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Prevalence and mean intensity of Anisakidae parasite in seafood caught in Mediterranean Sea focusing on fish species at risk of being raw-consumed. A Meta Analysis and Systematic Review.

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Summary

Objective: to assess the prevalence and the mean intensity of anisakids in seafood caught in Mediterranean sea, focusing on fish species at risk of being raw-consumed.

Design: Systematic review and meta-analysis of studies published 1960-2012.

Study selection: main criteria for inclusion of studies were: findings of anisakids larvae, both in muscles and viscera; fish species for human consumption, caught in Mediterranean Sea;

prevalence and mean intensity data for each species; sample size equal to or more than 40 fishes.

Results: twelve studies were identified. Among them four studies considered fish species which are often consumed raw or lightly preserved or not thoroughly cooked anchovy, pilchard and Atlantic mackerel. **Data synthesis:** all pooled analyses were based on random-effect model. Anisakids prevalence in fish muscle was 0.64% (P < 0.0001), in viscera was 1.34% (P < 0.0001); overall was 0.95% (P < 0.0001). Mean intensity in muscle was 2.31 (P = 0.0083), in viscera was 1.55 (P = 0.0174), overall was 1.81 (P < 0.0005). Heterogeneity indexes (I²) were significantly high with the exception of viscera mean intensity. **Conclusions**: anchovy, pilchard, Atlantic mackerel have a low prevalence and mean intensity of Anisakidae larvae both in viscera and in muscle. Mean Intensity is low as well.

Keywords: anisakids, Mediterranean seafood, systematic review, meta-analysis, fish consumed raw, zoonosis.

INTRODUCTION

Anisakids nematodes larvae (genera Anisakis. Pseudoterranova. Contracaecum. *Hysterothylacium*) are common parasites present in many marine fishes, crustaceans and squids: the problems caused by these parasites have impact both on public health (Hysterothylacium excepted), if they are not killed during food processing, and on commercial value of the product. Anisakiasis refers to infection of people with anisakids larvae. The Human is an accidental host in the life cycle of parasite and acquires the live larvae by eating raw or lightly preserved or undercooked seafood. Anisakiasis is a serious zoonotic disease with a number of forms depending on the localisation and the severity of lesions caused by worms. In Italy thirty one identified cases of human anisakiasis are reported over the period 1996 – 2012; in all the cases the patients referred raw or marinated fish consumption. Anisakiasis is misdiagnosed and underestimated, in fact the diagnosis is often obtained after surgery (Biondi et al., 2008; Filauro et al., 2011; Fumarola et al., 2009; Maggi et al., 2000; Mattiucci et al., 2011; Montalto et al., 2005; Moschella et al. 2004; Pampiglione et al., 2002; Pellegrini et al., 2005; Rea et al., 2008; Testini et al., 2003; Ugenti et al., 2007; Zullo et al., 2010). Although freezing and cooking assure to kill the anisakids larvae these treatments do not solve any health problem related to anisakidae presence, because of possible allergic reactions due to antigens which are freezing and cooking resistant (Pravettoni et al., 2012). The risk of allergic reactions after nonviable anisakids fish ingestion is a matter of some concern. Some authors reported that sensitized subjects did not show any symptom after administration of dead larvae (Alonso-Gómez et al., 2004; Sastre et al., 2000; Alonso et al., 1999). On the contrary, other researchers showed that 13 of 64 subjects with Anisakis sensitization history had adverse reaction after eating properly

cooked fish (Moneo et al., 2007). In the last study the authors believe that the intolerance recorded could be due to the high amounts of allergens. This systematic review and meta-analysis is aiming to assess prevalence (percentage of infected fishes number on total fishes number) and mean intensity (ratio of parasites number on infected fishes number) of anisakids larvae belonging to genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Hysterothylacium*) and hosted by fishes for human consumption captured in Mediterranean Sea.

METHODS

Eligibility criteria

Criteria for eligibility of studies are described here below. Presence of anisakids larvae (genera Anisakis, Pseudoterranova, Contracaecum, Hysterothylacium: not all these genera have the same human pathogenic potential but they are morphologically very similar on visual examination). Findings of larvae, both in muscles and viscera, detected by visual and/or enzymatic digestion methods (it is strategic to know the anatomical localisation owing to the epidemiologic implication of Anisakiasis). Fish species of legal size for human consumption, caught in Mediterranean Sea and not aquacultured. Prevalence and mean intensity data for each species. Sample size equal to or more than 40 fishes: this sample size was chosen as the best compromise between a suitable estimate of parasitological indices and the sample costs; in fact, a systematic underestimation of mean intensity was demonstrated with a small sample size (<40) (Marques and Cabral, 2007), although, in metazoan parasitology, the prevalence is a variable less affected by sample size than mean intensity, and a sample size of more than 15 specimens is enough to get a basic estimate of prevalence (Jovani and Tella, 2006); moreover

larger sample size is required for a robust estimate of prevalence. Papers published in English, French, Italian, Spanish, German and Portuguese are eligible.

Search strategy

Studies were identified by searching electronic databases and scanning reference lists of articles. This search was applied to PubMed, Embase, Web of Science, CABI, Scirus, Scopus, FSTA Food Science and Technology Abstract, Google Scholar, Medline in process, Medline Ovid, Agris FAO, Caris (FAO), SpringerLink, Annual Reviews, Conference Papers, CAB Reviews and CINAHL. Strategy has been developed using the following key words structure: "(anisakidae or anisakis or pseudoterranova or contracaecum or hysterothylacium) and (prevalence or mean intensity) and Mediterranean and (fish or seafood) and larvae". This syntax was occasionally recombined - without violating its logical sense - to meet settings of a particular database. Additionally hand searches of the reference lists of CB and of two degree theses were performed were performed.

