Food and Waterborne Parasitology 41 (2025) e00288

Contents lists available at ScienceDirect

Food and Waterborne Parasitology

journal homepage: www.elsevier.com/locate/fawpar

Decision-making and public health: How the prevalence of *Contracaecum* spp. larvae in market-size tilapia may influence fish sample-size to be inspected prior marketing

Nadav Davidovich [a,d,*], Perla Tedesco [b], Monica Caffara [b,**], Ekaterina Minkova [c], Ortal Aflalo [c], Shoshi Hadar [c], Vicotoria Baramboim [c], Gavriel Goldstein [c], Ofer Cohen [c], Shani Glasner [c], Michal Perry Markovich [a], Danny Morick [d,e], Aurora Lattanzi [b], Andrea Gustinelli [b]

[a] *Israeli Veterinary Services, Bet Dagan, Israel*
[b] *Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Via Tolara di Sopra 50, Ozzano Emilia, BO, Italy*
[c] *The Agency for the Veterinary Inspection of Food, Lod, Israel*
[d] *Morris Kahn Marine Research Station, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[e] *Department of Blue Biotechnologies and Sustainable Mariculture, The Leon H. Charney School of Marine Sciences, University of Haifa, Israel*

ARTICLE INFO

Keywords:
Contracaecum larvae
Seafood safety
Foodborne parasitic nematodes
Tilapia

ABSTRACT

Human consumption of raw or undercooked fish and fishery products may cause infection with foodborne parasitic nematodes, particularly of the family Anisakidae. *Contracaecum* species are cosmopolitan parasitic nematodes with numerous marine and freshwater fish species as intermediate or paratenic hosts, rarely reported as zoonotic agents. Tilapia, of great importance to human nutrition in many countries, can harbor larval stages of diverse *Contracaecum* species. Accurate examination of fish before marketing is crucial to ensure public health. We conducted a two-year survey of market-size tilapia farmed in Israel originating from 17 tilapia farms to assess the presence of *Contracaecum* larvae, including a retrospective calculation of infection prevalence and analysis of the accuracy of larval detection with various sample sizes. Between June 2020 and May 2022, *Contracaecum* larvae were found in 269/3605 (7.5 %) tilapia shipments. In 217 of these 269 (80.7 %) shipments, only a single larva was found. Among 380 *Contracaecum* larvae collected, only two were identified as *C. quadripapillatum*; all others were identified as *C. multipapillatum* E. The probability of tilapia being parasitized with *C. multipapillatum* E larvae is much higher than with *C. quadripapillatum*. Moreover, in the vast majority of tilapia shipments infected with *Contracaecum* larvae, only a single specimen was infected. Considering this relatively low prevalence, in a sample size of 30 fish tested for the presence of nematode larvae, there is only a 40 % probability of finding an infected specimen. Decision-makers should consider, among other factors, the sample size of tilapia to be inspected before marketing to reduce the chances of parasitized fish reaching the end consumer.

* Corresponding author at: Israeli Veterinary Services, Bet Dagan, Israel.
** Corresponding author.
E-mail addresses: Nadavd@moag.gov.il (N. Davidovich), monica.caffara@unibo.it (M. Caffara).

https://doi.org/10.1016/j.fawpar.2025.e00288
Received 22 June 2025; Received in revised form 8 September 2025; Accepted 11 September 2025
Available online 12 September 2025

1. Introduction

With the rising global population, farmed fish has become an important basic food product in the human diet due to its high nutritional content and relatively low environmental impact compared to terrestrial livestock and poultry farming (Han et al., 2017; Stentiford et al., 2020). However, foodborne parasitic nematodes may pose a risk to human health when consumed raw or undercooked (Suthar and Shamsi, 2021). Whereas for foodborne parasitic nematodes of mammalian origin (e.g., *Trichinella* spp.), science and legislation have established methods to diagnose and calculate sample size according to risk (Gajadhar et al., 2009), controlling foodborne parasitic nematodes of finfish origin may be more challenging for several reasons. In addition to cooking, freezing is one of the main methods that has been developed and standardized to ensure public health (FDA, 2019), but it does not provide a solution in cases of market demand for fresh products. Beyond the risk to humans of clinical infection with larval nematodes of the family Anisakidae, repeated exposure to larvae (dead or vital), or even small fragments of larvae, may provoke allergic reactions (Kochanowski et al., 2020; Robertson, 2025). Moreover, veterinary inspection of each individual fish is logistically challenging and often impractical at scale, and many fish species are marketed whole (non-eviscerated).

The family Anisakidae includes, among others, the three zoonotic genera *Pseudoterranova*, *Anisakis* and *Contracaecum*, in ascending order of species richness (Adroher-Auroux and Benítez-Rodríguez, 2020). With over 60 species (Buchmann and Mehrdana, 2016), the genus *Contracaecum* includes complexes of sibling species infecting the digestive tract of marine mammals (Shamsi et al., 2009; Zuo et al., 2018) and piscivorous birds, mainly of the families Pelecanidae (Landsberg, 1989; Mattiucci et al., 2010, 2020), Phalacrocoracidae, and Ardeidae, among others (Shamsi, 2019). In the heteroxenous life cycle of these nematodes, copepods act as first intermediate hosts (Køie and Fagerholm, 1995; Dziekońska-Rynko and Rokicki, 2007) while a variety of finfish from different families act as the second intermediate/paratenic host (Ángeles-Hernández et al., 2020; Paperna, 1964; Younis et al., 2017).

Historically, morphological examination of adult stages of *Contracaecum* was the main tool for species identification (Fagerholm, 1988), while the morphology of larval stages only allowed the distinction of *Contracaecum* from the related genera *Anisakis* and *Phocanema* (Olson Jr et al., 1983); with time, a variety of molecular tools opened new horizons for researchers with respect to parasite phylogeny and species identification, demonstrating that some identical morphotypes may represent more than one species (Mattiucci et al., 2020; Zuo et al., 2018; Shamsi et al., 2011). Subsequently, other studies, though focused primarily on adult stages, have reaffirmed the importance of integrated taxonomy for accurate diagnosis (Caffara et al., 2023; Shamsi et al., 2024).

