

UNIVERSITÀ DI PISA FISHLAB UNIVERSITÀ DEGLI STUDI DI TORINO
ECVPH AGM & Annual Scientific Conference 2018 ECVPH

1. INTRODUCTION

- **First commercial species** landed in the **EU** for **volume of products (15%)**, covers 110% of EU request
- **Third most commercialized species in the EU** (EUMOFA report, 2017)
- **Fresh products** and traditional **whole smoked products**
- Packets of **fillets, smoked, marinated or canned → ready to eat**

Herring represents one of the main host species of *Anisakis* spp., often called «herring worm», due to the association between the first confirmed human cases of anisakiasis and the consumption of lightly salted («*green*») herrings in the Netherlands
(Van Thiel et al., 1960; Van Thiel 1962).



Life cycle of *Anisakis* spp.

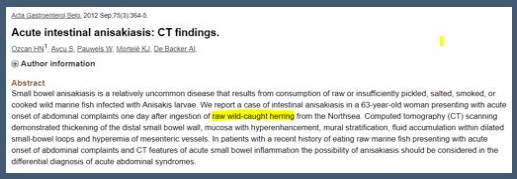
Anisakis spp. in **fresh herrings**:

- Levsen *et al.*, 2018
- Bao *et al.*, 2017
- Levsen *et al.*, 2017
- Unger *et al.*, 2014
- Campbell *et al.*, 2007
- Levsen *et al.*, 2005
- Tolonen & Karlsbakk, 2003
- Mattiucci & D'amelio, 1989
- Huang, 1988
- Hauck *et al.*, 1977
- Smith & Wotten, 1975

Anisakis spp. in **processed herrings**:

- Levsen & Lunestad, 2010
- Szostakowska *et al.*, 2005
- Panebianco & Lo Schiavo, 1985
- Hauck *et al.*, 1977
- Khalil, 1969

Reg. EU No 853/2004:
if a seafood product is consumed raw, or if treatments are not sufficient for larvae devitalization, a preventive cold treatment should be conducted



Acta Gastroenterol Belg. 2012 Sep;75(3):364-5.

Acute intestinal anisakiasis: CT findings.

Ozcan HN[1], Avcu S, Pauwels W, Mortelé KJ, De Backer AI

Author information

Abstract

Small bowel anisakiasis is a relatively uncommon disease that results from consumption of raw or insufficiently pickled, salted, smoked, or cooked wild marine fish infected with Anisakis larvae. We report a case of intestinal anisakiasis in a 63-year-old woman presenting with acute onset of abdominal complaints one day after ingestion of raw wild-caught herring from the Northsea. Computed tomography (CT) scanning demonstrated thickening of the distal small bowel wall, mucosa with hyperenhancement, mural stratification, fluid accumulation within dilated small-bowel loops and hyperemia of mesenteric vessels. In patients with a recent history of eating raw marine fish presenting with acute onset of abdominal complaints and CT features of acute small bowel inflammation the possibility of anisakiasis should be considered in the differential diagnosis of acute abdominal syndromes.

Z Gastroenterol. 2001 Feb;39(2):177-80.

[Anisakiasis of the stomach--a case report from Germany].

[Article in German]

Puth F[1], Hofe A, Zenglein D, Müller FW, Barten M, Reisinger EC, Liebe S

Author information

Abstract

Anisakiasis or "herring worm disease" is one of the most important parasitic diseases of the gastrointestinal tract in Japan. In 1988 Lorenz and Warzok published 8 cases of intestinal anisakiasis in Eastern Germany. In 1988 Spehn et al. reported a case of gastric anisakiasis in an AIDS patient. Here, we describe a case of gastric anisakiasis in Germany with an impressive serious clinical course. The symptoms--acute abdominal cramps, severe chest pain, diarrhoea, sub-febrile temperatures and leucocytosis--followed 4 h after consumption of raw herring, which was homemade pickled in vinegar. The conventional and the endoscopic ultrasonography showed a thickened gastric wall made of mainly thickened submucosa. The larvae of Anisakis in the gastric mucosa were found and extracted endoscopically. Acute and severe abdominal pain after eating raw fish is an indication for early gastroscopy. The endoscopical extraction of possible larvae is the only effective therapy, as anthelmintics against nematodes (mebendazole, albendazole, thiabendazole) are ineffective.

Z Gastroenterol. 2009 Oct;47(10):1059-61. doi: 10.1055/s-0028-1109468. Epub 2009 Oct 6.

[The first case of anisakiasis acquired in Austria].

[Article in German]

Kaspral C[1], Haditsch M, Wewalka F, Schatzlmayr W, Lenz K, Auer H

Author information

Abstract

Anisakiasis is caused by a fish parasite of the Nematode family. This kind of rare helminthozoonosis can mainly be found in countries where consumption of raw fish is traditionally high like Japan, the Netherlands, Pacific Islands, South Europe, Scandinavia, USA, and Canada. Man is the wrong hoste. Clinical manifestation depends on the localisation of penetration in the GI tract. In Japan, predominantly the stomach is affected in 97 % of cases, probably due to hypo- and achlorhydria, whereas mainly intestinal anisakiasis occurs in Europa. We report on a 67-year-old male patient with a gastric infestation of anisakiasis. The patient was on proton pump inhibitor which migh have caused the localisation of the infestation. The anisakis was an accidental endoscopic finding in a patient for control of an H. p.-positive gastric ulcer. Otherwise the patient was free of pain. The helminth (larva III) was endoscopically extracted. Thereafter, the patient remained in good health. Anisakis serology as well as repeated differential blood counts were without finding. The uneventful medical history and the normal blood findings indicate that our patient had a very early stage of infestation of anisakiasis. The patient reported no stay outside of Austria within the last years. However, he consumed on a regular basis "rolled pickled herring" produced by a well-known Viennese company for canned fish. This is the first documented case of this rare helminthozoonosis acquired in Austria.