Study selection

The literature search was conducted independently by 2 investigators (MC and CB). Two authors (MC and CB) independently selected potentially eligible studies for inclusion. Disagreements between reviewers were resolved by consensus; if no agreement could be reached, it was planned a third author (PC) would decide. The full eligible citations was examined in more detail.

Data Extraction

For each study, prevalence and mean intensity values were extracted by 2 authors using predefined data fields, as described here below.

We developed a data extraction sheet, pilot-tested it on five randomly selected included studies, and refined it accordingly. One review author (CB) extracted from included studies and entered in the data extraction form: host fish species, fish size and fishing area; anisakids category (raw group names were collected and recorded in categories defined according to the definition set by the authors) and its anatomical location; were also recorded: sample size, number of infested fishes, number of parasites, prevalence and mean intensity. Prevalence was recorded as number of infested fishes and total number of fishes; if the number of infested fishes was missing this outcome was derived by other suitable data; mean intensity was recorded as the ratio of total number of parasites on number of parasitized fishes; if any data were missing mean intensity derivation method was analogue to that used for prevalence.

A second author checked the extracted data (FC) to ensure data quality. Disagreements were resolved by discussion between the two review authors; if no agreement could be reached, it was planned a third author would decide (PC).

The primary outcomes were the anisakids category prevalence and mean intensity.

Methodological Quality Assessment

Due to the observational nature of considered studies no methodological quality assessment method (namely about the risk of bias in individual studies or across studies or other quality items) was set.

Data analysis

Punctual estimates and their 95% confidence intervals were calculated across all selected studies on statistical units that were every level combination of the following factors: host species by parasite category by anatomical location. Calculations were performed using the

"metaprop" and "metagen" procedures of "meta" R package (Schwarzer, 2010) with the normalizing natural logarithm conversion option and, where applicable, a correction factor for 0% outcomes was introduced (i.e. a value of 0.5 was added to both numerator and denominator). Using the same R package meta-analyses were performed using the random effects model, described by DerSimonian and Laird (1986), that was selected over the fixed effects model because it incorporates within and between study variability, which is applicable to this meta-analysis that was expected to yield a high degree of variability. The chosen level of significance for statistical tests was P<0.05. Heterogeneity, i.e. variability among records, was assessed by the I-squared (I²) statistic (Higgins et al., 2003). Ninety-five per cent (95%) prediction intervals were calculated by means of "metafor" R package (Viechtbauer, 2010). Adjusted prediction intervals of mean intensity were also calculated (Zhou et al, 2007) to give more sense to the interval values, meeting the left-truncation to 1 (or 0 referring to logarithm) condition of mean intensity distribution.

RESULTS

Searches yielded 1734 references, 1683 items from electronic databases, 51 items from hand searches of the reference lists of CB. Records excluded on the basis of title and abstract 1669, references left 65.

We excluded 53 studies because they didn't meet the adopted criteria.

Twelve studies were included in quantitative synthesis of systematic review; the process of studies selection is outlined in figure 1. Four of them considered fish species which are often consumed raw or lightly preserved or not thoroughly cooked.

Characteristics of included studies

Tables n.1a and 1b present characteristics of the 12 included articles, summarised below.

Population

The included studies comprised 17 fish species, the total number of analyzed subjects were 3209 fishes, captured in different local fishing areas. Fishes size ranged from 9 to 60 cm.

Infection

Eleven categories of anisakid parasites were considered, belonging to three genera: *Anisakis*, *Contracaecum* and *Hysterothylacium* (*Pseudoterranova* genus was not found); all parasites categories were present both in muscle and in viscera.

Outcomes

Anisakidae parasites prevalence and mean intensity were the primary outcomes assessed in all studies. No additional outcomes were considered or recorded.

Prevalence and mean intensity of anisakids

Prevalence and mean intensity of anisakids are here presented only on the following fish species at risk of being raw-consumed: *Engraulis encrasicolus*, *Sardina pilchardus* and *Scomber scombrus*. The full of the data are shown in appendix A (prevalence) and appendix B (mean intensity).

All pooled analyses were based on random-effect models, the amount of heterogeneity in metaanalysis and prediction 95% intervals were also calculated.

Anisakids prevalence in muscle was 0.64% (0.23 - 1.75), (P < 0.0001); in viscera 1.34% (0.52 - 3.43), (P < 0.0001); overall 0.95% (0.50 - 1.84), (P < 0.0001). Mean intensity in muscle was 2.31 (1.18 - 4.51), P = 0.0083; in viscera 1.55 (1.08 - 2.23), P = 0.0174; overall 1.81 (1.30 - 1.84).

2.53), P < 0.0005. Heterogeneity indices (I^2) and P values, respectively for muscle, viscera and overall, in the prevalence were: 78.40% (P<0.0001), 87.00%(P<0.0001) and 83.60% (P<0.0001); in the mean intensity were: 79.20% (P=0.0083), 44.90% (P=0.1419: not significant), 64.90% (P=0.009). Prevalence and mean intensity muscle 95% prediction intervals were, respectively, 0.02 - 16.74% and 0.68 (1.16) - 7.87 (6.65), within brackets the adjusted values. Prevalence and mean intensity viscera prediction intervals were, respectively, 0.07 - 24.77% and 0.85 (1.09) - 2.83 (2.60), within brackets the adjusted values. Prevalence and mean intensity overall were respectively 0.06 - 15.63% and 0.84 (1.12) - 3.89 (3.49), within brackets the adjusted values.

Forest plots of prevalence and mean intensity meta-analyses are shown respectively in figure 2 and figure 3.