In Israel, Paperna (1964) published the first report of *Contracaecum* larvae infecting different fish species in different water bodies by describing morphologically five types of *Contracaecum* larvae. Landsberg (1989) later reported infections with non-encapsulated *Contracaecum* larvae in the pericardial cavity of farmed tilapia hybrids in Israel. The occurrence of *Contracaecum* larvae in the pericardial cavity of warm water fish is relatively common (30–70 % prevalence) in cichlids (*Oreochromis* spp., *Tilapia* spp., and *Haplochromis* spp.) from African freshwater ecosystems (Hamouda and Younis, 2022; Paperna, 1974). Recently, *Contracaecum multipapillatum* E was described, morphologically and molecularly, for the first time in hybrid tilapia (*Oreochromis aureus* × *O. niloticus*) and red drum (*Sciaenops ocellatus*) farmed in polyculture in Kfar Ruppin, northeast Israel (Davidovich et al., 2022). The same species has also been reported in a few wild fish species in African waterbodies in Ethiopia, Egypt, and Kenya, by Otachi et al. (2014) and Younis et al. (2017): although these authors did not identify the larvae to the species level, the identification of *C. multipapillatum* E was subsequently confirmed by matching with available sequences in GenBank (Davidovich et al., 2022). Later still, an adult female *C. multipapillatum* E was collected from a great white pelican (*Pelecanus onocrotalus*) in Israel and described by molecular analysis (Caffara et al., 2023). Finally, Davidovich et al. (2023) detected *C. multipapillatum* E in the pericardial cavity of a single specimen of blue tilapia (*O. aureus*) from the Sea of Galilee (northeast Israel), together with *Contracaecum quadripapillatum* in *Carasobarbus canis* and *Luciobarbus longiceps* from the same area.

Over the years, scientific publications have described the presence of *C. multipapillatum* larvae in cultured and wild tilapia in Israel, as well as *C. multipapillatum* E in its definitive host. However, none of these papers focused on a long-term monitoring survey and the possible implication for the sample size of tested fish before marketing. Although anatomically *Contracaecum* larvae are located mainly in the pericardial and celomatic cavities, public health issues may arise, especially because *Contracaecum* larvae are stimulated to leave their host once it dies and as a result, may migrate to other tissues, including the flesh (Smith, 1999; Shamsi et al., 2018; Nonković et al., 2025).

In the last few decades, *Contracaecum* larvae, and especially *Contracaecum osculatum* (Nagasawa, 2012), have gained more attention as zoonotic agents (Shamsi, 2019; Shamsi and Butcher, 2011). However, to date, there has been no molecular identification of *Contracaecum* larvae recovered from human cases; this raises the possibility that other *Contracaecum* species may also have zoonotic potential and underlines the importance of accurate species-level identification, especially in the context of seafood safety and public health (Shamsi and Barton, 2023; Rahmati et al., 2020). Although rarely responsible for human clinical cases, this genus is distributed worldwide, and larval stages have been found in both marine and freshwater fish species (Shamsi, 2019).

For some *Contracaecum* species (including *C. multipapillatum* E), larval stages are clearly visible due to their relatively large size (3–4 cm, Davidovich et al., 2022), and thus more easily removed and discarded by food business operators. In accordance with the Israeli public health protection law (Food) of 2015, the Department of Supervision for Food of Animal Origin (DSFAO) within the Israeli Veterinary Services is responsible for state supervision of all fish-sorting stations. Public veterinarians in all fish-sorting stations in Israel perform organoleptic examination of all marketed seafood (mostly fish) products in accordance with the procedure for fish premarketing control (Israeli Veterinary Services, 2017), based on COUNCIL REGULATION (EEC) No. 103/76 of 19 January 1976 laying down common marketing standards for certain fresh or chilled fish. The fish examination includes, among other things, visual inspection of the abdominal cavity, internal organs, and musculature for the presence of zoonotic parasites. In the procedure for fish premarketing control, the minimum sample size for testing fish by cut is three specimens. Israeli legislation is also based on two later

EU legislative anchors addressing the risk of foodborne parasitic nematodes in products of animal origin, particularly: (1) Regulation (EC) No 853/2004, which lays down specific hygiene rules for food of animal origin, including measures for controlling parasites in fishery products intended for human consumption and (2) Regulation (EC) No 2074/2005, which outlines implementing measures, including those related to visual inspection requirements for parasites. According to EU legislation, correct application of the preventive procedures for managing the parasitological risk of anisakidosis (D'amico et al., 2014) is also important for freshwater fish species such as tilapia (Davidovich et al., 2022; Kaba et al., 2024).

The aims of the present study were to: (a) evaluate the prevalence of *Contracaecum* larvae over a two-year period among 17 tilapia farms in Israel; (b) identify the larvae to the species level; and (c) Assess the effectiveness and limitations of the current sampling strategy used during official surveillance for detecting *Contracaecum* larvae in market-size tilapia, and model the impact of different sample sizes on detection probability.