Live parasites

→ **hazard** for public health

→ product **injurious to health**

Dead (visible) parasites

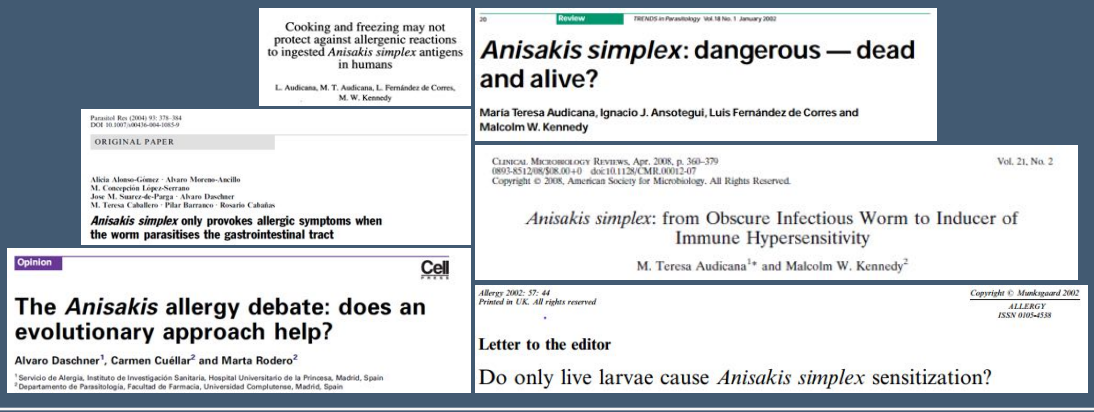
→ **defect** (CODEX STAN 244-2004)

→ product **unfit for human consumption**

Reg. 178/2002: both **unsafe** products → should not be placed on the market

Reg. 853/2004: products **obviously contaminated by parasites** should **not** be placed on the market

The **allergenic potential** of **dead** *Anisakis* spp. larvae is **debated**.
Contact with a live larva is generally deemed necessary for sensitization and for the trigger of an allergic reaction, but in a small number of sensitized patients allergic reactions may occur even following concatct with the proteins of dead larvae
(Nieuwenhuizen *et al*., 2016; Levsen & Lunestad, 2010; Daschner *et al*., 2008).



Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans

L. Audicana, M. T. Audicana, L. Fernández de Corres, M. W. Kennedy

Anisakis simplex: dangerous — dead and alive?

María Teresa Audicana, Ignacio J. Ansotegui, Luis Fernández de Corres and Malcolm W. Kennedy

ORIGINAL PAPER

Alicia Alonso-Gómez · Alvaro Moreno-Ancillo · M. Concepción López-Serrano · Jose M. Suarez-de-Parga · Alvaro Daschner · M. Teresa Caballero · Pilar Barranco · Rosario Cabañas

***Anisakis simplex* only provokes allergic symptoms when the worm parasitises the gastrointestinal tract**

Clinical Microbiology Reviews, Apr. 2008, p. 360–379 Vol. 21, No. 2
0893-8512/08/$08.00+0 doi:10.1128/CMR.00012-07
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Anisakis simplex: from Obscure Infectious Worm to Inducer of Immune Hypersensitivity

M. Teresa Audicana[1]* and Malcolm W. Kennedy[2]

Opinion

The *Anisakis* allergy debate: does an evolutionary approach help?

Alvaro Daschner[1], Carmen Cuéllar[2] and Marta Rodero[2]

[1] Servicio de Alergia, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, Madrid, Spain
[2] Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense, Madrid, Spain

Allergy 2002: 57: 44
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ALLERGY
ISSN 0105-4538

Letter to the editor

Do only live larvae cause *Anisakis simplex* sensitization?

2. OBJECTIVE

To assess the **occurrence, distribution** and **viability** of *Anisakis* spp. larvae in herring products sold in Italy

3. MATERIALS AND METHODS

3.1 SAMPLING

120 commercial products:

Whole herrings (N=50)		Filleted products (N=70)		
Smoked golden	Smoked silver	Smoked fillets	Marinated fillets	Canned fillets
N=25	N=25	N=25	N=30	N=15

- Bought in supermarkets in Pisa or sampled at the Border Inspection Point of Livorno (2016 – 2018)
- Registered with an internal unique code, photo, registration of label data

3.2 VISUAL INSPECTION

Direct observation by naked eye to detect **visible parasites**
(Reg. CE 853/2004; Reg. CE 2074/2005; Decision 93/140/EEC)

"**visible parasites**": non capsulated parasites longer than 10 mm, if capsulated larger than 3 mm
(Codex Alimentarius Commission, 1971)

- **Whole herrings**: eviscerated to inspect the viscera and the the visceral cavity
- **Marinated and canned fillets**: visual inspection also of marinating liquid or sauces if present

3.3 ARTIFICIAL DIGESTION

- Hcl and pepsin digestion (200 gr, 37°C, 20 min) using Trichineasy® (CTSV srl, Brescia), an integrated system of digestion and filtration validated for the analysis of fish tissue by the National Reference Centre for Anisakiasis (Cammilleri *et al.*, 2016).

