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Prevalence and Mean Intensity of Anisakidae Parasite in Seafood Caught in the Mediterranean Sea Focusing on Fish Species at Risk of Being Raw-consumed. A Meta Analysis and Systematic Review

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Objective: To assess the prevalence and mean intensity of anisakids in seafood caught in the Mediterranean Sea, focusing on fish species at risk of being raw-consumed. Design: Systematic review and meta-analysis of studies published from 1960–2012. Study selection: Main criteria for the inclusion of studies were as follows: Findings of anisakid larvae, in both muscles and viscera; fish species for human consumption caught in the Mediterranean Sea; prevalence and mean intensity data for each species; and sample size equal to or more than 40 fishes. Results: Twelve studies were identified. Among these, four studies considered the following three fish species that are often consumed raw or preserved lightly, or not cooked thoroughly: anchovy, pilchard, and Atlantic mackerel. Data synthesis: All pooled analyses were based on the random-effect model. Anisakids prevalence in fish muscle was 0.64% ($P < 0.0001$), in viscera it was 1.34% ($P < 0.0001$), and overall prevalence was 0.95% ($P < 0.0001$). Mean intensity in muscle was 2.31 ($P = 0.0083$), in viscera it was 1.55 ($P = 0.0174$), and overall it was 1.81 ($P < 0.0005$). Heterogeneity indices (I^2) were significantly high with the exception of viscera mean intensity. Conclusions: Anchovy, pilchard, and Atlantic mackerel have a low prevalence and mean intensity of anisakidae larvae in both viscera and muscles. Mean Intensity was also low.

Keywords Anisakids, Mediterranean seafood, systematic review, meta-analysis, fish consumed raw, zoonosis

INTRODUCTION

Anisakids nematodes larvae (genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, and *Hysterothylacium*) are common parasites present in many marine fishes, crustaceans, and squids: the problems caused by these parasites have impact on both public health (except *Hysterothylacium*) if they are not killed during food processing, and commercial value of the product. Anisakiasis refers to infection with anisakid larvae in people. Human

beings are accidental hosts in the life cycle of parasite, and acquire the live larvae by eating raw or preserved lightly, or undercooked seafood. Anisakiasis is a serious zoonotic disease with different forms depending on the localization and severity of lesions caused by worms. In Italy, 31 identified cases of human anisakiasis are reported over the period of 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 possible after surgery (Maggi et al., 2000; Pampiglione et al., 2002; Testini et al., 2003; Moschella et al. 2004; Montalto et al., 2005; Pellegrini et al., 2005; Ugenti et al., 2007; Biondi et al., 2008; Rea et al., 2008; Fumarola et al., 2009; Zullo et al., 2010; Filauro et al., 2011; Mattiucci et al., 2011).. Although freezing and cooking assure to kill the

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anisakids larvae, these treatments do not solve any health problem related to presence of anisakidae because of possible allergic reactions due to antigens resistant to freezing and cooking (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 et al., 1999; Sastre et al., 2000; Alonso-Gómez et al., 2004). On the contrary, other researchers showed that 13 of the 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 number of infected fishes to total number of fishes) and mean intensity (ratio of number of parasites to number of infected fishes) of anisakid larvae belonging to genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, and *Hysterothylacium*) and hosted by fishes for human consumption captured in the Mediterranean Sea.

METHODS

Eligibility Criteria

Criteria for the eligibility of studies are described as follows: (1) Presence of anisakids larvae (genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, and *Hysterothylacium*: not all these genera have the same human pathogenic potential but are morphologically very similar on visual examination). (2) Findings of larvae, in both muscles and viscera, detected by visual and/or enzymatic digestion methods (it is strategic to know the anatomical localization because of the epidemiologic implication of anisakiasis). (3) Fish species of legal size for human consumption, caught in the Mediterranean Sea and not aquacultured. (4) Prevalence and mean intensity data for each species. (5) Sample size equal to or more than 40 fishes: this sample size was chosen as the best compromise between a suitable estimate of parasitologic indices and the sample cost; in fact, a systematic underestimation of mean intensity was demonstrated with a small sample size (<40 fishes; Marques and Cabral, 2007), although in metazoan parasitology, the prevalence is a variable less affected by the sample size than the 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. (6) Papers published in English, French, Italian, Spanish, German, and Portuguese were 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. In addition, hand searches of the reference lists by Cristian Bernardi and that of two degree-theses were also performed.