2. Material and methods

2.1. Fish sampling and larva identification

Since June 2020, intensified sampling has been applied by the DSFAO on any tilapia batch, regardless of its origin, as follows: an initial sample size of 30 randomly selected specimens for each tilapia batch—10-fold higher (n = 3 vs. 30). If the test results are negative for larvae referable to the genus *Contracaecum*, the fish can be marketed whole. If the test results are positive (a single *Contracaecum* larva), the public veterinarian will test another 30 randomly selected tilapia specimens by cut (total sample size = 60). If two or more *Contracaecum* larvae are detected, all fish in the infected batch are eviscerated and filleted, followed by careful inspection using a white light transilluminator (Fig. 1). Using this sampling scheme, between June 2020 and May 2022, 17 tilapia farms were assessed for the presence of *Contracaecum* larvae. Details of the number of shipments and fish sampled, along with the locations of the fish farms, including prevalence of infection, are reported in Table 1 and Fig. 2. Larvae were isolated using a dissecting needle and tweezers, counted, and preserved in 70 % ethanol for downstream analyses. The larvae were observed under a light microscope (Leica Microsystems, Wetzlar, Germany) to record total length and a small portion (about 5 mm) was cut from the central part of the larvae for molecular analysis. All of the collected larvae were subjected to molecular identification following Caffara et al. (2023). Briefly, the middle portions of the collected larvae were subjected to a fast DNA extraction method using Chelex®100 (Sigma-Aldrich, Darmstadt, Germany): 300 µl of 5 % Chelex®100 solution in sterile DNA/RNA free molecular grade water was added and heated at 95 °C for 10 min. and then centrifuged at full speed for five min. The supernatant was removed and diluted 1:10 for downstream molecular analyses. The ITS rDNA was amplified with primers NC5_f (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and NC2_r (5′-TTA GTT TCT TCC TCC GCT −3′) (Zhu et al., 1998) and then 10 µl were subjected to PCR-RFLP with the restriction enzymes *Msp*I (Zhu et al., 2007) and *Ssp*I (Caffara et al., 2023) to distinguish among species of *Contracaecum*. After digestion (37 °C for 90 min) the specimens were electrophoresed on a 2 % agarose gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, Carlsbad, CA, USA) in 0.5× TBE

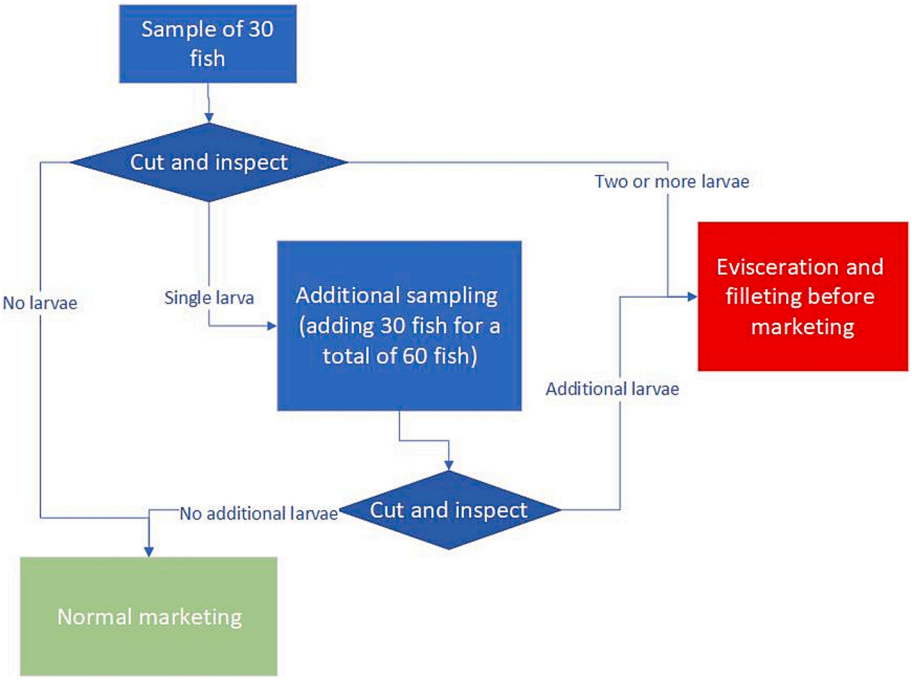

Fig. 1. Decision tree for tilapia sampling during the intensified sampling period.

Table 1
Analyzed tilapia and number of specimens parasitized by *Contracaecum* larvae with prevalence of positive shipments and fish, mean intensity and mean abundance.

Farm	Positive shipments/ total shipments	%	Positive fish/no. of fish tested	%	No. of larvae	Mean intensity	Mean abundance	*Contracaecum* spp.
A	11/44	25	18/680	2.6	16	0.9	0.02	*C. multipapillatum* E
B	4/74	5.4	8/270	3	8	1	0.03	*C. multipapillatum* E
C	12/143	8.4	16/790	2	15	0.9	0.02	*C. multipapillatum* E
D	26/205	12.7	30/1585	1.9	30	1	0.02	*C. multipapillatum* E *C. quadripapillatum*
E	3/253	1.2	3/180	1.7	3	1	0.02	*C. multipapillatum* E
F	6/183	3.3	7/310	2.3	9	1.3	0.03	*C. multipapillatum* E
G	22/365	6	27/1530	1.8	26	1	0.02	*C. multipapillatum* E
H	3/250	1.2	5/180	2.8	3	0.6	0.02	*C. multipapillatum* E
I	8/143	5.6	9/464	1.9	13	1.4	0.03	*C. multipapillatum* E
J	14/434	3.2	15/850	1.8	15	1	0.02	*C. multipapillatum* E
K	27/259	10.4	38/1689	2.2	38	1	0.02	*C. multipapillatum* E *C. quadripapillatum*
L	13/188	6.9	13/780	1.7	13	1	0.02	*C. multipapillatum* E
M	12/212	5.7	13/720	1.8	13	1	0.02	*C. multipapillatum* E
N	83/336	24.7	108/5083	2.1	117	1.1	0.02	*C. multipapillatum* E
O	16/234	6.8	46/990	4.6	46	1	0.05	*C. multipapillatum* E
P	2/165	1.2	2/120	1.7	2	1	0.02	*C. multipapillatum* E
Q	7/117	6	13/410	3.2	13	1	0.03	*C. multipapillatum* E
Total	**269/3605**	**7.5**	**371/16631**	**2.2**	**380**			

for 90 min. In each digestion reaction, previously Sanger-sequenced specimens were included as positive controls.

2.2. Data analysis

Data collected by public veterinarians at all fish-sorting stations were recorded using a data-collection website interface (https://foodinspection.moag.gov.il). Prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997). To estimate the probability of detecting at least one positive specimen across different sample sizes and assumed prevalence levels, we used a binomial probability model. This computation was implemented in R (version 4.4.2; R Core Team, 2024) using cumulative distribution function of the binomial distribution via pbinom function. We calculated the probability for sample sizes ranging from 1 to 100 for five possible prevalence values: the mean and mode of observed prevalence in the study dataset, and fixed prevalence values of 20 %, 5 %, and 1 %. The results were visualized using ggplot2 package (Wickham, 2016). Data were analyzed using the R statistical computing environment in the RStudio integrated development environment (Team, 2023).