Whole herrings:
Separated digestion of **viscera and muscle** → **manually filetted** herrings, fillets included skin and belly flaps

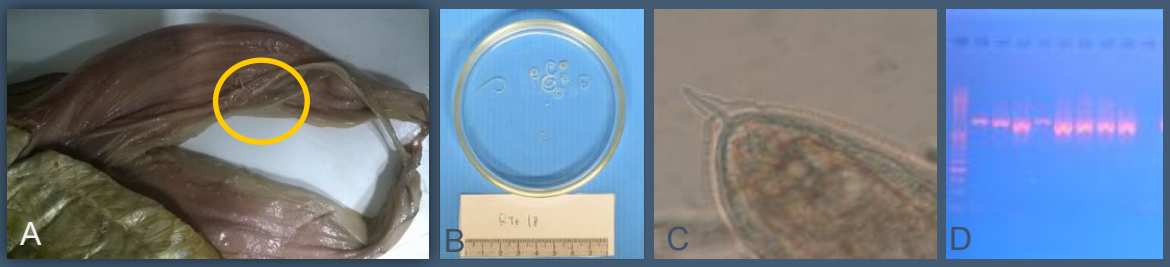
Fillets:
Digestion of the whole packet content (tissues and liquid/sauce when present)

- After the digestion, the **material** was **filtered** (sieve: 180 µm), subdivided in Petri plates and **examined** under **natural** and **UV** (365 nm) light.

3.4 COLLECTION OF THE LARVAE AND ASSESSMENT OF THEIR VIABILITY

Visible larve detected **during the visual inspection or after the digestion and filtration:**

- counted and identified to genus level by microscopy
- assessment of viability → **spontaneous or stimulated movements** (CODEX STAN 244-2004 Standard for salted atlantic herring and salted sprat)



Visible larvae during visual inspection (A), after digestion and filtration (B), microscopy (C) and amplicons from PCR (D)

- stored (70% alcohol, 4°C), keeping separate larvae from the viscera and the muscle
- molecular analysis → **COX2** (long and short fragment)

3.5 STATISTICAL ANALYSIS

Groups of comparison	Parameters included in the analysis									
	Positivity rate		MA		MI		N of larvae/product		Larval density/gram	
	V	M	V	M	V	M	V	M	V	M
Whole herrings (1) / Fillets (2)	-	χ2	-		-		-	MW	-	MW
Whole golden (1a) / Whole silver (1b)	χ2		MW		MW		MW		MW	
Smoked fillets (2a) / Marinated fillets (2b) / Canned fillets (2c)	-	χ2	-		-		-	KW	-	KW

- Depending on the product type, parameters calculated at visceral and muscle level, in the 2 categories of products (whole herrings/fillets) and in the subcategories
- The differences in the examined categories and subcategories were investigated using Chi-squared, Mann-Whitney and Kruskal-Wallis tests. Significant when p>0.05

4. RESULTS AND DISCUSSION

4.1 OCCURRENCE AND DISTRIBUTION OF THE LARVAE

1715 *Anisakis* spp. **larvae** from **56** products → **46.7%** of the analyzed products positive for at least 1 larva

1559 larvae (**91%**) in the **viscera** of 49 of the 50 **whole herrings**

- Prevalence (P): 98%
- Mean abundance (MA): 31,2
- Mean ntensity (MI): 31,8
- Range: 0 - 172

In agreement with other studies:

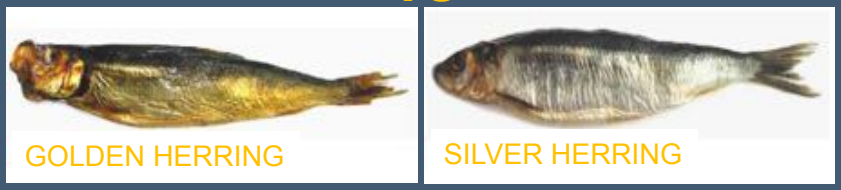
- Levsen et al., 2018
- Levsen and Lunestad, 2010
- Etc..