DISCUSSION

Summary of evidence

The rigorous sample size criteria adopted reduced the eligible papers, which should lead to robust results and conclusion. The studies enclosed in this work should provide high-quality evidence for meta-analysis. The careful examination of the papers revealed that the majority of the studies did not justify their sample size choice. Some studies were excluded due to lack of data; others were excluded because of the low quality of information, such as prevalence and mean intensity of larvae without any anatomical reference. Results of meta-analyses show an overall central estimate of anisakids prevalence in the order of 1 percent, this value being of the same order of magnitude both in muscle and in viscera. Mean intensity data yield a value that

surrounds 2, this value is similar to that of viscera, whereas it is slightly lower than that of muscle. After a question raised from a Referee, a kind of sensitivity analysis (s.a.) was performed to assess the impact of larvae detection on results, as is known that visual method has lower recovery rate than enzymatic one: so meta-analyses were performed on the main data split in two subsets ("visual" and "enzymatic" groups), yielding output (data not shown) not giving a clue that results could be affected by the variable considered. On the contrary, the same s.a., when applied to appendix data, yielded higher outcome values in favour of visual method; this effect, being in the opposite direction than that expected, could be likely due to the very heterogeneous fish species considered in studies.

CONCLUSION

<u>Implications for practice</u>

The three considered species (anchovy, mackerel and pilchard) have been found lightly parasitized, but it is of primary importance for food safety that they undergo, before consumption, to a preventive treatment in order to kill all possible live larvae. The absence of genus *Pseudoterranova* in Mediterranean sea is confirmed since no record was found in the studies included (see tables 1a and 1b). In routine control of fishery products an high sample size is necessary in species with low prevalence to detect the positive lot.

Implications for research

The muscle prevalence near zero and the viscera prevalence near 1% of the Anisakidae larvae in the target species is suggesting to researchers to improve sample size because large sample size (100-1000) are required to detect very low prevalences (Gregory et al., 1991). Gutiérrez-Galindo

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et al. (2010) recorded no presence of Anisakidae larvae in anchovy and pilchard, on the contrary in mackerel caught in the same area was recorded a 11% mean prevalence. Should be very interesting to understand if this evidence is an effect of low sample size or there is a biological phenomenon.

A total of 53 papers were not included in the analysis because they did not meet the eligibility criteria, this underlines the necessity to adopt a more rigorous experimental design in parasitological research, particularly most of works were excluded because the parasitological raw data were not stated even though the authors declared to record them. In our opinion, the importance of raw data publication is undervalued by researchers in this field since to access raw data would allow statistical comparison and elaboration. Moreover this work indicates further research is necessary on anisakids in those Mediterranean fish species proved to be highly parasitized in other fisheries areas, such as European sea bass, monkfish or hake.

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Table 1a. Included studies. Host fish species, size, fish area, anatomical localization of larvae, type of parasite and sample size.

Source	Host fish species	Host fish size (length, cm)	Anatomical localization of larvae	Type of larva	Fishing area	Sample size	
Valero et.	Merluccius	40-52	viscera	Anisakis spp,	Mediterraneanoff southern	63	
Al.(2006)-a	merluccius	40-52	muscle	Anisakis spp,	11		
	Engraulis encrasicolus	12.3-16.5	viscera muscle	No anisakids		153	
	Sardina pilchardus	11.5 - 19	viscera muscle	No anisakids		160	
Gutierrez-			muscle	Anisakis type I			
Galindo et			viscera	Anisakis type I	Tarragona (NE Spain)		
al.(2010)	Scomber scombrus	20.5-37.0	viscera	Hysterothylacium aduncum	Turragona (TL Spain)	447	
			viscera	Contracaecum sp,			
	Trachurus		muscle	Anisakis type I			
	trachurus	16.2-30.2	viscera	Anisakis type I		155	
	ir acruir us		viscera	Contracaecum sp,			
Keser et	Pomatomus	Not stated	viscera	Hysterothylacium aduncum	Dardanelles at Çanakkale,	41	
Al.(2007)	saltatrix	Not stated	viscera	Hysterothylacium aduncum	Turkey		
Rello et al.(2009)	Engraulis encrasicolus	9,0-18,8	muscle	Anisakis type I	NW AlboránSea	72	
Figus et Al.(2005)	Serranus cabrilla	10,5-19,5	viscera	Hysterothylacium sp,	Gulf of Cagliari. south- west Mediterranean sea	71	
	Micromesistius poutassou	17-28	muscle	Anisakis pegreffii		301	
Valero et			viscera	Anisakis pegreffii	Mediterranean region		
Al.(2000)			viscera	Anisakis physeteris	ofsouthern Spain		
711.(2000)	poniusson		viscera	Hysterothylacium aduncum	oisoutiem spain		
			viscera	Anisakis physeteris		272	
			viscera	Anisakis simplex s,l,			
	Phycis blennoides	27-50	viscera	Hysterothylacium fabri L3			
	1 nycis biennoides		viscera	Hysterothylacium fabri L4			
			viscera	Hysterothylacium fabri L4			
Farjallah et			viscera	Anisakis physeteris	eastern Mediterranean		
Al.(2006)			viscera	Anisakis simplex s,l,	coasts in Tunisia		
	Phycis phycis	30-60	viscera	Hysterothylacium aduncum L3		320	
			viscera	Hysterothylacium aduncum L4			
			viscera	Hysterothylacium fabri L3			
			viscera	Hysterothylacium fabri L4			
Rello et Al.(2008)	Sardina pilchardus	12.2-21	viscera	Hysterothylacium aduncum	Northern Alboran sea	99	
			muscle	Hysterothylacium aduncum	rvortiiem Atuoran sea	77	

	viscera	Hysterothylacium aduncum	Western Balearic Sea	115	1
	muscle	Hysterothylacium aduncum	western Baleanc Sea	113	

Table 1b. Included studies. Host fish species, size, fish area, anatomical localization of larvae, type of parasite and sample size.