3. Results

3.1. Fish inspections

A total of 3605 tilapia batches were analyzed during a two-year period. Inspections conducted on tilapias from 17 farms revealed an overall prevalence of 2.2 % (16,631 fish tested, 371 positive) for *Contracaecum* larvae: a total of 380 larvae were collected, with a mean intensity of 1 (range: 0.6–1.4) and a mean abundance of 0.02 (range: 0.02–0.05). In Table 1, the cumulative results for each farm are reported. In 217 of the 269 tilapia shipments in which larvae were detected during the study (80.7 %), a single *Contracaecum* larva was found. In 48 of these shipments, two or more larvae were detected (Table 2).

3.2. Molecular identification

Out of 380 larvae collected, 378 (99.5 %) were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses as *C. multipapillatum* E; only two (0.5 %) were identified as *C. quadripapillatum* (Table 1).

3.3. Evaluation of surveillance scheme

To evaluate the applied surveillance scheme, we calculated the probability of detecting one (Fig. 3A) or more (Fig. 3B) infected fish, assuming different infection rates and sample sizes. Considering the low prevalence of *Contracaecum* larvae in the current study, sampling 30 fish from each shipment yields a 40 % probability of finding a parasitized specimen (Fig. 3A) and a 10 % probability of forwarding the shipment to processing and cleaning. Increasing the sample size to 60 specimens would result in redirecting 25 % of the shipments to processing and cleaning (Fig. 3B).

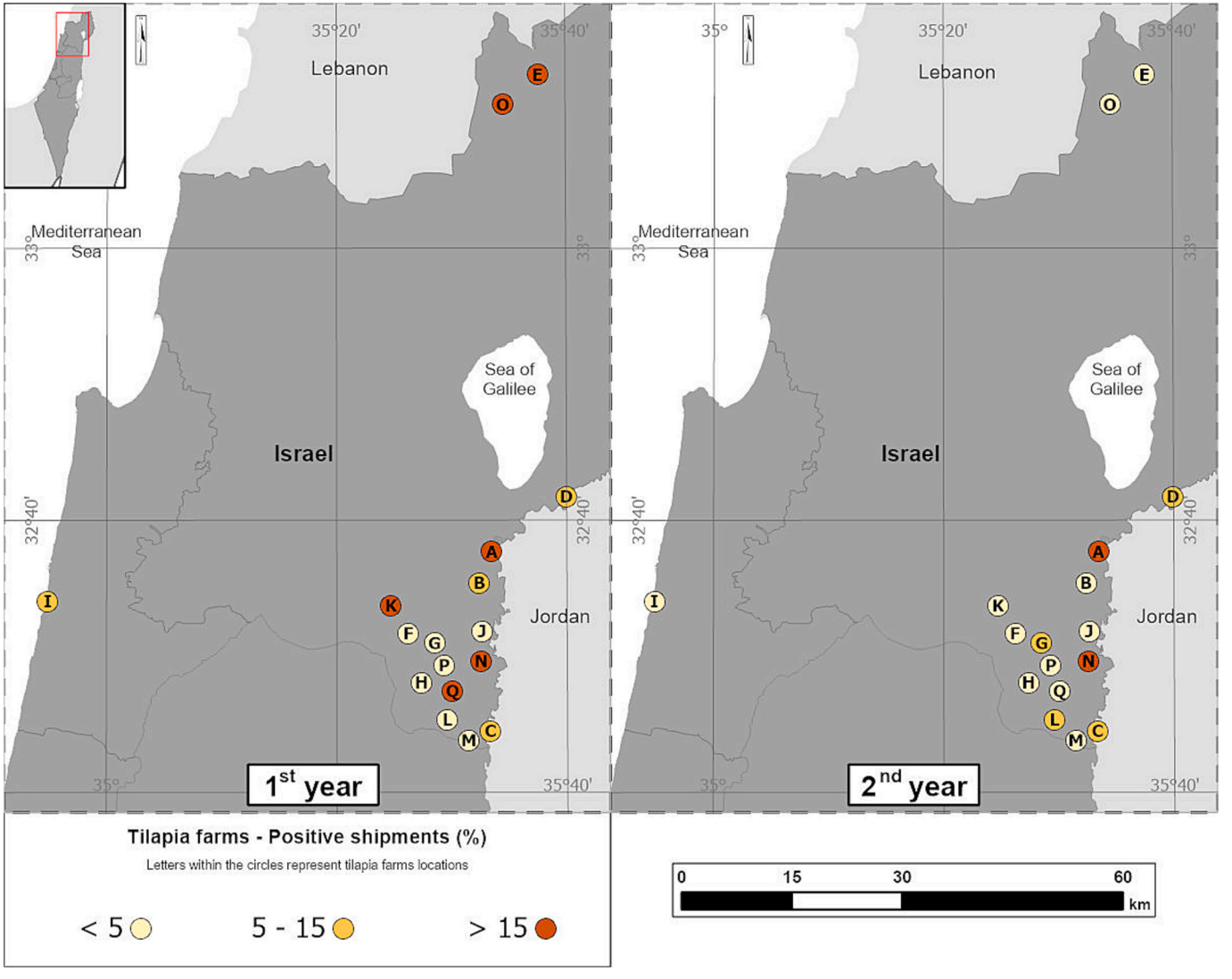

Fig. 2. Northern Israel with farm localities and prevalence of infection during the 1st and 2nd year of study.

Table 2
Distribution of tilapia infected by *Contracaecum* larvae relative to number of positive shipments.