Study Selection

The literature search was conducted independently by two investigators (Maddalena Castelletti and Cristian Bernardi). Two authors (Maddalena Castelletti and Cristian Bernardi) independently selected potentially eligible studies for inclusion. Disagreements between reviewers were resolved by consensus; if no agreement could be reached, it was planned that a third author (Patrizia Cattaneo) would decide. Full eligible citations were examined in more detail.

Data Extraction

For each study, prevalence and mean intensity values were extracted by two authors using predefined data fields as follows.

We developed a data extraction sheet, pilot-tested it on five randomly selected/included studies, and refined it accordingly. One review author (Cristian Bernardi) extracted from the 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 the categories defined according to the definition set by the authors); and its anatomical location. Sample size, number of infested fishes, number of parasites, prevalence, and mean intensity were also recorded. Prevalence was recorded as the number of infested fishes and the 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 to the number of parasitized fishes; if any data were missing, mean intensity derivation method was analogue to that used for prevalence.

The first author (Fabio Colombo) checked the extracted data to ensure data quality. Disagreements were resolved by discussion between the two review authors; if no agreement could be reached, it was planned that a third author would decide (Patrizia Cattaneo).

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

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 (CI) were calculated across all selected studies on statistical units that were every level combination of the following factors: host species of 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); this 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^2 statistic (Higgins et al., 2003). Ninety-five percent 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 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 reference lists by Cristian Bernardi. Records excluded based on titles and abstracts were 1669, and references left were 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 selection of studies is outlined in Figure 1. Four of these considered the fish species that were often consumed raw or preserved lightly, or not cooked thoroughly.

Characteristics of Included Studies

Table presents the characteristics of the 12 included articles, as summarised below.

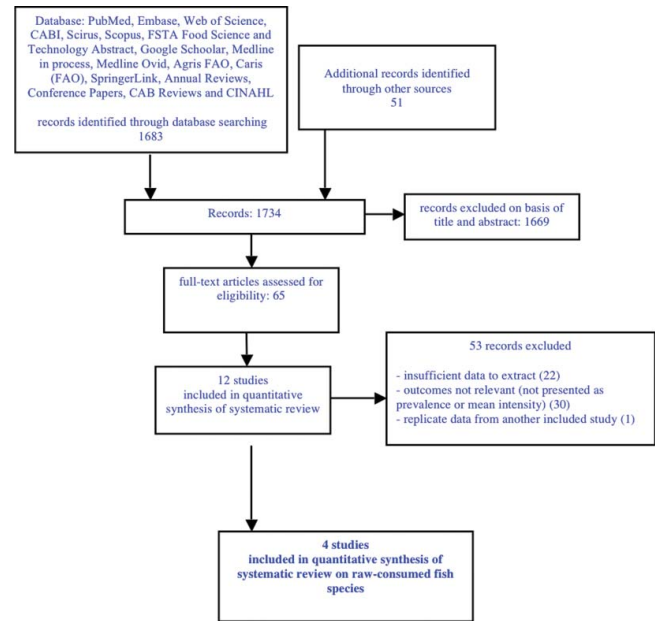


Figure 1 Selection of articles for meta-analysis.

Population

The included studies comprised 17 fish species, the total number of analyzed subjects were 3209 fishes, captured in different local fishing areas. Size of fishes 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 categories of parasites were present in both muscles and viscera.

Outcomes

Prevalence and mean intensity of anisakidae parasites were the primary outcomes assessed in all the studies. No additional outcomes were considered or recorded.