Source	Host fish species	Host fish size (length, cm)	Anatomical localization of larvae	Type of larva	Fishing area	Sample size
	Diplodus vulgaris	Not stated	viscera			72
	Mullus surmuletus		viscera			68
Ternengo et	Pagellus erythrinus	Not stated	viscera	Hysterothylacium	Bonifacio Strait Marine Reserve(Corsica Island,	47
	Phycis phycis	Not stated	viscera	- fabri	Mediterranean Sea)	40
	Scorpaena scrofa	Not stated	viscera			57
	Symphodus tinca	Not stated	viscera			58
			muscle	Anisakis physeteris		
		Ī	muscle	Anisakis simplex		
		Ī	viscera	Anisakis physeteris		
	Physic blannoides	18-55	viscera	Anisakis simplex s.l.		209
	Phycis blennoides	10-33	viscera	Hysterothylacium aduncum		209
			viscera	Hysterothylacium fabri	Mediterranean coasts of eastern Andalucia (Southern Spain)	
	Phycis phycis	24-58	viscera	Anisakis physeteris		
			viscera	Anisakis simplex s.l.		
			viscera	Hysterothylacium aduncum		58
			viscera	Hysterothylacium fabri		
	Xiphias gladius	Not stated	viscera	Anisakids	Tyrrhenian and Ionian sea	66
	Merluccius merluccius	Not stated	viscera			45
	Micromesistius	NI.4 .4.4.1	viscera			<i>E</i> 1
NI	poutassou	Not stated	muscle	1		51
Normanno et al. (2011)	Boops boops	Not stated	viscera	Anisakids	Adriatic sea	54
	Trachurus	Not state 1	viscera	1		62
	trachurus	Not stated	muscle	1		62
	Sardina pilchardus	Not stated	viscera			53

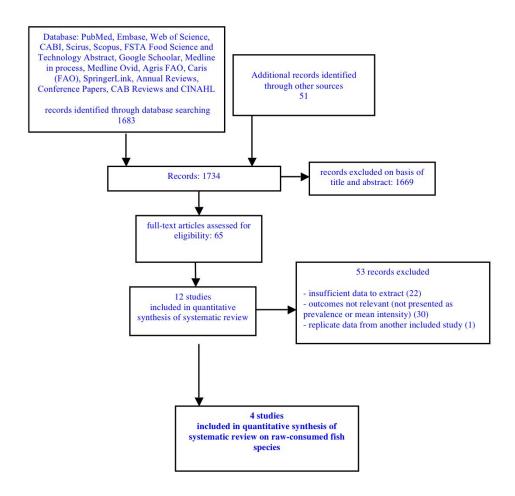


Fig 1 Selection of articles for meta-analysis

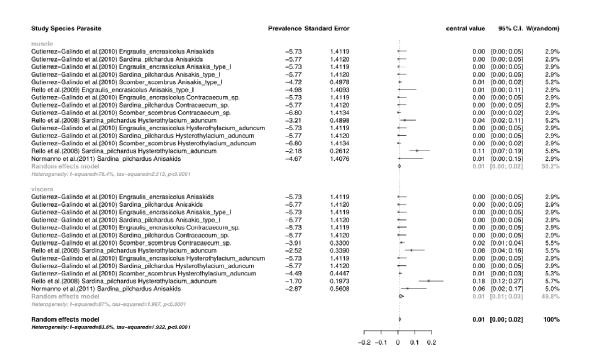


Fig 2 Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species at risk of being raw-consumed.

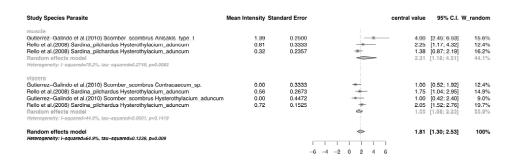


Fig 3 Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species at risk of being raw-consumed.