No. of infected fish within a shipment	No. of shipments	%
1	217	80.7
2	37	13.8
3	6	2.2
4	1	0.4
5	4	1.5
>6	4	1.5
Total	**269**	

4. Discussion

The objectives of the present investigation were to assess the prevalence of *Contracaecum* larvae over a two-year period across 17 tilapia farms in Israel, to identify the larvae at the species level, and to evaluate the effectiveness and limitations of the current sampling strategy used in official surveillance for detecting *Contracaecum* larvae in market-size tilapia. We also modeled how different sample sizes affect the probability of detection. Our two-year survey revealed extremely variable prevalence among farms, and even between the first and second year of sampling (Fig. 2). Although the visual inspection method carried out in the present investigation is a commonly applied approach, it is important to note that smaller larvae, such as those of *Contracaecum rudolphii s. l.*, can easily be overlooked by visual methods alone; consequently, it is not possible to exclude that *Contracaecum* spp. diversity, together with prevalence and intensity values reported herein may be underestimated. As concerns the species identified in the current study, Caffara et al. (2023) showed that the great white pelican (*Pelecanus onocrotalus*) is a suitable definitive host of both *C. multipapillatum* E and *C. quadripapillatum*, although both of these species were found at low prevalence in the examined birds. Therefore, the number of pelicans that migrate through Israel twice a year and their feeding behavior may impact the number of infected tilapias within a pond.

According to the Israeli procedure for fish premarketing control (Israeli Veterinary Services, 2017), the official cut examination of farmed fish includes at least three specimens. In June 2020, after a second case of heavily infected tilapia and red drum was found (53.8 % and 40.9 %, respectively), the DSFAO decided to increase the number of tilapia specimens tested by cut in all tilapia shipments to 30 fish. In contrast to ecological studies, official controls of fish before marketing should consider several aspects, such as time spent examining individual specimens of a given batch, localization of the larva in the host, larval size, and the cost of marketing filleted fish. In our study, we critically evaluated whether, in this case, the decision to intensify tilapia sampling and to eviscerate and market all fish as fillets when two or more larvae were found was justified. Apart from collecting and analyzing data over a two-year period, we evaluated different surveillance schemes; considering the relatively low prevalence of *Contracaecum* larvae detected in the current study, sampling 30 individuals from a given shipment would yield a 40 % probability of finding a parasitized tilapia and a 10 % probability of making the decision to forward the shipment to processing and cleaning. Increasing the sample size to 60 individuals would lead to the decision to redirect 25 % of shipments to processing and cleaning. It is important to acknowledge that even with intensified sampling strategies, there is no absolute guarantee that all infected specimens will be detected prior to marketing. Host infection within and between batches is inherently variable, and the probability models presented here estimate only the likelihood of detecting at least one infected fish based on assumed prevalence. Therefore, the proposed procedures should be regarded as a risk-reduction tool rather than a complete safeguard. Similar challenges have been reported for other foodborne parasitic nematodes, such as *Anisakis simplex* in marine fish and *Gnathostoma* spp. in freshwater fish, where visual inspection and sample-based surveillance substantially reduce, but do not eliminate, the possibility of contaminated fish reaching consumers (Karl et al., 2011; Diaz, 2015). In both marine and freshwater contexts, the most effective strategies combine targeted sampling with additional preventive measures, such as consumer education, post-harvest processing, and, where relevant, freezing protocols for high-risk products. Incorporating these broader lessons into tilapia surveillance programs could improve efficiency while maintaining robust public health protection.

Beyond our national findings, several studies have emphasized that the effectiveness of parasite detection schemes is strongly dependent on sample size and inspection methodology (Shvydka et al., 2018). Shamsi and Suthar (2016) highlighted that limited sampling can substantially underestimate the prevalence of *Contracaecum* larvae in fish populations, stressing the importance of considering larval distribution patterns when designing surveillance protocols. Similar concerns were raised in food safety contexts, where Guillier et al. (2011) demonstrated that inappropriate or insufficient sampling strategies in fishery products may compromise the reliability of official inspection systems. From a methodological perspective, stochastic modeling approaches have also been applied to optimize sampling in food control; for instance, Guardone et al. (2016) proposed probabilistic tools for estimating detection power under different prevalence assumptions, underscoring that risk-based frameworks can improve efficiency compared to fixed sample numbers. Comparable issues are recognized in aquaculture parasitology, where even well-designed surveillance may fail to detect rare or unevenly distributed infections. Marques et al. (2010) noted that parasite aggregation and host variability in fish farms often require adaptive sampling schemes rather than uniform standards. Taken together, these studies reinforce our observation that while intensified sampling increases detection sensitivity, it should ideally be embedded in a flexible, risk-based inspection framework that accounts for host–parasite ecology, logistic feasibility, and public health priorities.

In conclusion, this two-year national-scale surveillance study offers the first comprehensive evaluation of *Contracaecum* spp. larvae in farmed tilapia in Israel and provides essential data to inform public health policy for other tilapia farming countries. Despite the detection of *C. multipapillatum* E in multiple farms, the overall prevalence was low, and in most positive shipments, only a single larva was found. The probability of detecting infected fish using the current sample size of 30 specimens per batch remains limited, with only