Prevalence and Mean Intensity of Anisakids

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

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

Prevalence of anisakids in muscles was 0.64% (0.23–1.75; $P < 0.0001$); in viscera 1.34% (0.52–3.43; $P < 0.0001$); overall prevalence was 0.95% (0.50–1.84; $P < 0.0001$). Mean intensity in muscles was 2.31 (1.18–4.51; $P = 0.0083$); in

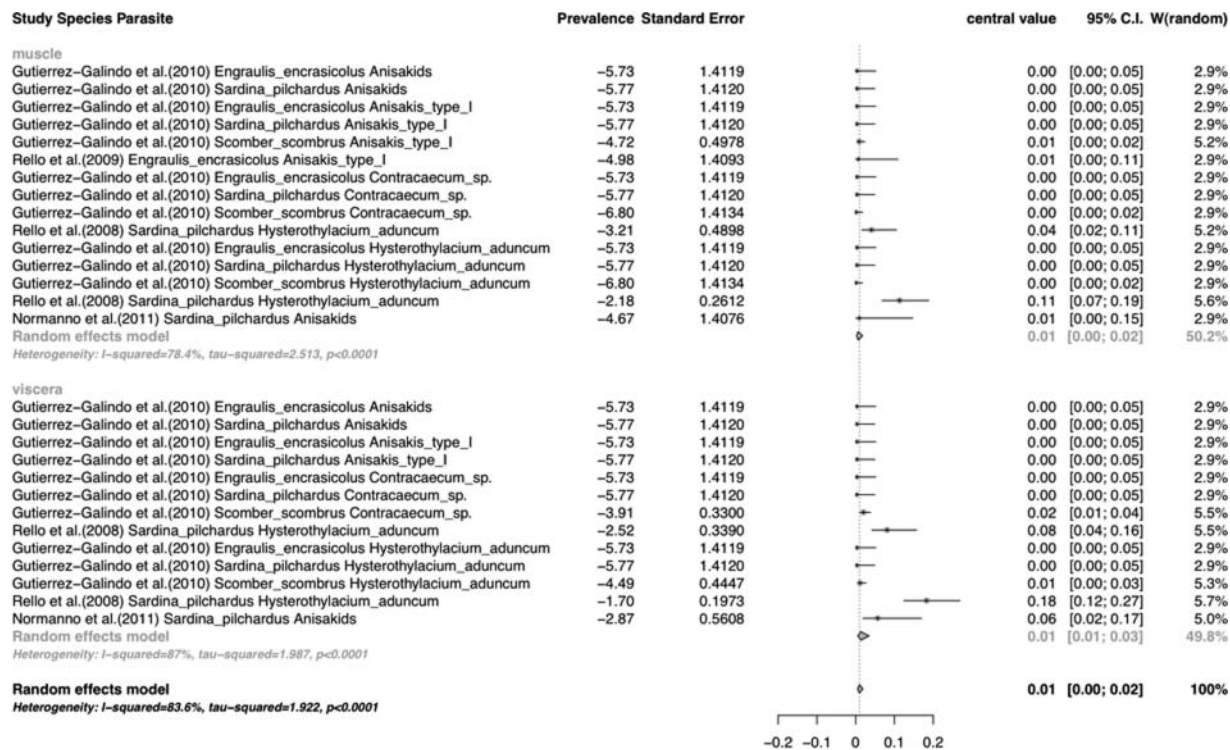


Figure 2 Forest plot of meta-analysis of anisakids prevalence on Mediterranean fish species at risk of being raw-consumed.

viscera 1.55 (1.08–2.23; $P = 0.0174$); overall Mean intensity was 1.81 (1.30–2.53; $P < 0.0005$). Heterogeneity indices (I^2) and P -values, respectively for muscles, viscera and overall, in the prevalence were: 78.40% ($P < 0.0001$), 87.00% ($P < 0.0001$), and 83.60% ($P < 0.0001$); these values in the mean intensity respectively were: 79.20% ($P = 0.0083$), 44.90% ($P = 0.1419$, not significant), and 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); the adjusted values are given in parentheses. Prevalence and mean intensity viscera prediction intervals were respectively 0.07 – 24.77% and 0.85 (1.09) – 2.83 (2.60); the adjusted values are given in parentheses. Overall prevalence and mean intensity respectively were 0.06 – 15.63% and 0.84 (1.12) – 3.89 (3.49); the adjusted values are given in parentheses.