Most of the larvae located in the viscera
(Mattiucci et al., 2017)

4.1 OCCURRENCE AND DISTRIBUTION OF THE LARVAE: VISCERA (whole herrings)

		visceral larvae						
subcategories	n products	pos	% pos	n larvae	MA	MI	range	Mean density
whole golden (1)	25	24	96	738	29,5	30,8	0-172	
whole silver (2)	25	25	100	821	32,8	32,8	5-76	

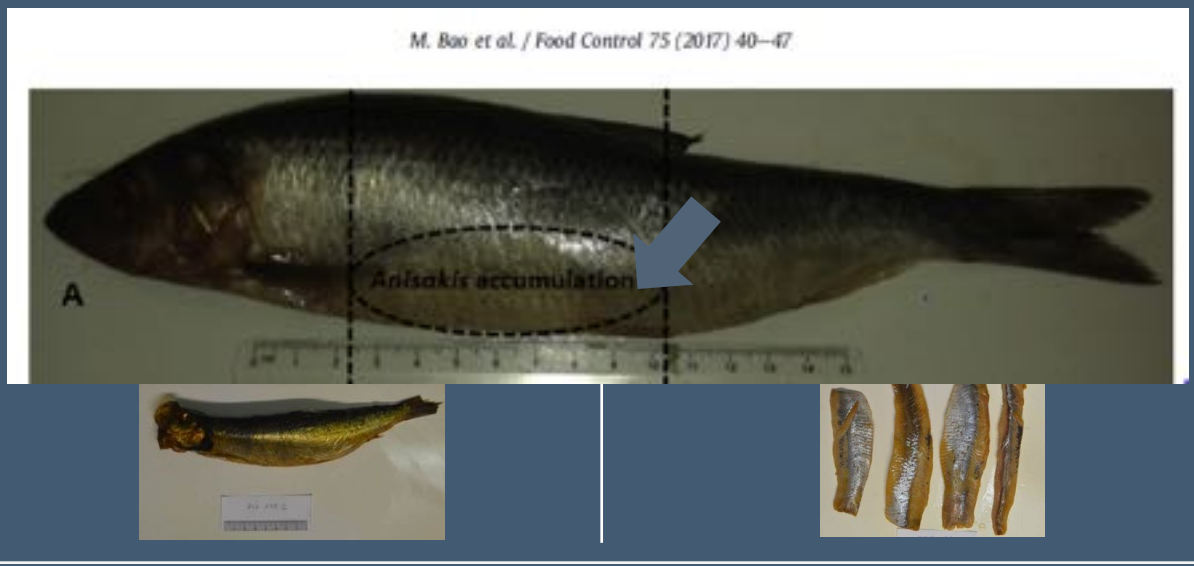
VS



GOLDEN HERRING SILVER HERRING

No significant differences between the 2 sub-categories

4.1 OCCURRENCE AND DISTRIBUTION OF THE LARVAE: MUSCLE (whole- fillets)



4.1 PRESENCE AND DISTRIBUTION OF THE LARVAE: MUSCLE (whole-fillets)

Results divided according the analyzed subcategories – muscle larvae

Type of product	Sub-categories	n	pos	% pos	n larvae	larvae/tot products	larvae/pos products	range	Mean density
Whole herrings	Golden	25	16	64,0	78	3,1	4,9	0-15	
	Silver	25	15	60,0	71	2,8	4,7	0-17	
Fillets	Smoked	25	3	12,0	4	0,2	1,3	0-2	
	Marinated	30	3	10,0	3	0,1	1,0	0-1	
	Canned	15	0	0,0	0	0,0	0,0	0	

- No significant differences among subcategories of the same type of product
- Canned fillets completely negative: possible influence of the lower number (other 15 analyzed in the last month, still negative)

4.2 MOLECULAR IDENTIFICATION OF THE LARVAE

- **All identified as *Anisakis simplex***
- No differences between larvae from the viscera and the muscle
- Results in agreement with other studies → only *A. simplex* in the Atlantic herrings (Bao et al., 2017, Cross et al., 2007; Levsen & Lunestad, 2010; Mattiucci & Nascetti, 2006; Tolonen & Karlsbakk, 2003)

4.3 LARVAE VIABILITY

- **All devitalized → no risk for human anisakiasis**

CONCLUSIONS

- Characterization of the **occurrence** and **distribution** of *Anisakis* spp. **in products made of herring**
- **Products with parasites are frequently found on the market**
- **High prevalence of *Anisakis simplex* in whole herrings**, both in the **viscera** and in the **fillets** manually obtained (in agreement with Levsen e Lunestad, 2010)
- **All the larvae were dead** → correct management of the risk of anisakiasis

- Fishery products that are **obviously contaminated with parasites** should **not** be placed on the market for human consumption (Reg. EC 853/2004).
- **Massive infections** may create **disgust** in consumers or contribute to **depricing** the product (Bao et al., 2017; Levsen & Lunestad, 2010; Reg. 178/2002)

- **Significant difference** between the infection of **fillets manually obtained** (whole herrings) and products made of **fillets**

→ **different level of exposure to dead larvae** according to consumers' preferences (in agreement with Levsen & Lunestad, 2010)

Thank you for your attention

Fishing herrings in Sweden, 1555

Great Yarmouthar, UK, 1890 ca

THE JOURNAL OF PARASITOLOGY
Vol. 63, No. 3, June 1977, p. 515-519

OCCURRENCE AND SURVIVAL OF THE LARVAL NEMATODE *ANISAKIS* SP. IN THE FLESH OF FRESH, FROZEN, BRINED, AND SMOKED PACIFIC HERRING, *CLUPEA HARENGUS PALLASI**

A. K. Hauck
Fish Pathology Laboratory, State of Alaska, Department of Fish and Game, Anchorage, Alaska 99502

TABLE I. *Proportions of 220 Pacific herring with* Anisakis *larvae in the flesh, time from death to necropsy, and average processing temperatures.*

	Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
No. of fish	39	120	20	21	20
Flesh infected (%)	38.5	42.5	50.0	57.1	95.0
Time (hr)	8	2*	8	26	26
Average temperature (C)	20	−20	18	21	30

* Time between death and being frozen.