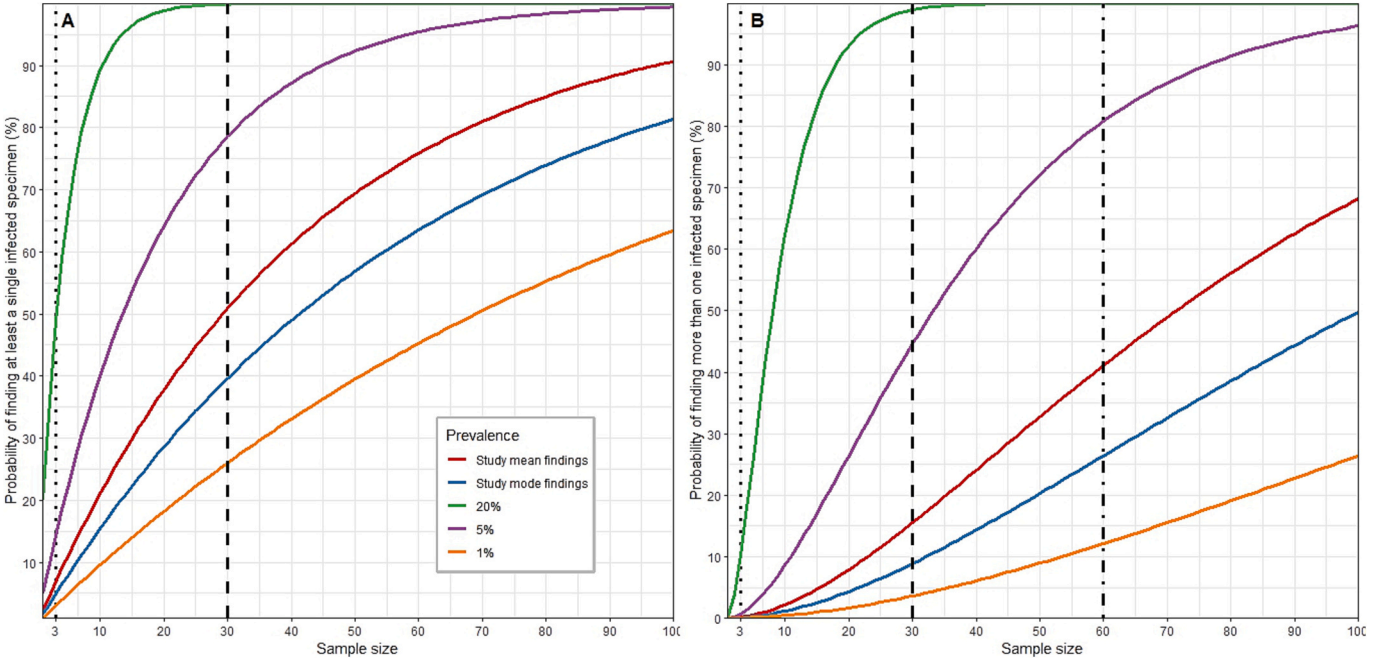

Fig. 3. (A) Calculation of the probability of finding at least a single infected specimen (%) considering an increase in sample size. (B) Calculation of the probability of finding more than one infected specimen (%) considering an increase in sample size.

a 40 % chance of identifying an infected specimen. While intensified sampling enhances detection sensitivity, it must be balanced against logistical constraints and the lack of evidence for the zoonotic potential of *C. multipapillatum* E and *C. quadripapillatum*. In light of this, we recommend shifting from blanket intensified sampling to a risk-based approach, focusing enhanced surveillance on farms or ponds with historically high prevalence. This targeted strategy would optimize resource allocation while maintaining seafood safety standards. Our findings highlight the need to continually refine inspection protocols based on epidemiological evidence to ensure efficient and science-driven public health interventions.

CRediT authorship contribution statement

Nadav Davidovich: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Perla Tedesco:** Writing – review & editing, Methodology, Investigation. **Monica Caffara:** Writing – review & editing, Methodology, Investigation. **Ekaterina Minkova:** Investigation. **Ortal Aflalo:** Investigation. **Shoshi Hadar:** Investigation. **Vicotoria Baramboim:** Investigation. **Gavriel Goldstein:** Investigation. **Ofer Cohen:** Investigation. **Shani Glasner:** Investigation. **Michal Perry Markovich:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Danny Morick:** Writing – review & editing. **Aurora Lattanzi:** Investigation, Formal analysis. **Andrea Gustinelli:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adroher-Auroux, F.J., Benítez-Rodríguez, R., 2020. Anisakiasis and *Anisakis*: an underdiagnosed emerging disease and its main etiological agents. Res. Vet. Sci. 132, 535–545. https://doi.org/10.1016/j.rvsc.2020.08.003.

Ángeles-Hernández, J.C., Gómez-deAnda, F.R., Reyes-Rodríguez, N.E., Vega-Sánchez, V., García-Reyna, P.B., Campos-Montiel, R.G., Zepeda-Velázquez, A.P., 2020. Genera and species of the Anisakidae family and their geographical distribution. Animals 10 (12), 2374.

Buchmann, K., Mehrdana, F., 2016. Effects of anisakid nematodes *Anisakis simplex* (s.l.), *Pseudoterranova decipiens* (s.l.) and *Contracaecum osculatum* (s.l.) on fish and consumer health. Food Waterborne Parasitol. 4, 13–22. https://doi.org/10.1016/j.fawpar.2016.07.003.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. Revisited. J. Parasitol. 83, 575. https://www.jstor.org/stable/3284227?origin=crossref.

Caffara, M., Tedesco, P., Davidovich, N., Locke, S.A., Gustinelli, A., King, R., Nuytten, M., Nuzzo, M., Fioravanti, M.L., 2023. Advancing understanding of the taxonomy and diversity of the genus *Contracaecum* in the great white pelican (*Pelecanus onocrotalus*). Parasitol. Res. 122, 315–331. https://doi.org/10.1007/s00436-022-07732-z.

D'amico, P., Malandra, R., Costanzo, F., Castigliego, L., Guidi, A., Gianfaldoni, D., Armani, A., 2014. Evolution of the *Anisakis* risk management in the European and Italian context. Food Res. Int. 64, 348–362. https://doi.org/10.1016/j.foodres.2014.06.038.

Davidovich, N., Tedesco, P., Caffara, M., Yasur-Landau, D., Gustinelli, A., Drabkin, V., Minkova, E., Aflalo, O., Morick, D., Fioravanti, M.L., 2022. Morphological description and molecular characterization of *Contracaecum* larvae (Nematoda: Anisakidae) parasitizing market-size hybrid tilapia (*Oreochromis aureus* x *Oreochromis niloticus*) and red drum (*Sciaenops ocellatus*) farmed in Israel. Food Waterborne Parasitol. 26, e00147. https://doi.org/10.1016/j.fawpar.2022.e00147.

Davidovich, N., Tedesco, P., Caffara, M., Luci, V., Cantori, A., Morick, D., Fioravanti, M.L., Gustinelli, A., 2023. Low prevalence of *Contracaecum* third-stage larvae parasitizing sea of Galilee fisheries: 1-year survey after 57 years of no information. Food Waterborne Parasitol. 32, e00204. https://doi.org/10.1016/j.fawpar.2023.e00204.