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

DISCUSSION

Summary of Evidence

The rigorous sample size criteria adopted reduced the eligible papers, which should lead to robust results and conclusions. The studies covered in this work should provide high-quality evidence for meta-analysis. A careful examination of the papers revealed that the majority of the studies did not justify the choice of their sample size. Some studies were excluded due to lack of data; others due to the low quality of

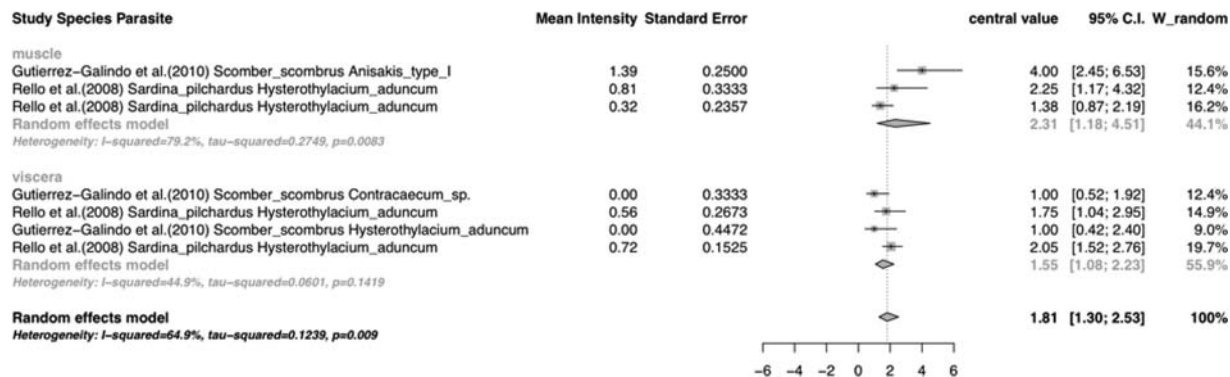


Figure 3 Forest plot of meta-analysis of anisakids mean intensity on Mediterranean fish species at risk of being raw-consumed.

Table 1 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 al. (2006)	<i>Merluccius merluccius</i>	40–52	Viscera Muscle	<i>Anisakis spp</i>	Mediterranean off southern Spain	63
Gutiérrez-Galindo et al. (2010)	<i>Engraulis encrasicolus</i>	12.3–16.5	Viscera	<i>Anisakis spp</i>	Tarragona (NE Spain)	153
			Muscle	No anisakids		
	<i>Sardina pilchardus</i>	11.5–19	Viscera	No anisakids		
	<i>Scomber scombrus</i>	20.5–37.0	Muscle	<i>Anisakis type I</i>		
			Viscera	<i>Anisakis type I</i>		447
			Viscera	<i>Hysterothylacium aduncum</i>		
			Viscera	<i>Contracaecum spp</i>		
	<i>Trachurus trachurus</i>	16.2–30.2	Muscle	<i>Anisakis type I</i>		
Keser et al. (2007)			Viscera	<i>Anisakis type I</i>	Dardanelles at Çanakkale, Turkey	41
	<i>Pomatomus saltatrix</i>	Not stated	Viscera	<i>Contracaecum spp</i>		
			Viscera	<i>Hysterothylacium aduncum</i>		
			Viscera	<i>Hysterothylacium aduncum</i>		
Rello et al. (2009)	<i>Engraulis encrasicolus</i>	9.0–18.8	Muscle	<i>Anisakis type I</i>	NW Alborán Sea	72
	<i>Serranus cabrilla</i>	10.5–19.5	Viscera	<i>Hysterothylacium spp</i>		71
Valero et al. (2000)	<i>Micromesistius poutassou</i>	17–28	Muscle	<i>Anisakis pegreffii</i>	Mediterranean region of southern Spain	301
Farjallah et al. (2006)			Viscera	<i>Anisakis pegreffii</i>	Eastern Mediterranean coasts in Tunisia	272
			Viscera	<i>Anisakis physeteris</i>		
			Viscera	<i>Hysterothylacium aduncum</i>		
			Viscera	<i>Anisakis physeteris</i>		
			Viscera	<i>Anisakis simplex s.l</i>		
			Viscera	<i>Hysterothylacium fabri L3</i>		
			Viscera	<i>Hysterothylacium fabri L4</i>		320
			Viscera	<i>Hysterothylacium fabri L4</i>		
			Viscera	<i>Anisakis physeteris</i>		
			Viscera	<i>Anisakis simplex s.l</i>		
			Viscera	<i>Hysterothylacium aduncum L3</i>		
			Viscera	<i>Hysterothylacium aduncum L4</i>		
Rello et al. (2008)			Viscera	<i>Hysterothylacium fabri L3</i>	Northern Alboran Sea	99
			Viscera	<i>Hysterothylacium fabri L4</i>		
			Viscera	<i>Hysterothylacium aduncum</i>		
			Muscle	<i>Hysterothylacium aduncum</i>		
			Viscera	<i>Hysterothylacium aduncum</i>		
			Muscle	<i>Hysterothylacium aduncum</i>		
Temengo et al. (2009)			Viscera	<i>Hysterothylacium aduncum</i>	Bonifacio Strait Marine Reserve (Corsica Island, Mediterranean Sea)	72
	<i>Diplodus vulgaris</i>	Not stated	Viscera	<i>Hysterothylacium fabri</i>		
	<i>Mullus surmuletus</i>	Not stated	Viscera			
	<i>Pagellus erythrinus</i>	Not stated	Viscera			
	<i>Phycis phycis</i>	Not stated	Viscera		Western Balearic Sea	40
			Viscera			