Study Species Parasite	Prevalence In(Pr	evalence)Standard Error	central value	95% C.L.	W(random)
muscle					
Valero et al. (2000) Micromesistius_poutassou Anisakids	-5.71	0.9983 -		[0.00; 0.02]	0.8%
Gutierrez-Galindo et al.(2010) Engraulis_encrasicolus Anisakids Gutierrez-Galindo et al.(2010) Sardina_plichandus Anisakids	-5.73 -5.77	1.4119 = 1.4120 =	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Engraulis_encrasicolus Anisakis_type_l	-5.73	1.4119	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Sardina_pilchardus Anisakis_type_l	-5.77	1.4120	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Scomber_scombrus Anisakis_type_I	-4.72	0.4978 •	0.01	[0.00; 0.02]	1.5%
Gutierrez-Galindo et al.(2010) Trachurus_trachurus Anisakis_type_I	-5.04	0.9968 +	0.01	[0.00; 0.05]	0.8%
Rello et al.(2009) Engraulis_encrasicolus Anisakis_type_l Valero et al.(2000) Micromesistius_poutassou Anisakis_pegreffii	-4.98 -5.71	1.4093 ÷	0.01 0.00	[0.00; 0.11] [0.00; 0.02]	0.5% 0.8%
Valero et al. (2006) Phycis_blennoides Anisakis_physeteris	-5.71 -4.65	0.7037	0.00	[0.00; 0.04]	1.2%
Valero et al.(2006) Phycis_phycis Anisakis_physeteris	-4.76	1.4082	0.01	[0.00; 0.14]	0.5%
Valero et al. (2000) Micromesistius_poutassou Anisakis_physeteris	-6.40	1.4130 -	0.00	[0.00; 0.03]	0.5%
Farjallah et al.(2006) Phycis_blennoides Anisakis_phyaeteris	-6.30	1.4129 -	0.00	[0.00; 0.03]	0.5%
Farjallah et al.(2006) Phycis_phycis Anisakis_physeteris Valero et al.(2006) Phycis_blennoides Anisakis_simplex	-6.46 -5.34	1.4131 - 0.9976 •	0.00	[0.00; 0.02]	0.5%
Valero et al. (2006) Phycis_phycis Anisakis_simplex	-5.34 -4.76	1,4082 ←	0.00	[0.00; 0.03]	0.5%
Fariallah et al. (2006) Phycis blennoides Anisakis simplex s.l.	-6.30	1,4129	0.00	[0.00: 0.03]	0.5%
Farjallah et al. (2006) Phycis phycis Anisakis simplex s.l.	-6.46	1,4131 *	0.00	[0.00; 0.02]	0.5%
Valero et al. (2006) A Merluccius_merluccius Anisakis_sp.	-2.76	0.4639	0.06	[0.02; 0.16]	1.5%
Gutierrez-Galindo et al.(2019) Engraulis_encrasicolus Contracaecum_sp. Gutierrez-Galindo et al.(2019) Sardina_plichardus Contracaecum_sp.	-5.73 -5.77	1.4119 = 1.4120 =	0.00	[0.00; 0.05] [0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2019) Scomber_scombrus Contracaecum_sp.	-6.80	1.4134	0.00	[0.00: 0.02]	0.5%
Gutierrez-Galindo et al.(2010) Trachurus_trachurus Contracaecum_sp.	-5.74	1.4119 -	0.00	[0.00; 0.05]	0.5%
Valero et al. (2006) Phycis_blennoides Hysterothylacium_aduncum	-6.04	1.4125 -	0.00	[0.00; 0.04]	0.5%
Valero et al. (2006) Phycis_phycis Hysterothylacium_aduncum	-4.76	1.4082 ←	0.01	[0.00; 0.14]	0.5%
Valero et al. (2000) Micromesistius_poutassou Hysterothylacium_aduncum Bello et al. (2003) Speline, elichordus Historothylacium, aduncum	-6.40 -3.21	1.4130 • . 0.4698 +	0.00	[0.00; 0.03]	0.5%
Relio et al. (2008) Sandina_plichardus Hysterothylacium_aduncum Gutierrez-Galindo et al. (2010) Engraulis encrasicolus Hysterothylacium, aduncum	-5.73	1,4119 =	0.00	10.00: 0.051	0.5%
Gutierrez-Galindo et al.(2010) Sardina_pilchardus Hysterothylacium_aduncum	-5.77	1,4120 =	0.00	[0.00: 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Scomber_scombrus Hysterothylacium_aduncum	-6.80	1.4134 -	0.00	[0.00; 0.02]	0.5%
Relio et al.(2008) Sardina_pilchardus Hysterothylacium_aduncum	-2.18	0.2612	0.11	[0.07; 0.19]	1.9%
Valero et al. (2006) Phycis_blennoides Hysterothylacium_tabri Valero et al. (2006) Phycis_phycis Hysterothylacium_fabri	-6.04 -4.76	1.4125	0.00	[0.00; 0.04] [0.00; 0.14]	0.5%
Normanno et al. (2011) Merluccius_merluccius Anisakids	-4.76 -4.51	1.4064	0.01	[0.00:0.14]	0.5%
Normanno et al. (2011) Micromesistius: poutassou Anisakids	-3.93	0.9901	0.02	[0.00: 0.14]	0.8%
Normanno et al. (2011) Boops_boops Anisakids	-4.69	1.4077	0.01	[0.00; 0.14]	0.5%
Normanno et al.(2011) Trachurus_spp. Anisakids	-4.13	0.9919		[0.00; 0.11]	0.8%
Normanno et al. (2011) Sardina_pilchardus Anisakids	-4.67	1.4076	0.01	[0.00; 0.15]	0.5%
Random effects model Woterogeneity: I-squaredet 7.7%, tau-squaredet 1.806, p-tb.0001		•	0.01	[0.00; 0.01]	26.3%
proceedings in aguarente 7,7%, ara-aquarenti 1,800, pcs.0001					
viscera					
Gutierrez-Galindo et al. (2010) Engraulis_encrasicolus Anisakids	-5.73	1.4119 =	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Sardina_pilchardus Anisakids	-5.77	1.4120 -	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Engraulis_encrasicolus Anisakis_type_l	-5.73 -5.77	1.4119 × 1.4120 ×	0.00	[0.00; 0.05]	0.5% 0.5%
Gutierrez-Galindo et al.(2010) Sardina_pilchardus Anisakis_type_ll Valero et al.(2006) Phycis_phycis Anisakis_physeteris	-5.77 -3.37	0.6948	0.00	[0.01; 0.13]	1.2%