TABLE II. *Larval* Anisakis *infection in samples of fresh, frozen, brined, cold smoked-whole, and cold smoked-gibbed herring (total number of larvae (T); percent of larvae in flesh (%F); percent of viable larvae in flesh (%VF)).*

	Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
T	753	2099	542	232	285
%F	3.5	5.3	6.6	20.7	44.2
%VF	96.2	—	100.0	87.5	14.4*

* Twenty percent of larvae in the gibbed fish were viable.

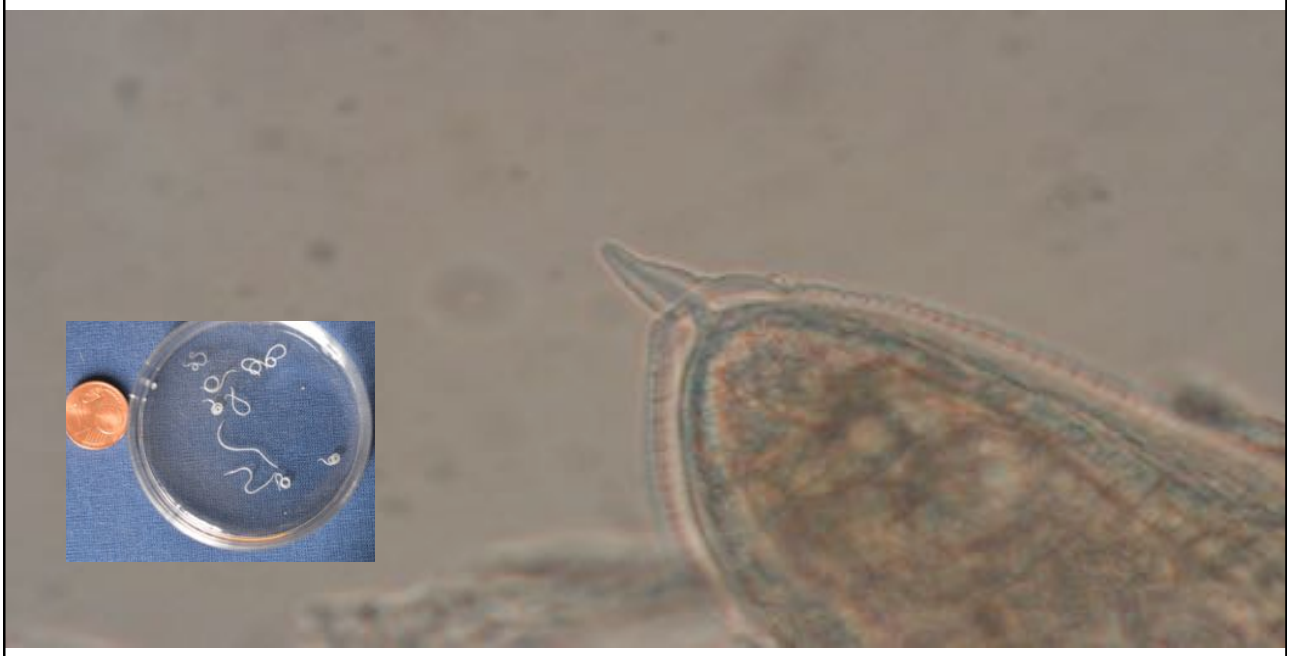
Thank you for your attention

L'attività di pesca dell'aringa ha radici molto antiche

Pesca delle aringhe in Scania (Svezia), 1555

Porto di Great Yarmouthar, UK, 1890 ca

- Necessario implementare controllo qualità nei prodotti venduti non eviscerati (aringhe affumicate intere) così come proposto anche per le acciughe salate (Guardone et al., 2018)



Allergy 2002: 57: 44
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ALLERGY
ISSN 0105-4538

Letter to the editor

Do only live larvae cause *Anisakis simplex* sensitization?

A recent paper in *Allergy* (1) describing the prevalence and risk factors for IgE sensitization to *Anisakis simplex* in Galicia, Spain, showed that only 0.43% of a large adult sample was sensitized and stated that sensitization is only possible by live larvae. This seroprevalence is lower than previously reported in Spain (2) and elsewhere (3).

As part of an ongoing investigation involving children and adolescents (6–18 years) routinely observed at a

Our prevalence estimates, based on a small sample size, are clearly larger than those found in a population with a similar genetic background. Plausible explanations for this might be the differences in eating habits and fish intake, cohort effects or differences in test performances.

In Portugal the consumption of raw fish is unusual especially in the paediatric population. Therefore, cultural differences concerning fish consumption (4) tests remains to be proven. This method may not detect lower specific IgE levels and the higher risk of sensitization related to raw fish consumption might result from higher IgE levels in raw fish consumers.

Sensitization to *A. simplex* caused only by live larvae is not supported by our findings. Further studies are needed for a better understanding of the role of *A. simplex* sensitization and its importance as a major allergen to public health.

All these fish species may be infected by larvae of anisakids

Anisakidae

Raphidascarididae

Anisakis spp.

Pseudoterranova spp.

Contracaecum spp.

Hysterothylacium spp.