Table 1 Included studies: host fish species, size, fish area, anatomical localization of larvae, type of parasite, and sample size (*Continued*)

Source	Host fish species	Host fish size (length, cm)	Anatomical localization of larvae	Type of larva	Fishing area	Sample size	
Valero et al. (2006)	<i>Scorpaena scrofa</i>	Not stated	Viscera	<i>Anisakis physeteris</i>	Mediterranean coasts of eastern Andalusia (Southern Spain)	57	
	<i>Symphodus tinca</i>	Not stated	Viscera			58	
	<i>Phycis blennoides</i>	18–55	Muscle			209	
	<i>Phycis phycis</i>	24–58	Muscle	<i>Anisakis simplex</i>		58	
			Viscera	<i>Anisakis physeteris</i>			
			Viscera	<i>Anisakis simplex s.l.</i>			
			Viscera	<i>Hysterothylacium aduncum</i>			
			Viscera	<i>Hysterothylacium fabri</i>			
			Viscera	<i>Anisakis physeteris</i>			
			Viscera	<i>Anisakis simplex s.l.</i>			
Viscera			<i>Hysterothylacium aduncum</i>				
Giannetto and Gaglio (2006) Normanno et al. (2011)	<i>Xiphias gladius</i>	Not stated	Viscera	<i>Hysterothylacium fabri</i>	Tyrrhenian and Ionian Sea Adriatic sea	66	
	<i>Merluccius merluccius</i>	Not stated	Viscera	Anisakids		45	
	<i>Micromesistius poutassou</i>	Not stated	Viscera	Anisakids		51	
	<i>Boops boops</i>	Not stated	Muscle			54	
			Viscera				62
			muscle				
	<i>Trachurus trachurus</i>	Not stated	Viscera				
	<i>Sardina pilchardus</i>	Not stated	Viscera			53	

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%, this value being of the same order of magnitude in both muscles and viscera. Mean intensity data yield a value that is around 2, this value is similar to that for viscera, whereas it is slightly lower than that for muscles. After a question was raised by a referee, a kind of sensitivity analysis (s.a.) was performed to assess the impact of larvae detection on results. As it is known that visual method has a lower recovery rate than enzymatic method, 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 considered variable. On the contrary, the same sensitivity analysis when applied to appendix data yielded higher outcome values in favor of the visual method; this effect, being in the opposite direction than expected, could be likely due to the very heterogeneous fish species considered in studies.

CONCLUSIONS

Implications of Practice

The three considered species (anchovy, pilchard, and Atlantic mackerel) have been found lightly parasitized, but this is of primary importance for food safety that they undergo before consumption to a preventive treatment to kill all possible live larvae. The absence of genus *Pseudoterranova* in the Mediterranean Sea is confirmed since no record was found in the included studies (see Table 1. In routine control of fishery products, a 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 anisakidae larvae in the target species

recommend researchers to improve sample size because large sample size (100–1000) is required to detect very low prevalence (Gregory et al., 1991). Gutiérrez-Galindo et al. (2010) recorded no presence of anisakidae larvae in anchovy and pilchard; on the contrary, in Atlantic mackerel caught in the same area, they recorded a mean prevalence of 11%. It would be very interesting to understand whether this evidence was 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, because most of the 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 publication of raw data is undervalued by researchers in this field since to access raw data would allow statistical comparison and elaboration. Moreover, this work indicates that further research is necessary on anisakids in the Mediterranean fish species that proved to be highly parasitized in other fishery areas, such as European sea bass, monkfish, or hake.