Valero et al. (2000) Micromesistius_poutassou Anisakis_physeteris	-3.63	0.3488 +	0.03	[0.01: 0.05]	1.7%
Farjallah et al.(2006) Phycis_blennoides Anisakis_physeteris	-1.97	0.1505 +	0.14	[0.10; 0.19]	2.0%
Farjallah et al.(2006) Phycis_phycis Anisakis_physeteris	-2.77	0.2165	0.06	[0.04; 0.10]	1.9%
Valero et al. (2006) Phycis_phycis Anisakis_simplex	-4.06	0.9913	0.02	[0.00; 0.12]	0.8%
Farjallah et al.(2006) Phycis_blennoides Avisakis_simplex_s.l. Farjallah et al.(2006) Phycis_phycis Anisakis_simplex_s.l.	-2.83 -3.00	0.2425 + 0.2437 +	0.06	[0.04; 0.09]	1.9%
Giannetto et al. (2006) Phycis_phycis Arisakis_amplex_st. Giannetto et al. (2006) Xiphias_gladius Anisakis_ap.	-0.20	0.0580	- 0.82	[0.73: 0.82]	2.1%
Valero et al. (2006)A Mertuccius_mertuccius Anisakis_sp.	-0.89	0,1503	→ 0.41	[0.31: 0.55]	2.0%
Gutierrez-Galindo et al.(2010) Engraulis_encrasicolus Contracaecum_sp.	-5.73	1.4119 -	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Sardina_pilchardus Contracaecum_sp.	-5.77	1.4120 -	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Scomber_scombrus Contracaecum_sp.	-3.91	0.3300 -	0.02	[0.01; 0.04]	1.8%
Gutierrez-Galindo et al.(2010) Trachurus_trachurus Contracaecum_sp. Keser et al.(2007) Pomatomus_saltatrix Hysterothylacium_aduncum	-5.04 -1.92	0.9968 0.3772	- 0.01 - 0.15	[0.00; 0.05]	0.8% 1.7%
Keser et al.(2007) Trachurus_trachurus Hysterothylacium_adunoum	-1.54	0.2955	- 0.21	[0.12; 0.38]	1.8%
Valero et al.(2006) Phycis_blennoides Hysterothylacium_aduncum	-0:67	0.0675	+ 0.51	[0.45; 0.58]	2.1%
Valero et al. (2006) Phycis_phycis Hysterothylacium_aduncum	-2.96	0.5622 ←	0.06	[0.02; 0.16]	1.4%
Valero et al.(2000) Micromesistius_poutassou Hysterothylacium_aduncum	-3.76	0.3735	0.02	[0.01; 0.05]	1.7%
Relio et al.(2008) Sardina_plichardus Hysterothylacium_aduncum Gutierrez-Galindo et al.(2010) Engraulis_encrasicolus Hysterothylacium_aduncum	-2.52 -5.73	0.3390 ÷	0.08	[0.04; 0.16]	1.8%
Gutierrez-Galindo et al.(2010) Sardina_pilchandus Hysterothylacium_aduncum	-5.77	1,4120 =	0.00	[0.00; 0.05]	0.5%
Gutierrez-Galindo et al.(2010) Scomber_scombrus Hysterothylacium_aduncum	-4.49	0.4447	0.01	[0.00; 0.03]	1.6%
Relio et al.(2008) Sardina_pilchardus Hysterothylacium_aduncum	-1.70	0.1973 +	0.18	[0.12; 0.27]	2.0%
Farjallah et al. (2006) Phycis_phycis Hysterothylacium_aduncum_L3	-4.38 -3.28	0.4969	0.01	[0.00; 0.03]	1.5%
Farjallah et al.(2006) Physis_physis Hysterothylacium_aduncum_L4 Ternengo et al.(2009) Diplodus_vulgaris Hysterothylacium_fabri	-3.28 -2.48	0.3832	0.04	[0.02; 0.07]	1.8%
Ternengo et al. (2009) Mullus_surmuletus Hysterothylacium_fabri	-0.23	0.0617	- 0.79	[0.70: 0.90]	2.1%
Ternengo et al. (2009) Pagellus_erythrinus Hysterothylacium_tabri	-0.48	0.1149	0.62	[0.49; 0.77]	2.0%
Ternengo et al. (2009) Phycis_phycis Hysterothylacium_tabri	-0.36	0.1035	0.70	[0.57; 0.86]	2.0%
Ternengo et al. (2009) Scorpaena_scrota Hysterothylacium_tabri	-1.00	0.1734	0.37	[0.26; 0.52]	2.0%
Ternengo et al. (2009) Symphodus_finca Hysterothylacium_fabri	-0.17	0.0983	0.84	[0.76; 0.94]	2.1%
Valero et al. (2006) Phycis_blennoides Hysterothylacium_tabri Valero et al. (2006) Phycis_phycis Hysterothylacium_fabri	-3.73 -0.56	0.4418 0.1143	0.02 	[0.01; 0.06]	1,6% 2,0%
Fariallah et al.(2006) Phycis blennoides Hysterothylacium fabri L3	-3.53	0.3483 +	0.03	[0.01:0.06]	1.7%
Farjallah et al.(2006) Phycis_phycis Hysterothylacium_fabri_L3	-2.77	0.2165 +	0.06	[0.04; 0.10]	1.9%
Farjallah et al.(2006) Phycis_blennoldes Hysterothylacium_fabri_L4	-2.20	0.1722 +	0.11	[0.08; 0.15]	2.0%
Farjallah et al.(2006) Phycis_phycis Hysterothylacium_tabri_L4	-0.95	0.0703	+ 0.39	[0.34; 0.44]	2.1%
Figus et al.(2005) Serranus_cabrilla Hysterothylacium_sp. Normanno et al.(2011) Mertuccius_mertuccius_Anisakids	-0.14 -0.37	0.0452 0.1002	0.87 0.89	[0.80; 0.95] [0.57; 0.84]	2.1%
Normanno et al. (2011) Mertuccius_mertuccius_Anisaxids Normanno et al. (2011) Micromesistius_poutassou Anisakids	-0.37 -0.13	0.1002 0.0511	- 0.89 - 0.88	[0.57; 0.84]	2.0%
Normanno et al.(2011) Boops_boops Anisakids	-3.30	0.6939 ←	0.04	[0.01; 0.14]	1.2%
Normanno et al.(2011) Trachurus_spp. Anisakids	0.00	0.0114	* 1.00	[0.98; 1.02]	2.1%
Normanno et al. (2011) Sardina_pilchardus Anisakids	-2.87	0.5608	0.06	[0.02; 0.17]	1.4%
Random effects model Woteregeneity: F-aguared=08.2%, tau-aguared=0.8005, p=0.0001		•	0.12	[0.10; 0.16]	73.7%
enverogenessy: r-squareumse.ess, rau-aquareum.num, pencuum					
Random effects model		į.	0.06	[0.04; 0.07]	100%
Heterogenalty: I-aquaradn97.5%, tau-aquaradn0.649, p-t0.0001					
		-1 -0.5 0	0.5 1		

Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species.

Valor of al. (2000) Moromeseinia, poutascou Arisakós, ypc. 1.59 1.500 1.000	Study Species Parasite	Mean Intensity	Standard Error		central value	95% C.I.	W_random
Valorio col. 14(2000) Micromosciptics, poutlascour, Anisakids 0.00 1.0000 1.000 1.00 1.014, 7.10 1.3% Coulterez-Galindo col. 14(2010) Forther, sombinum Anisakids, type_I 1.39 0.0500 1.0000 1.0000 1.00 1.010 1.14, 7.10 1.3% Valerio et al. (2000) Micromossibus, poutlassou, Anisakids, projecteris 0.00 1.0000 1.0000 1.00 1.010 1.14, 7.10 1.3% Valerio et al. (2000) Physic, blemofoldes Anisakis, physeteris 0.00 0.7071 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.000000 1.000000 1.0000000 1.0000000000							
Cultimers - Galindo et al. (2010) Roomber - soombrus Anisakis , ptpe 1		0.00	4.0000		4.00	[0.44: 7.40]	4.00/
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.				T.			
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.							
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.							
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.				<u></u>			
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.				-			
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.				-			
Normann or tal (2011) Micromesistius_poutassou Anisakids 0.00 1.0000 1.0000 1.000 1.057; 2.55 17.7% 1.3% Plandom effects model Heterogeneity: I-aquared=0.1572, ps.0.0783 17.7% 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.		0.81	0.3333	+	2.25		2.4%
Plandom effects model	Relio et al. (2008) Sardina_pilchardus Hysterothylacium_aduncum	0.32	0.2357	•	1.38	[0.87; 2.19]	2.5%
Vis.caria		0.00	1.0000	+			
Valoro ot al. (2006) Phyotic, phyotic Anicakic, physeteris 0.41 Valoro ot al. (2006) Phyotic, phyotic Anicakic, physeteris 0.22 0.3162 1.05 1.07, 2.32 2.4% Farjallah et al. (2006) Phyotic, bhyotic Anicakic, physeteris 0.18 0.00 0.1622 1.00 1.07, 1.37 2.6% Farjallah et al. (2006) Phyotic, bhyotic Anicakic, physeteris 0.18 0.00 1.000 1.000 1.001 1.01 1.01 1.01 1.05 1.00 1.01 1.01 1.01 1.01 1.03 1.03 1.03 1.00				•	1.66	[1.09; 2.55]	17.7%
Valoro et al.(2006) Physis, physics Anicakis, physeteris 0.21 0.41 0.5774 - 1.50 0.48; 4.66] 2.0% Valoro et al.(2006) Micromestatis, pourassour Anicakis, physeteris 0.22 0.3162 - 1.25 1.667; 2.32 2.4% Valoro et al.(2006) Physis, blennoides Anicakis, physeteris 0.18 0.2041 - 1.20 1.080; 1.79 2.6% Farjallah et al.(2006) Physis, physic Anicakis, physeteris 0.18 0.2041 - 1.20 1.080; 1.79 2.6% Valoro et al.(2006) Physis, physic Anicakis, simplex 0.00 0.10000 - 1.00 1.04; 7.10 1.3% Valoro et al.(2006) Physis, physic Anicakis, simplex 0.00 0.2500 - 1.00 1.04; 7.10 1.3% Valoro et al.(2008) Physis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.3333 - 1.00 0.52; 1.25 0.25% Valoro et al.(2007) Aphysis Physic Physics Physi	Heterogeneity: I-squared=41.9%, tau-squared=0.1572, p=0.0783						
Valoro et al.(2006) Physis, physics Anicakis, physeteris 0.21 0.41 0.5774 - 1.50 0.48; 4.66] 2.0% Valoro et al.(2006) Micromestatis, pourassour Anicakis, physeteris 0.22 0.3162 - 1.25 1.667; 2.32 2.4% Valoro et al.(2006) Physis, blennoides Anicakis, physeteris 0.18 0.2041 - 1.20 1.080; 1.79 2.6% Farjallah et al.(2006) Physis, physic Anicakis, physeteris 0.18 0.2041 - 1.20 1.080; 1.79 2.6% Valoro et al.(2006) Physis, physic Anicakis, simplex 0.00 0.10000 - 1.00 1.04; 7.10 1.3% Valoro et al.(2006) Physis, physic Anicakis, simplex 0.00 0.2500 - 1.00 1.04; 7.10 1.3% Valoro et al.(2008) Physis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.2500 - 1.00 0.51; 1.83 2.5% Valoro et al.(2009) Aphysis, physic Anicakis, simplex 0.00 0.3333 - 1.00 0.52; 1.25 0.25% Valoro et al.(2007) Aphysis Physic Physics Physi							