(Fagerholm, 1991)

AUTORE	FAMIGLIA	SUBFAMIGLIA	GENERE
Hartwich G., 1974	Anisakidae	Anisakinae	*Anisakis, Phocanema* (= *Pseudoterranova*), *Terranova, Sulcascaris, Duplicaecum, Goleicepa, Contracaecum, Phocascaris*
		Goezinae	*Goezia*
		Raphidascaridinae	*Raphidascaris, Raphidascarioides, Thynnascaris* (= *Hysterothylacium*), *Lappetascaris, Aliascaris, Heterotyphlum, Paranisakis, Paranisakiopsis*
Fagerholm H.P., 1991	Anisakidae	Anisakinae	*Anisakis, Pseudoterranova, Terranova, Sulcascaris, Pavitrachelius, Pulchrascaris, Paranisakiopsis*
		Contracaecinae	*Contracaecum, Goleicepa, Phocascaris*
	Raphidascarididae		*Raphidascaris, Raphidascarioides, Hysterothylacium, Lappetascaris, Heterotyphlum, Paranisakis, Goezia, Spinitectoscaris, Parahetertyphlum*

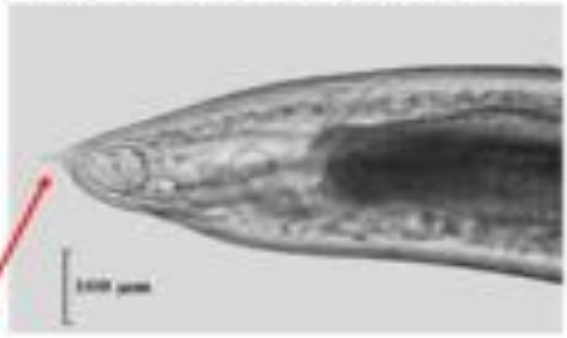
tab. 2.1 – Confronto tra la classificazione proposta da Hartwich G., 1974 e quella proposta da Fagerholm H.P., 1991 (modificata da Lymbery A.J. and Cheah F.Y., 2007)

Classificazione dei nematodi famiglia Anisakidae molto complessa. Pochissime differenze specie-specifiche dei caratteri morfologici aventi un significato tassonomico (sistema escretorio, canale alimentare, papilla caudale del maschio, papille caudali nel maschio, posizione della vulva e lunghezza degli spiculi). Inoltre, applicabili solo agli individui adulti e non osservabili nelle larve .

Negli ultimi anni, MAE *e successivamente PCR hanno evidenziato che alcune specie sono complessi di specie criptiche, che differisco tra loro per struttura genetica, distribuzione geografica e preferenza di ospite. L'attuale classificazione tassonomica è basata sui caratteri morfologici integrati da quelli genetici e tende a supportare la classificazione proposta da Fagerholm.*

Morfotipo I

Anisakis sp. Larva Type I (sensu Berland, 1961)



terminazione caudale con "mucron"

ventricolo di forma allungata

Normativa di riferimento

- Legge 283/1962, art 5, punto D
- Ordinanza Ministeriale 12 maggio 1992
- Regolamento CE/853/04 Sezione VIII Capitolo III , Capitolo V
- Regolamento CE/2074/05, Allegato II, Sezione I, Capitolo I, II
- Regolamento CE/1020/08, Allegato II

Il mancato rispetto della normativa vigente è soggetto all'emanazione di sanzioni amministrative (art. 6 comma 8 del D.lgs 193/2007) e penali (art. 5 punto d della Legge 283/1962) da parte dell'Autorità competente che esercita l'attività di vigilanza lungo tutta la filiera.

A cura della Regione Piemonte
Direzione Sanità
in collaborazione con
l'Università degli Studi di Torino
Facoltà di Medicina Veterinaria

Informate i vostri clienti

Quando vendete pesce fresco al consumatore finale:

- se il consumatore desidera consumarlo crudo o marinato consigliate l'applicazione domestica del trattamento di congelamento;
- se vendete o somministrate preparazioni tipo "sushi" o "sashimi", riforniteVi di prodotto per il quale sia garantito il preventivo congelamento, in caso contrario dovrete applicarlo presso il vostro esercizio.

REGIONE PIEMONTE

CONTROLLO DEL RISCHIO ANISAKIS NEI PRODOTTI DELLA PESCA

Precauzioni e obblighi degli operatori del settore alimentare

Che cos'è l'Anisakidosi

L'anisakidosi è una zoonosi provocata da larve di parassiti appartenenti a vari generi tra cui *Anisakis*, *Pseudoterranova*, *Contracoecum*. Le forme adulte di questo parassita si trovano nel tratto digerente dei mammiferi marini (pinnipedi, balene e delfini) di tutto il mondo, mentre molte specie di pesci sono infestate dalle larve. Ad oggi la parassitosi è stata evidenziata in oltre 123 specie di pesci ed anche in quattro specie di cefalopodi, tra cui calamari.

I gadidi (merluzzo nordico, nasello, potassolo, molo, ecc.), gli sgombri, il pesce sciabola, la rana pescatrice, il pesce San Pietro, ma anche sardine, acciughe, triglie, pagelli sono le specie più spesso infestate, mentre nei pesci piatti l'infestazione è più rara. Il numero di larve e le specie di anisakidi variano a seconda delle zone geografiche dei luoghi di pesca, anche se nessuna zona ad oggi può considerarsi priva di rischio.