Diaz, J.H., 2015. Gnathostomiasis: an emerging infection of raw fish consumers in *Gnathostoma* nematode-endemic and nonendemic countries. J. Travel Med. 22 (5), 318–324.

Dziekońska-Rynko, J., Rokicki, J., 2007. Life cycle of the nematode *Contracaecum rudolphii* Hartwig, 1964 (sensu lato) from northern Poland under laboratory conditions. Helminthologia 44, 95–102. https://doi.org/10.2478/s11687-007-0013-9.

Fagerholm, H.P., 1988. Patterns of caudal papillae in *Contracaecum osculatum* (Nematoda) and some related species from different regions of the world. Int. J. Parasitol. 18 (8), 1039–1051. https://doi.org/10.1016/0020-7519(88)90074-4.

FDA, 2019. Chapter 5: Parasites. In: Fish and Fishery Products Hazards and Controls Guidance. Food and Drug Administration, pp. 91–98. http://www.fda.gov/downloads/food/guidanceregulation/ucm252393.pdf.

Gajadhar, A.A., Pozio, E., Gamble, H.R., Nöckler, K., Maddox-Hyttel, C., Forbes, L.B., Vallée, I., Rossi, P., Marinculić, A., Boireau, P., 2009. *Trichinella* diagnostics and control: mandatory and best practices for ensuring food safety. Vet. Parasitol. 159, 197–205. https://doi.org/10.1016/j.vetpar.2008.10.063.

Guillier, L., Thébault, A., Gauchard, F., Pommepuy, M., Guignard, A., & Malle, P. (2011). A risk-based sampling plan for monitoring of histamine in fish products, J. Food Prot., 74(2), 302-310.

Guardone, L., Malandra, R., Costanzo, F., Castigliego, L., Tinacci, L., Gianfaldoni, D., Armani, A., 2016. Assessment of a sampling plan based on visual inspection for the detection of anisakid larvae in fresh anchovies (*Engraulis encrasicolus*). A first step towards official validation? Food Anal. Methods 9 (5), 1418–1427.

Hamouda, A.H., Younis, A.E., 2022. Molecular characterization of zoonotic anisakid *Contracaecum* spp. larvae in some fish species from Lake Nasser, Egypt. Aquac. Res. 53, 2548–2561. https://doi.org/10.1111/are.15774.

Han, F., Huang, X., Mahunu, G.K., 2017. Exploratory review on safety of edible raw fish per the hazard factors and their detection methods. Trends Food Sci. Technol. 59, 37–48. https://doi.org/10.1016/j.tifs.2016.11.004.

Israeli Veterinary Services, 2017. Pre-marketing Control of Locally Grown Edible Fish: Procedure. https://www.gov.il/he/departments/policies/moag-pro-082 (2017) (in Hebrew).

Kaba, T., Shurbe, M., Wondimu, A., 2024. Awareness of fish-borne zoonoses and prevalence of *Contracaecum* in *Oreochromis niloticus* and *Lates niloticus* collected from Lake Chamo, Arba Minch, Ethiopia. Zoonoses Public Health 71, 790–798. https://doi.org/10.1111/zph.13145.

Karl, H., Baumann, F., Ostermeyer, U., Kuhn, T., Klimpel, S., 2011. *Anisakis simplex* (ss) larvae in wild Alaska salmon: no indication of post-mortem migration from viscera into flesh. Dis. Aquat. Org. 94 (3), 201–209.

Kochanowski, M., Dąbrowska, J., Różycki, M., Karamon, J., Sroka, J., Cencek, T., 2020. Proteomic profiling reveals new insights into the allergomes of *Anisakis simplex*, *Pseudoterranova decipiens*, and *Contracaecum osculatum*. J. Parasitol. 106, 572–588. https://doi.org/10.1645/19-75.

Køie, M., Fagerholm, H.P., 1995. The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. Parasitol. Res. 81, 481–489. https://doi.org/10.1007/BF00931790.

Landsberg, J.H., 1989. Parasites and associated diseases of fish in warm water culture with special emphasis on intensification. In: Shilo, M., Sarig, S. (Eds.), Fish Culture in Warm Water Systems: Problems and Trends. CRC Press, pp. 195–252.

Marques, J.F., Santos, M.J., Cabral, H.N., 2010. Aggregation patterns of macroendoparasites in phylogenetically related fish hosts. Parasitology 137 (11), 1671–1680.

Mattiucci, S., Paoletti, M., Solorzano, A.C., et al., 2010. (2010). *Contracaecum gibsoni* n. sp. and *C. Overstreeti* n. sp. (Nematoda: Anisakidae) from the Dalmatian pelican *Pelecanus crispus* (L.) in Greek waters: genetic and morphological evidence. Syst. Parasitol. 75, 207–224. https://doi.org/10.1007/s11230-009-9220-8.

Mattiucci, S., Sbaraglia, G.L., Palomba, M., Filippi, S., Paoletti, M., Cipriani, P., Nascetti, G., 2020. Genetic identification and insights into the ecology of *Contracaecum rudolphii* a and *C. Rudolphii* B (Nematoda: Anisakidae) from cormorants and fish of aquatic ecosystems of Central Italy. Parasitol. Res. 119, 1243–1257. https://doi.org/10.1007/s00436-020-06658-8.

Nagasawa, K., 2012. The biology of *Contracaecum osculatum* sensu lato and *C. Osculatum* a (Nematoda: Anisakidae) in Japanese waters: a review. Biosphere Sci. 51, 61–69. https://www.hiroshima-u.ac.jp/system/files/7855/07nagasawa.pdf.

Nonković, D., Tešić, V., Šimat, V., Karabuva, S., Medić, A., Hrabar, J., 2025. Anisakidae and anisakidosis: a public health perspective. Pathogens 14, 217. https://doi.org/10.3390/pathogens14030217.

Olson Jr., A.C., Lewis, M.D., Hauser, M.L., 1983. Proper identification of anisakine worms. Am. J. Med. Technol. 49 (2), 111–114.