Valero et al. (2000) Micromesistus, poutassou Anisakis, physeteris 0.22 0.3162 1.25 0.67; 2.32 2.4% Farjallah et al. (2006) Phytois, phytois Anisakis, physeteris 0.18 0.2041 1.20 0.78; 1.37 2.6% Farjallah et al. (2006) Phytois, phytois Anisakis, physeteris 0.18 0.2041 1.20 0.80; 1.79 2.6% Valero et al. (2006) Phytois, phytois Anisakis, physeteris 0.18 0.2041 1.20 0.80; 1.79 2.6% Valero et al. (2006) Phytois, phytois Anisakis, pimplex, s.l. 0.00 0.2500 1.00 0.81; 1.63 2.5% Farjallah et al. (2008) Phytois, phytois Anisakis, pimplex, s.l. 0.00 0.2500 1.00 0.81; 1.63 2.5% Giannetto et al. (2009) Sphios, phytois Anisakis, pimplex, s.l. 0.00 0.2500 1.00 0.81; 1.63 2.5% Giannetto et al. (2009) Sphios, phytois Anisakis, s.p. 2.94 0.0312 1.90 0.787; 20.20 2.7% Valero et al. (2006) Metricucus, metrucius Anisakis, s.p. 0.46 0.1562 1.90 0.787; 20.20 2.7% Cultierrez-Galindo et al. (2010) Scomber, scombrus Contracaecum, s.p. 0.00 0.3333 1.00 0.52; 1.92 2.4% Cultierrez-Galindo et al. (2010) Trachrus L'actionus Contracaecum, s.p. 0.00 0.3033 1.00 0.52; 1.92 2.4% Cultierrez-Galindo et al. (2007) Trachrus L'actionus Contracaecum, s.p. 0.00 0.0000 1.00000 1.00000 1.0000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 1.000000 1.000000 1.0000000000		0.44	0.5774	. 1	4.60	10.49: 4.661	2.0%
Farjallah et al.(2006) Phytojs. Delnoriodes Anisakis, physeteris 0.18 0.2041 1.20 1.08, 1.79 2.6% Farjallah et al.(2006) Phytojs. Delnoriodes Anisakis, simplex 0.18 0.2041 1.20 1.08, 1.79 2.6% Valero et al.(2006) Phytojs. Delnoriodes Anisakis, simplex 0.00 0.10000 1.00 0.14; 7.10 1.3% Farjallah et al.(2006) Phytojs. Delnoriodes Anisakis, simplex 0.00 0.2500 1.00 0.81; 1.63 2.5% Farjallah et al.(2008) Phytojs. Delnoriodes Anisakis, simplex 0.00 0.2500 1.00 0.81; 1.63 2.5% Gaineretto et al.(2009) Replication of the anisakis 0.00 0.2500 1.00 0.81; 1.63 2.5% Valero et al.(2009) Replication of et al.(2010) Somber - scombrous Contracaccum.sp. 0.00 0.3333 1.00 0.52; 1.92 2.4% 0.0312 1.5% 1.16; 2.14 2.6% Culterrez-Galindo et al.(2010) Trachurus trachurus Contracaccum.sp. 0.00 0.1000 1.000 1.00 0.14; 7.10 1.3% Keser et al.(2007) Trachurus, trachurus Hysterothylacium. aduncum 0.29 0.3536 1.33 0.07; 2.67 2.4% Valero et al.(2006) Phytojs. Delmonides Hysterothylacium, aduncum 1.27 0.1768 3.66 2.51; 5.00 2.0% Valero et al.(2006) Phytojs. Delmonides Hysterothylacium aduncum 0.10 0.5774 1.00 0.32; 3.10 2.0% Valero et al.(2000) Micromesistius poutassou Hysterothylacium aduncum 0.56 0.2673 1.75 1.04; 2.95 2.5% Relio et al.(2008) Sardina, plichardus Hysterothylacium, aduncum 0.56 0.2673 1.75 1.04; 2.95 2.5% Relio et al.(2009) Sardina, plichardus Hysterothylacium, aduncum 0.72 0.1525 2.05 1.52; 2.70 2.0% Relio et al.(2009) Sardina, plichardus Hysterothylacium, aduncum 0.75 0.1525 2.05 1.52; 2.70 2.0% Relio et al.(2009) Sardina, plichardus Hysterothylacium, aduncum 0.75 0.1525 2.05 1.52; 2.70 2.0% Relio et al.(2009) Sardina, plichardus Hysterothylacium, aduncum 0.75 0.1525 2.05 1.52; 2.70 2.0% Relio et al.(2009) Sardina, plichardus Hysterothylacium, aduncum 0.75 0.1525 2.05 1.52; 2.70 2.0% Relio et al.(2009)				1			
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Farjallah et al.(2006) Phytois_ phytois Anisakis_sp. 0.00 0.2500 - 1.00 (0.81; 1.63) 2.5% Calienterot et al.(2009) Kiphias_gladius Anisakis_sp. 0.46 0.1562 - 1.58 1.16; 2.14 2.6% Calienterot_Galindo et al.(2010) Csomber_scombrous Contracaecum_sp. 0.00 0.3333 - 1.00 10.52; 1.92 2.4% Calienterot_Galindo et al.(2010) Csomber_scombrous Contracaecum_sp. 0.00 0.3333 - 1.00 10.52; 1.92 2.4% Calienterot_Galindo et al.(2010) Trachurus_trachurus Contracaecum_sp. 0.00 0.000 - 1.00 1.04; 7.10 1.3% Kasar et al.(2007) Pramatomus_pathtrix hysterothylacium_aduncum 0.29 0.3536 - 1.31 1.067; 2.67 2.4% Keser et al.(2007) Trachurus_trachurus_							
Valero et al.(2006) Ameritocius Ariisakis sp. 0.46 0.1562 1.88 1.16 2.14 2.5%		0.00	0.2500		1.00	[0.61; 1.63]	2.5%
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Gulferrez-Galindo et al. (2010) Trachurus Larchrurus Contracaecum.sp. 0.00					1.58	[1.16; 2.14]	
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Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species.