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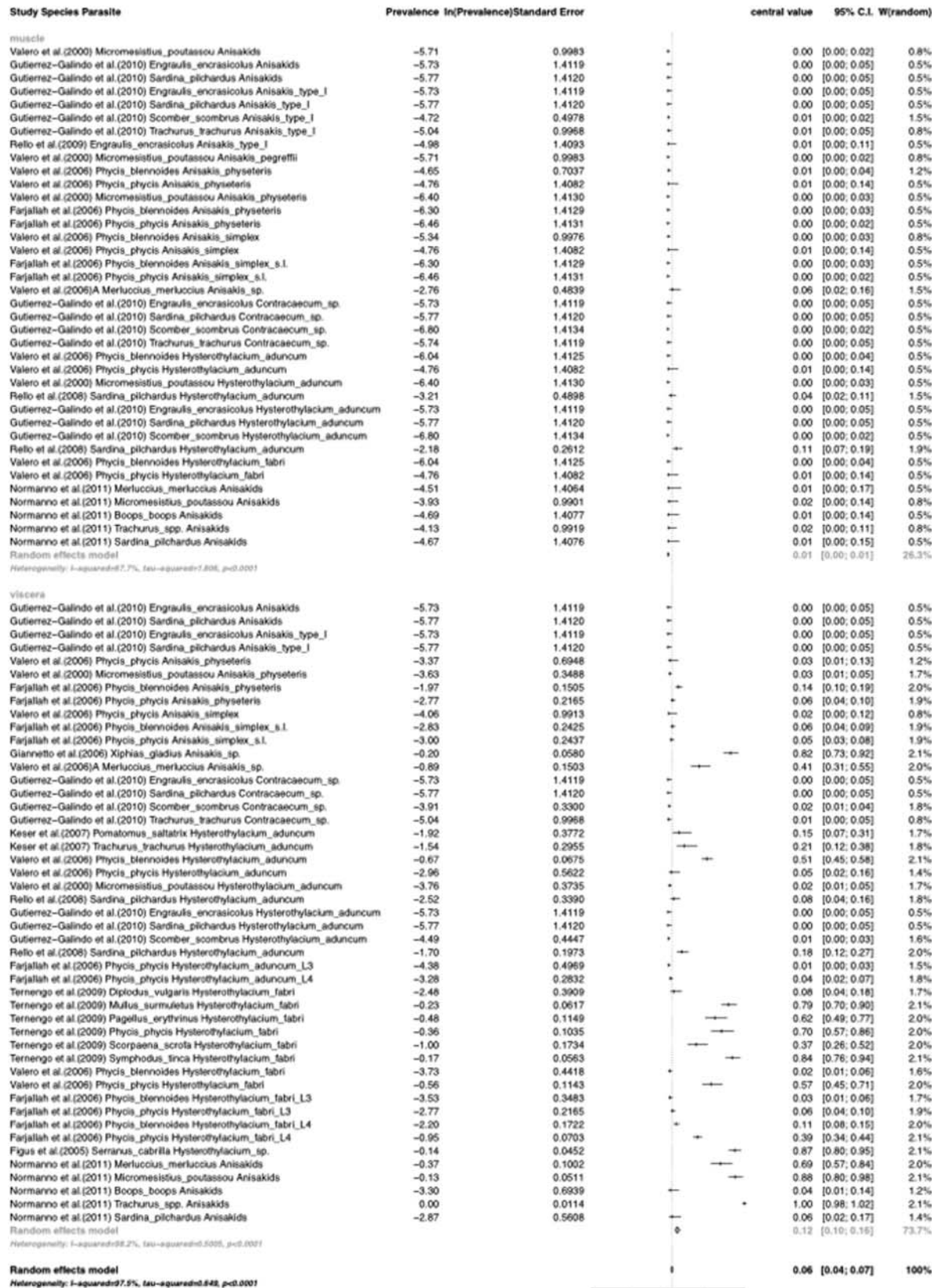
FUNDING

This review was not funded.