Otachi, E.O., Szostakowska, B., Jirsa, F., Fellner-Frank, C., 2014. Parasite communities of the elongate tigerfish *Hydrocynus forskahlii* (Cuvier 1819) and redbelly tilapia *Tilapia zillii* (Gervais 1848) from Lake Turkana, Kenya: influence of host sex and size. Acta Parasitol. 60, 9–20. https://doi.org/10.1515/ap-2015-0002.

Paperna, I., 1964. The metazoan parasite fauna of Israel inland water fishes. Bamidgeh Bull. Fish Cult. Israel 16, 3–66.

Paperna, I., 1974. Larval *Contracaecum* in the pericardium of fishes from east African lakes. Proc. Helminthol. Soc. Wash. 41, 252.

R Core Team, 2024. R: A Language and Environment for Statistical Computing Accessed. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rahmati, A.R., Kiani, B., Afshari, A., Moghaddas, E., Williams, M., Shamsi, S., 2020. World-wide prevalence of Anisakis larvae in fish and its relationship to human allergic anisakiasis: a systematic review. Parasitol. Res. 119, 3585–3594. https://doi.org/10.1007/s00436-020-06892-0.

Robertson, L.J., 2025. Transmission of parasites to people via food: how can we determine their impact and why do we need to know? Food Waterborne Parasitol. 39, e00258. https://doi.org/10.1016/j.fawpar.2025.e00258.

Shamsi, S., 2019. Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. Paras. Epidemiol. Control 4, e00087. https://doi.org/10.1016/j.parepi.2019.e00087.

Shamsi, S., Barton, D.P., 2023. A critical review of anisakidosis cases occurring globally. Parasitol. Res. 122 (8), 1733–1745. https://doi.org/10.1007/s00436-023-07881-9.

Shamsi, S., Butcher, A.R., 2011. First report of human anisakidosis in Australia. Med. J. Aust. 194, 199–200. https://doi.org/10.5694/j.1326-5377.2011.tb03772.x.

Shamsi, S., Suthar, J., 2016. A revised method of examining fish for infection with zoonotic nematode larvae. Int. J. Food Microbiol. 227, 13–16.

Shamsi, S., Norman, R., Gasser, R., Beveridge, I., 2009. Redescription and genetic characterization of selected *Contracaecum* spp. (Nematoda: Anisakidae) from various hosts in Australia. Parasitol. Res. 104, 1507–1525. https://doi.org/10.1007/s00436-009-1357-5.

Shamsi, S., Gasser, R.B., Beveridge, I., 2011. Mutation scanning-coupled sequencing of nuclear ribosomal DNA spacers as a tool for the specific identification of different *Contracaecum* (Nematoda: Anisakidae) larval types. Mol. Cell. Probes 25 (1), 13–18. https://doi.org/10.1016/j.mcp.2010.09.003.

Shamsi, S., Turner, A., Wassens, S., 2018. Description and genetic characterization of a new *Contracaecum* larval type (Nematoda: Anisakidae) from Australia. J. Helminthol. 92 (2), 216–222. https://doi.org/10.1017/S0022149X17000360.

Shamsi, S., Nelson, L., Gordon, A., Markham, K., Francis, N., Suthar, J., Zhu, X., 2024. Multidisciplinary approach to the diagnosis of *Contracaecum magnipapillatum* infections in Australian black noddies, Anous minutus (Charadriiformes: Laridae). Parasitol. Res. 123 (1), 90. https://doi.org/10.1007/s00436-023-08050-8.

Shvydka, S., Sarabeev, V., Estruch, V.D., Cadarso-Suárez, C., 2018. Optimum sample size to estimate mean parasite abundance in fish parasite surveys. Helminthologia 55 (1), 52.

Smith, J.W., 1999. Ascaridoid nematodes and pathology of the alimentary tract and its associated organs in vertebrates, including man: a literature review. Helminthol. Abstracts 68 (2), 49–96.

Stentiford, G.D., Bateman, I.J., Hinchliffe, S.J., Bass, D., Hartnell, R., Santos, E.M., Devlin, M.J., Feist, S.W., Taylor, N.G.H., Verner-Jeffreys, D.W., van Aerle, R., Peeler, E.J., Higman, W.A., Smith, L., Baines, R., Behringer, D.C., Katsiadaki, I., Froehlich, H.E., Tyler, C.R., 2020. Sustainable aquaculture through the one health lens. Nat. Food 1, 468–474. https://doi.org/10.1038/s43016-020-0127-5.

Suthar, J., Shamsi, S., 2021. The occurrence and abundance of infective stages of zoonotic nematodes in selected edible fish sold in Australian fish markets. Microb. Pathog. 154, 104833. https://doi.org/10.1016/j.micpath.2021.104833.

Team, P. (2023). RStudio: integrated development environment for R. Posit Software. *PBC*. http://www.posit.co.

Wickham, H., 2016. Programming With ggplot2. ggplot2. Use R! Springer, Cham. https://doi.org/10.1007/978-3-319-24277-4_12.

Younis, A.E., Saad, A.I., Rabei, J.M., 2017. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in four teleostean species from Lake Nasser, Egypt: morphological and molecular studies. J. Basic Appl. Zool. 78, 9. https://doi.org/10.1186/s41936-017-0012-4.

Zhu, X., Gasser, R.B., Podolska, M., Chilton, N.B., 1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. Int. J. Parasitol. 28 (12), 1911–1921.

Zhu, X.Q., D'Amelio, S., Gasser, R.B., Yang, T.B., Paggi, L., He, F., Li, A.X., 2007. Practical PCR tools for the delineation of Contracaecum rudolphii A and Contracaecum rudolphii B (Ascaridoidea: Anisakidae) using genetic markers in nuclear ribosomal DNA. Mol. cell. probes 21 (2), 97–102.

Zuo, S., Kania, P.W., Mehrdana, F., Marana, M.H., Buchmann, K., 2018. *Contracaecum osculatum* and other anisakid nematodes in grey seals and cod in the Baltic Sea: molecular and ecological links. J. Helminthol. 92, 81–89. https://doi.org/10.1017/S0022149X17000025.