Nel pesce le larve del parassita, lunghe da 1 a 3 cm, di colore biancastro o giallastro, e con diametro di 0,8 mm circa si trovano spesso arrotolate in spirale nella cavità addominale, nell'intestino, sul fegato e sulle gonadi, talvolta nel muscolo.

Effetti sulla salute

Nell'uomo la malattia insorge, spesso in forma acuta, con forti dolori allo stomaco dopo poche ore dal pasto (1-8 ore), a seguito di ingestione di larve vitali presenti in pesci che non abbiano subito trattamenti idonei per l'inattivazione del parassita (congelamento a -20°C per 24 ore o trattamenti con il calore ad almeno 60°C).

Più rare sono le forme di orticaria: tali forme allergiche, ancora oggetto di studio, possono anche essere gravi, se la persona è particolarmente sensibile.

Occasionalmente, si segnalano anche forme intestinali con dolori addominali che compaiono dopo alcuni giorni dall'ingestione del parassita, talvolta associate ad ostruzioni o perforazioni intestinali.

Quali sono gli obblighi dell'operatore del settore alimentare ?

Chi produce, commercializza o prepara per la ristorazione pesce o prodotti a base di pesce è il primo responsabile della sicurezza sanitaria degli alimenti immessi sul mercato (Reg. CE/853/04). In particolare tali prodotti non devono contenere parassiti. Questo pericolo deve essere preso in considerazione nei piani di autocontrollo sanitario (piani HACCP, Buone Pratiche di Lavorazione, tracciabilità, gestione delle non conformità). La normativa vigente prevede i seguenti obblighi per gli operatori:

- controllo visivo, effettuato su un numero rappresentativo di campioni, per assicurare l'assenza di parassiti visibili.
- congelamento a –20°C per almeno 24 ore per i seguenti prodotti a rischio:
  - i prodotti della pesca consumati crudi o quasi crudi;
  - i prodotti della pesca che hanno subito un trattamento di affumicatura a freddo, nel corso del quale la temperatura interna del prodotto non ha superato i 60° (nelle specie di aringa, salmone selvatico, sgombro, spratto);
  - i prodotti della pesca marinati e/o salati, se il trattamento è insufficiente a distruggere le larve (*).

Il controllo dev'essere eseguito dall'addetto, in modo continuativo, al momento dell'estrazione dei visceri e del lavaggio.

(*) Se si vuole evitare il congelamento, lavorazioni particolari, come salagione e marinatura, devono essere eseguite solo in condizioni controllate nel rispetto di specifici trattamenti e parametri.

Il controllo dei filetti o dei tranci di pesce dev'essere effettuato dagli operatori durante la preparazione successiva alla sfilettatura o all'affettatura.

Quali altre misure possono ridurre il rischio?

Per l'operatore del settore alimentare che commercializza prodotti della pesca freschi:

- qualificare i propri fornitori, richiedendo che forniscano, laddove sia possibile, prodotti già eviscerati. Infatti l'eviscerazione praticata il più vicino possibile al momento della cattura consente di eliminare i parassiti con il pacchetto intestinale. E' dimostrato che gli anisakidi migrano nella cavità addominale, sul fegato, e nel muscolo, solo alcune ore dopo la morte del pesce.
- richiedere al proprio fornitore precise indicazioni scritte che sia stato effettuato un "controllo visivo" per la ricerca dei parassiti a livello di intestino, fegato e gonadi, secondo le modalità indicate nel Regolamento CE/2074/2005, su un numero significativo di campioni.
  Queste certificazioni sono particolarmente importanti per quelle specie abitualmente vendute non eviscerate (acciughe, triglie per esempio), dove non è stato possibile mettere in atto un sistema preventivo per il controllo del parassita.
- formare accuratamente il personale che esegue i controlli.

Per l'operatore che prepara prodotti della pesca destinati ad essere consumati crudi (sushi, sashimi) o che vengono sottoposti solo ad un debole trattamento conservativo (pesce affumicato, marinato, salato, agrodolce) è necessario garantire l'inattivazione delle larve eventualmente presenti nei seguenti modi:

- mediante utilizzo di pesce già congelato all'origine;
- sottoponendo i prodotti acquistati freschi, o dopo la preparazione, a un trattamento di congelamento in profondità (-20°C per almeno 24 ore).


ACCIUGHE SALATE SPAGNOLE
0.150kg
RTE 24


Ready to eat **fish** products are particularly popular. Among them, products made of differently prepared raw **anchovies** are highly appreciated throughout Italy.

Other fish species frequently used
for such products are
herrings, **sardines and mackerel.**

All these fish species may be infected by larvae of anisakids

Anisakidae

Anisakis spp.

Pseudoterranova spp.

Contracaecum spp.

(Fagerholm, 1991)

Nel pesce, la localizzazione delle larve è prevalentemente viscerale, dove si ritrovano incistate a spirale sulla superfice esterna degli organi (principalmente fegato, gonadi e mesentere) (Bao *et al.*, 2017), ma è possibile che queste migrino anche a livello muscolare, costituendo un pericolo per l'uomo che ingerisce queste carni. Questa migrazione può essere avvenire:

- post-mortem: in seguito ad un'eviscerazione tardiva oppure un'infestazione massiva; è favorita da una mancata conservazione della catena del freddo
- intra-vitam: meno frequente (Cipriani *et al.*, 2016; Levsen *et al.,* 2010; Karl *et al.,* 2002; Pravettoni *et al.*, 2012).