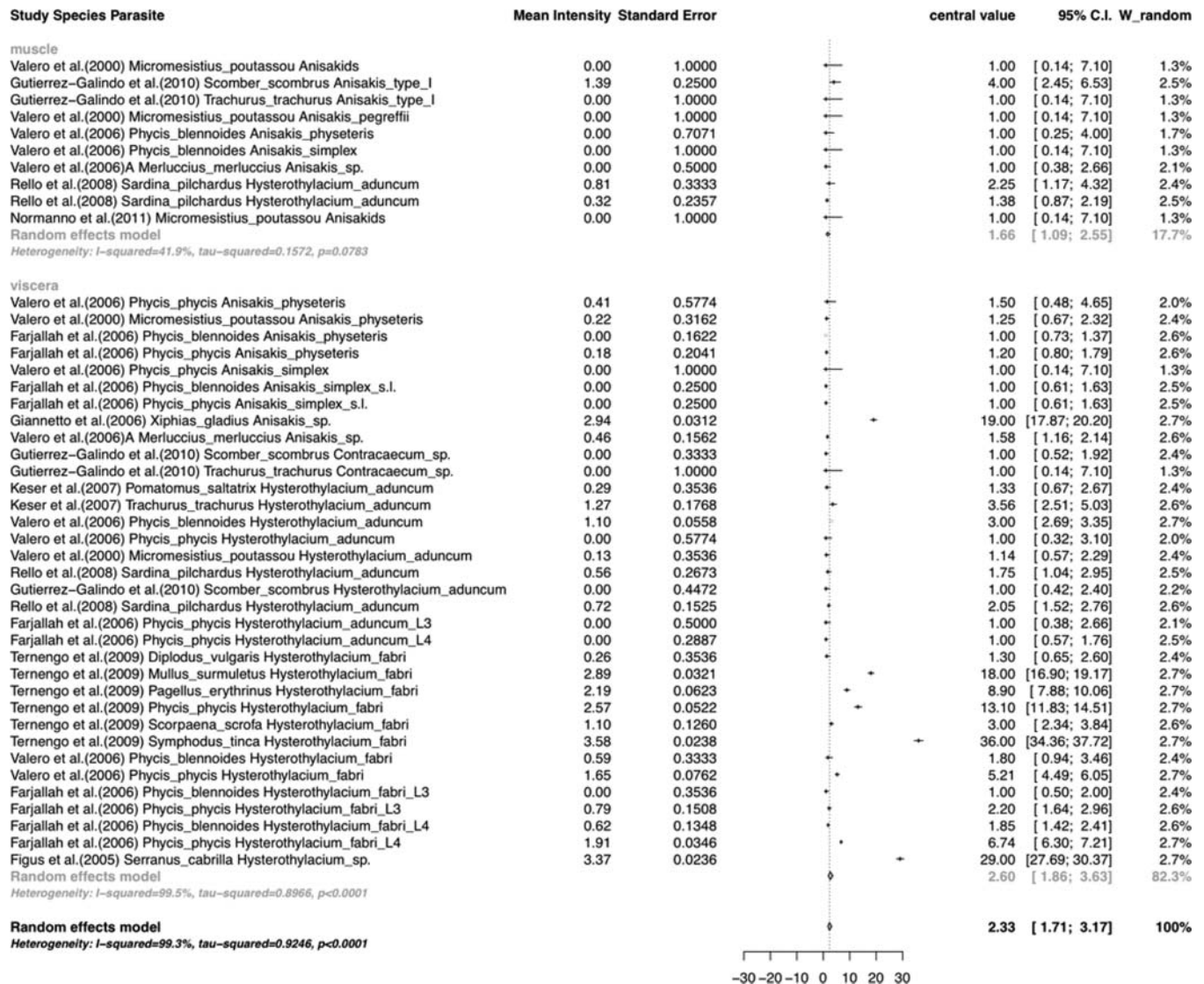
STATEMENT OF INTEREST

The authors declare no real or perceived conflict of interest.

Appendix A



Appendix B



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