Ad oggi, le uniche specie in cui è stata evidenziata la capacità di migrare nel muscolo sono *A. simplex* s.s. e *A. pegreffii* (Cipriani et al., 2017). A livello muscolare, le larve si vanno a localizzare prevalentemente a livello delle aree ventrali (*belly flaps*) (Pierce *et al.*, 2017; Cipriani *et al.*, 2015, Levsen *et al.,* 2010, 2014).

Anisakis larvae in the muscle (post digestion)

Anisakis larvae in the visceral cavity

Guardone *et al.* - Occurrence of *Anisakis* spp. larvae in products made of herring (*Clupea harengus*) - SOIPA 2018

3.5 MOLECULAR IDENTIFICATION OF THE LARVAE

Representative subsample (n=150)

DNA extraction → protocol described by Armani et al., 2014, modified according as Guardone et al., 2016

Qualitative and quantitative evaluation of extracted DNA → total DNA elettrophoresis and spettrophotometric analysis

DNA amplification: amplification of a long (629 pb) and of a short (246 pb, internal) fragment of COX2 gene

- primers amplifying the long fragment: 211 R and 210F (Nadler e Hudspeth, 2000)
- primers amplifying the short fragment: An-F2 e An-R2 (this study)

Sequencing and sequences analysis

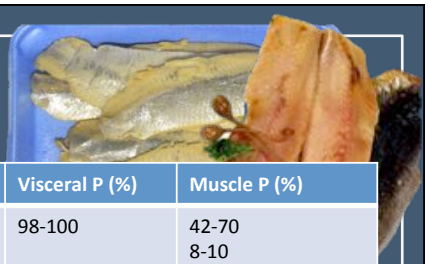
1. INTRODUCTION

Herring represent one of the main host species of *Anisakis* spp. *Anisakis* spp. is often called «herring worm», due to the association between the first confirmed human cases of anisakiasis and the consumption of undercooked herrings («*green herrings*») in the Netherlands
(Van Thiel et al., 1960; Van Thiel 1962).

Anisakis spp. in **fresh herring**: Prevalence values

References	Origin (N)	Method	Visceral P (%)	Muscle P (%)
Bao et al. 2017	North Sea (209)	Press + candling	76	0
Levsen et al. 2017	North Sea (1252) Norw. spring spawn. (726) Baltic Sea (695)	UV-press	81.2 92.6 65.5	17.4 37.1 14.8
Levsen et al., 2005	Norw. spring spawn. (78)	Digestion + UV	94.9	23.1-43.6
Tolonen & Karlsbakk 2003	Norw. spring spawn. (220)	Dissection under microscope	100	rare
Smith & Wotten, 1975	North Sea (38 + 38 + 38, 3 experiments)	Digestion	-	39.5-73.7

Anisakis spp. in **processed herrings**:



Reference	Tipe of products (n)	Origin	Method	Visceral P (%)	Muscle P (%)
Levsen & Lunestad, 2010	"manual" fillets (250) "industrial" fillets (250)	Norwegian Sea	Digestion Uv-press	98-100	42-70 8-10
Szostakowska et al., 2005	Fillets: marinated, salted, spiced, ready to eat (140, 39 types) Non eviscerated salted herrings (34) and smoked (40)	Poland (market)	Digestion	-	6 out of 39 types
Panebianco e Lo Schiavo, 1985	Salted herrings (40) Smoked herrings (30)	The Netherlands (market)	Digestion	85.7%	3.3%
Hauck et al., 1977	Brined Cold smoked «Gibbed» cold smoked	Pacific (Pacific herring)		-	50 57.1 95
Khalil, 1969	Cured and smoked herring			5% (all viable)	

3. MATERIALS & METHODS

parasite
Precedente Avanti

2.2.2.4 Heavily salted fish

The salt content of the fish muscle is above 20 g salt /100 g in water phase.

2.2.3 Storage temperatures

The products shall be kept frozen or refrigerated at a time/temperature combination which ensures their safety and quality in conformity with Sections 3 and 5. Very lightly salted fish must be kept frozen after processing.

2.3 PRESENTATION

Any presentation of the product shall be permitted provided that it:

2.3.1 meets all requirements of this standard, and

2.3.2 is adequately described on the label to avoid confusing or misleading the consumer.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 FISH

Salted Atlantic herring and salted sprats shall be prepared from sound and wholesome fish which are of a quality fit to be sold fresh for human consumption after appropriate preparation. Fish flesh shall not be obviously infested by parasites.

8. DEFINITION OF DEFECTIVES

8.1 The sample unit shall be considered as defective when it exhibits any of the properties defined below.

8.1.1. Foreign matter

The presence in the sample unit of any matter which has not been derived from fish, does not pose a threat to human health, and is readily recognized without magnification or is present at a level determined by any method including magnification that indicates non-compliance with good manufacturing and sanitation practices.

8.1.2 Parasites

The presence of readily visible parasites in a sample of the edible portion of the sample unit detected by normal visual inspection of the fish flesh (see Annex III).